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**APPETITE REGULATING
NEUROPEPTIDES IN ALCOHOL
ADDICTION: FOCUS ON MELANIN
CONCENTRATING HORMONE AND ITS
MCH1-RECEPTOR**

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APPETITE REGULATING NEUROPEPTIDES IN ALCOHOL
ADDICTION: FOCUS ON MELANIN CONCENTRATING
HORMONE AND ITS MCH1-R
THESIS FOR DOCTORAL DEGREE (Ph.D)

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ABSTRACT

Current treatment options for alcohol use disorders are limited in number and have limited efficacy. It is therefore important to find new, more effective medications. In this thesis, the work focused on the involvement of the hypothalamic neuropeptide Melanin Concentrating Hormone (MCH) and its MCH-1 receptor (MCH1-R) in alcohol related behaviors in rodents.

In the initial study, the selective MCH1-R antagonist GW803430 was evaluated in animal models of motivation to obtain alcohol and relapse to alcohol-seeking in rats using operant self-administration. GW803430 potently attenuated alcohol self-administration and cue-induced reinstatement while reinstatement induced by a foot-shock stressor was unaffected. To extend these findings, GW803430 was assessed in states of escalated alcohol consumption. In rats consuming high amounts of alcohol during intermittent access GW803430 treatment significantly reduced intake of both alcohol and feed while in low drinking rats only food intake was decreased. Following protracted abstinence induced by intermittent access, alcohol self-administration was significantly attenuated by GW803430. In contrast, GW803430 had no effect on escalated alcohol self-administration induced by vapor exposure. These studies provide evidence for a combined effect of the MCH1-R antagonist on consumption of alcohol through effects both on appetite for calories, and rewarding alcohol actions.

In order to evaluate effects on sugar, which also has addiction-like properties and activate pathways in the brain overlapping those for drugs of abuse, we assessed GW803430 on sucrose and saccharin self-administration. While sucrose consumption was significantly decreased by GW803430, no effect was seen on saccharin intake, suggesting that MCH1-R blockade primarily regulates calorie intake. However, GW803430 also reduced cue-induced seeking and enhanced motivation to obtain sucrose under a progressive ratio schedule. Reward from palatable food and drugs of abuse can activate overlapping neurobiological mechanisms, and the findings further indicate that the MCH1-R may have a dual role on appetite for calories and reward.

Based on the findings that the MCH1-R regulates the rewarding properties of both alcohol and sucrose, alcohol reward was investigated in mice in the conditioned place preference (CPP) model to avoid calorie intake as a confounding variable. Genetic deletion of the MCH1-R prevented alcohol induced CPP and this finding was replicated in wildtype C57BL/6 mice treated with GW803430. Downstream signaling mechanisms of the MCH1-R after acute alcohol administration were further investigated. Immunohistochemistry showed

that acute alcohol administration induced phosphorylation of the dopamine and cAMP regulated phospho protein 32 (p-DARPP-32) downstream of the MCH1-R.

In conclusion, our results suggest a role of MCH and its MCH1-R both in calorie intake and in regulation of alcohol reward.

LIST OF SCIENTIFIC PAPERS

- I. A. Cippitelli, **C. Karlsson**, J L. Shaw, A. Thorsell, D R. Gehlert and M. Heilig. Suppression of alcohol self-administration and reinstatement of alcohol seeking by melanin-concentrating hormone receptor 1 (MCH1-R) antagonism in Wistar rats.
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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
AUD	Alcohol use disorder
CeA	Central amygdala
CRH	Corticotropin-releasing hormone
CPA	Conditioned place aversion
CPP	Conditioned place preference
DA	Dopamine
DARPP-32	Dopamine and cyclic adenosine monophosphate phosphoprotein 32 kilo dalton
ERK	Extracellular signal regulated kinases
GABA	γ -aminobutyric acid
GPCR	G-protein coupled receptor
HPA	Hypothalamic-pituitary-adrenal
LH	Lateral hypothalamus
MCH	Melanin concentrating hormone
MCH1-R	Melanin concentrating hormone 1 receptor
MCH2-R	Melanin concentrating hormone 2 receptor
NAc	Nucleus accumbens
NAcSh	Nucleus accumbens shell
PVN	Paraventricular nucleus
VTA	Ventral tegmental area

1 INTRODUCTION TO ALCOHOL ADDICTION

Alcohol is consumed worldwide, mainly for its pleasurable effects. Although occasional consumption does not necessarily have harmful effects, drinking pattern and the amount of alcohol consumed is directly related to the individual's health (Antai et al. 1993). Harmful alcohol use has a significant causal role in numerous types of chronic diseases. The top five categories listed by the world health organization (WHO) are cancers, cardiovascular diseases, diabetes, neuropsychiatric disorders, gastrointestinal diseases, and infectious diseases (World Health Organization 2004). Further, frequent intoxication is associated with increased risk-taking and impulsivity which, in turn, may result in harmful consequences such as motor vehicle accidents causing harm to others as well as the intoxicated individual (Cherpitel 1993).

In addition to causing increased healthcare costs due to physical and psychological health problems, alcohol use and abuse also contribute to detrimental societal and economic consequences such as decreased productivity and criminality (Rehm et al. 2009, World Health Organization 2004). WHO reports that 3.3 million deaths per year are caused by alcohol, an equivalent of 5.9% of all deaths globally. Alcoholism also significantly contributes to the global disease burden, 5.1%, which is calculated as disability adjusted life years, DALYs, lost (World Health Organization 2004).

Excessive drinking has historically been considered as a character flaw or lack of discipline, a view that has only partly changed over time as alcoholism has slowly become accepted as a disease. Approximately 2 billion people worldwide consume alcohol, and between 5-10% of them will escalate their use and develop addiction (World Health Organization 2004). An interplay between biological, psychological, and social processes has been linked to progression of alcohol addiction (Altman et al. 1996, Pellmar et al. 2002). Heritability contributes approximately 50 - 60% of the risk for developing this condition, leaving the remaining variance in risk to influences of shared as well as individual environmental factors (Goldman et al. 2005).

Although heritability increases the risk of developing alcohol addiction, the interaction with the environment is important to determine whether addiction will in fact arise. Environmental factors such as stress and trauma can increase the vulnerability (Goeders 2003, Volpicelli et al. 1999). Cues associated with alcohol, including social settings and people are also important factors contributing to progression to addiction (Donovan and Marlatt 1980).

1.1 ALCOHOL ADDICTION

Alcohol addiction, hereafter equated with “alcoholism”, is a chronic, relapsing disorder that affects numerous organ systems. The Swedish physician, Magnus Huss (1849), was among the first to coin the term “alcoholism”, which has since been replaced with other terms such as alcohol abuse and alcohol dependence. In modern times, a classic description of an “alcohol dependence syndrome” was introduced by Edwards and Gross (Edwards and Gross 1976).

1.1.1 Diagnostic criteria for alcoholism

Alcoholism is a chronic relapsing disorder characterized by multiple symptoms. For a diagnosis, certain criteria have to be met, which in the US are described in a classification system, the Diagnostic and Statistical Manual of Mental Disorders (DSM) (American Psychiatric Association 1994). In Europe and other parts of the world, the International Statistical Classification of Diseases and Related Health Problems (ICD) (World Health Organization 2004) is widely used. Recently, the fifth edition of the DSM (DSM-5) was published (American Psychiatric Association 2013), which includes several changes from the previous version, DSM-IV (American Psychiatric Association 1994). In the DSM-5, diagnostic criteria for alcohol use disorders are grouped in four blocks (Table 1).

The first four criteria describe impaired control over substance use, such as having problems cutting down on drinking and consuming more alcohol over time than was intended. Craving for alcohol or a strong desire to drink has been added as a new criterion.

Criteria 5-7 focus on social impairments from consuming alcohol and have not been changed from the previous version. They involve the negative outcomes of alcohol use affecting social and professional life and describe negative impact on work, school or other activities, as well as hobbies the individual is willing to give up for drinking alcohol.

In the third grouping, criteria 8-9 describe the risky use of alcohol. Alcohol use may put the individual in situations that could be of physical risk such as driving under intoxication, or being violent as a result from excessive drinking. Existing physiological and psychological problems may also be exacerbated by alcohol drinking.

The last two criteria, 10-11, describe symptoms of tolerance and withdrawal. Tolerance is a hallmark of addiction where more alcohol is needed over time to produce the wanted effect.

Tolerance also includes a lesser sensitivity to the sedative properties of alcohol and adaptations in motor coordination. However, individual variability in response to alcohol can sometimes make it difficult to determine if tolerance has developed. Withdrawal is the last criterion, a syndrome that occurs when alcohol exposure is terminated after excessive use that has led to the development of tolerance. The withdrawal symptoms can be both physical and psychological. Symptoms include tremor, anxiety, sweating and may in severe cases induce delirium. In order to alleviate these symptoms it is likely that the individual will continue to consume alcohol to get rid of the aversive feelings (American Psychiatric Association 2013).

In DSM-IV, a diagnosis of alcohol dependence was based on meeting three out of seven criteria within a year. Based on a separate set of criteria, an individual who did not meet criteria for alcohol dependence could instead be diagnosed with alcohol abuse (American Psychiatric Association 1994). This has been modified in DSM-5, where a single category of “alcohol use disorder (AUD)” has replaced “abuse” and “dependence”, and is now graded as “mild”, “moderate” or “severe” based on number of criteria met within a year. The presence of two or three symptoms corresponds to mild AUD, four to five moderate AUD, and six or more indicate severe AUD. Withdrawal is not necessary for a diagnosis but is highly associated with severe AUD (American Psychiatric Association 2013). Additionally, according to the DSM- 5, a diagnosis of alcohol use disorder with or without physical dependence is used.

CRITERIA FOR ALCOHOL USE DISORDER ACCORDING TO DSM-5

1. More alcohol is consumed than intended and over a longer period of time
2. Wanting to cut down or stop using alcohol but not managing to
3. Spending a lot of time getting, using, or recovering from use of alcohol
4. Cravings and urges to use alcohol

5. Problems keeping up with work, home or school, because of alcohol use
6. Continuing to use, even when it causes problems in relationships
7. Giving up important social, occupational or recreational activities because of alcohol use

8. Alcohol is used continuously, despite it puts you in danger
9. Alcohol use is continued, even when physical or psychological problem which could have been caused or made worse by alcohol use

10. Needing more alcohol to obtain the desired effect (tolerance)
11. Development of withdrawal symptoms, which can be relieved by consuming more alcohol

Table 1. Diagnostic criteria for alcohol use disorder according to DSM-5. The recent update was published by American Psychiatric Association in November 2013 (American Psychiatric Association 2013).

1.1.2 Alcohol addiction starts with positive reinforcement by alcohol

In the early stages of the addictive process, drinking to intoxication is primarily driven by positive reinforcement by alcohol, i.e. a motivation to obtain alcohol for its pleasurable effects (Brown et al. 1980). At this stage, impulsive reward craving for alcohol is driving further binge drinking (Figure 1), leading to heavy use, and periods of sobriety (Heilig and Koob 2007, Koob 1992, Koob and Volkow 2009). Reward craving is highly associated with environmental stimuli, or cues, previously experienced during alcohol use, such as social context, specific individuals or places (Heinz et al. 2003). This conditioned craving is an important factor in the process of developing addiction, because of its ability to induce relapse to alcohol seeking and intake even after long periods of abstinence during which these

behaviors have been extinguished, a phenomenon referred to as “reinstatement” (Grüsser et al. 2004, Sinha and Li 2007).

Although alcohol does not have a unique target site in the nervous system, such as a receptor or transporter, it ultimately impacts numerous neurochemicals and receptor systems throughout the brain which impact the homeostasis of the brain [see below; (Covarrubias et al. 2006, Ronis et al. 2007, Spanagel 2009)]. It is therefore challenging to identify which of these systems contributes the most to the transition from normal to compulsive drug use. Several major neurotransmitter systems have been implicated in the regulation of reward from alcohol. These include dopamine (DA), endogenous opioids, γ -aminobutyric acid (GABA) and glutamate systems, all of which have been extensively studied in relation to alcoholism (Cowan and Lawrence 1999, Holmes et al. 2013, Koob 1992, Xiao and Ye 2008). These systems interact, and mediate alcohol reward in part through actions within the ventral tegmental area (VTA) and the nucleus accumbens (NAc), a circuit broadly implicated in reinforcement and drug addiction (Brodie et al. 1999, Di Chiara and Imperato 1988, Di Chiara et al. 2004, Spanagel and Weiss 1999). In addition to its rewarding effects, acute alcohol intoxication also results in anxiolytic, sedative and ataxic effects, which are likely associated with enhancement of GABA and inhibition of glutamate transmission (Koob 1992) .

As positively reinforcing, or “rewarding” brain mechanisms are pathologically engaged by alcohol use, the nervous system responds with processes of compensatory, opponent actions, or “neuroadaptations” in order to maintain homeostasis (Solomon and Corbit 1974). At this early stage in the addictive process, however, opponent neuroadaptations go back to normal once the individual has recovered from intoxication. Underlying mechanisms driving reward and reinforcement will be further discussed in chapter 1.2.

1.1.3 Progression into alcohol addiction is through negative reinforcement

Excessive drinking over a time course of 5-10 years is thought to be necessary for developing alcohol addiction (Mann et al. 2005, Zilberman et al. 2004, Åmark 1951). At this point, opponent processes in the brain triggered by heavy alcohol use become long lasting, or even permanent, resulting in the emergence of tolerance for the pleasurable effects of alcohol, and a negative emotional state once alcohol intake is discontinued, a process that has been referred to as affective allostasis (Koob and Le Moal 2001, McEwen 2000, Valdez and Koob 2004). Tolerance for pleasurable alcohol effects is caused by dysregulation of multiple

neurochemicals in the brain, which involves down regulation of DA and GABA receptors in response to prolonged enhanced release (Biggio et al. 2003, Funk et al. 2006, Grobin et al. 1998). Once alcohol consumption is discontinued, the neuroadaptations that counteracted alcohol effects become unopposed, resulting in a loss of homeostasis or, rather “allostasis”, i.e. the new pathological equilibrium that has been established in presence of alcohol.

Withdrawal from alcohol comprises both a physiological and an affective component. Much more is known about former than the latter. Physiological withdrawal is associated with generally increased excitability due to enhanced glutamate release following impaired GABA-inhibition of glutamatergic neurons (Gallegos et al. 1999, Heilig et al. 2010). Increased levels of extracellular glutamate and changes in expression of glutamate receptors have been observed during abstinence (Holmes et al. 2013). In acute withdrawal (in general within 3 days after alcohol is discontinued), physical signs of withdrawal appear, characterized by tremor, increased heart rate and sweating, and even include the risk for epileptic seizures and delirium tremens (Heilig et al. 2010, Mayo-Smith et al. 2004, Victor and Adams 1952). Affective processes associated with withdrawal symptoms are more persistent, last into protracted abstinence, and are likely more relevant for maintaining addiction. The negative emotional state associated with protracted abstinence is characterized by elevated anxiety, low mood and enhanced stress sensitivity. The desire to alleviate these highly aversive symptoms sets up a powerful incentive to resume alcohol use, [Figure 1; (Heilig and Koob 2007, Heilig et al. 2011, Stewart et al. 2001)]. Thus, craving for alcohol is now driven by negative reinforcement (Koob and Le Moal 2005), and consumption becomes compulsive, i.e. insensitive to negative consequences. Although several neuroadaptive processes have been suggested to account for the switch from positively to negatively reinforced alcohol seeking and use, the exact mechanisms behind this transition are still largely unknown. Understanding the neurobiology underlying these behavioral changes is important in order to develop new, more efficient treatments for alcohol addiction.

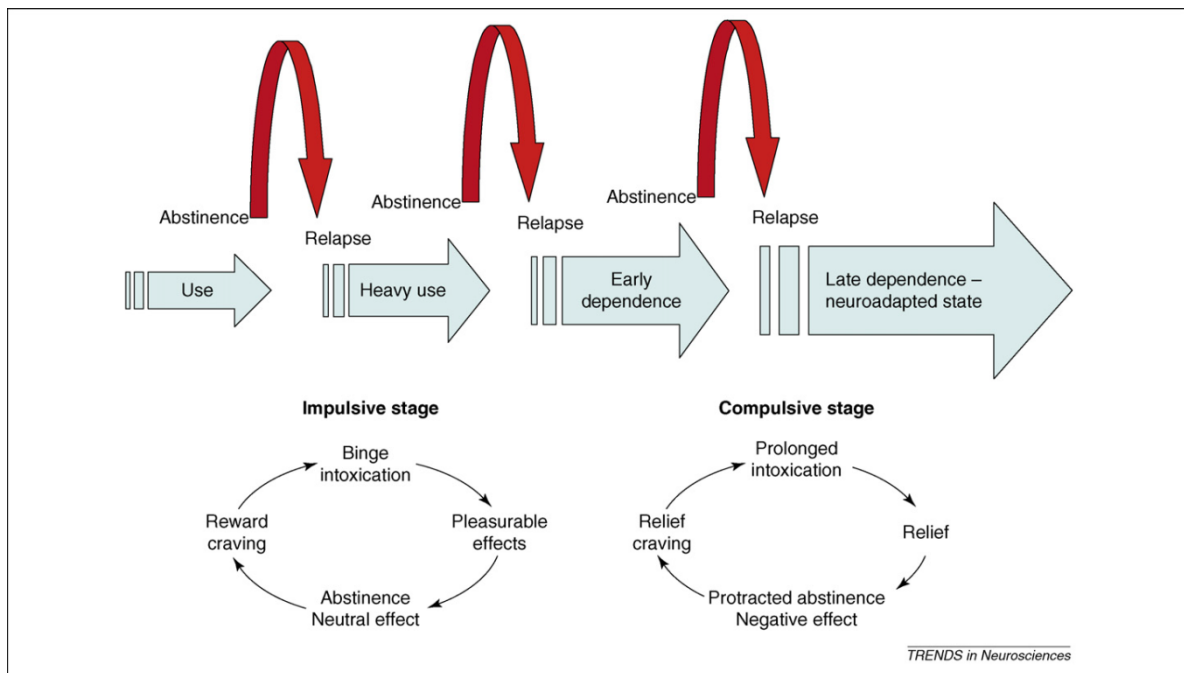


Figure 1. Transition from alcohol use to alcohol addiction. A schematic picture of the addiction cycle. At the early impulsive stage, alcohol is consumed for its positively reinforcing effects, driven by reward craving. However, after prolonged cycles of intoxication interspersed by sobriety, a shift occurs. Alcohol use becomes compulsive, and alcohol is now consumed to alleviate the highly aversive the symptoms that are pronounced in the chronic state. The picture is reproduced with permission from Elsevier (Heilig and Koob 2007).

1.1.4 Available treatments for alcohol addiction

Despite the fact that millions of people are suffering from alcohol use disorders and the socioeconomic burden they cause, only a limited number of medications for this group of conditions is available. As of today, three medications are approved by the Food and Drug Administration (FDA) in the United States, and the situation in Sweden is similar with one additional medication approved, nalmefene (Selincro), a novel opioid antagonist. The treatments available to prevent craving and relapse all have limited efficacy.

Disulfiram (Antabuse) was the very first treatment introduced on the market for treatment of alcohol addiction. Disulfiram works by inhibiting the enzyme acetaldehyde dehydrogenase, thereby blocking the metabolism of alcohol (Kitson 1977). This results in an accumulation of acetaldehyde, which causes highly aversive symptoms such as sweating, flushing and nausea if alcohol is consumed after taking medication. The resulting acetaldehyde accumulation may also have toxic effects on several organ systems, including the liver (Peachey and Naranjo 1983). It is therefore important that a patient receiving disulfiram is well informed about the actions of this medication, and aware of the risks associated with alcohol use while on it. The

aim of using disulfiram is to establish an association between alcohol and aversive feelings that will help the patient refrain from drinking, but the medication has no effect on withdrawal symptoms or the desire to drink. Presumably as a result of this, compliance is low, and if disulfiram is provided in an unsupervised fashion, controlled studies indicate that it is not superior to placebo to promote abstinence (Jørgensen et al. 2011).

Naltrexone (Revia, Vivitrol), was the second drug to be approved by FDA for alcohol addiction. Naltrexone is an opioid receptor antagonist, which at clinically used doses is thought to preferably bind to μ - and κ -opioid receptors. Although its mechanism of action is not completely clear, naltrexone most likely modulates alcohol-induced dopamine transmission, thereby decreasing reward and pleasure obtained from alcohol. Studies show that naltrexone attenuates the positively reinforcing, pleasurable effects of alcohol in social drinkers and in alcoholics who “slip”, and sometimes drink alcohol (O'Malley et al. 1996, Volpicelli et al. 1995). However, the effect of the treatment is limited and has variable outcome. Naltrexone is thought to work better in people carrying a specific variant of the gene encoding the μ -opioid receptor, *OPRM1* (Chamorro et al. 2012, Garbutt et al. 2014, Kroslak et al. 2007). In addition, it has been reported that males respond better to naltrexone treatment than women do.

Acamprosate (Campral) is the latest medication approved for treatment of alcohol addiction in the US. (It should be noted that acamprosate was approved before naltrexone as treatment of alcoholism in Sweden). Instead of inducing aversive feelings or reducing positive reinforcement induced by alcohol as described above, acamprosate reduces craving in alcoholic patients and promotes abstinence (Paille et al. 1995). The chemical structure of acamprosate is similar to GABA, but despite initial hypotheses, it does not seem that acamprosate directly affects GABAergic neurotransmission. The exact mechanism of action through which acamprosate exerts its therapeutic effects in fact remains unclear, but studies indicate that the primary action of acamprosate is on excitatory glutamatergic neurotransmission (Harris et al. 2002), resulting in reduced glutamate levels in the brain. These findings have been translated into humans using magnetic resonance spectroscopy (Umhau et al. 2010). Based on preclinical experiments, it was recently suggested that actions of acamprosate may in part or even fully be a results of its function to act as a carrier of calcium into the nervous system (Spanagel et al. 2013), although this proposition remains controversial.

1.2 NEUROBIOLOGY OF ALCOHOL ADDICTION

Alcohol is a “dirty drug” that affects multiple neurocircuits and a multitude of receptor systems (Nestler 2005). In addition, different stages of alcohol addiction may also involve different regions and signaling systems which make this condition challenging to study (Koob and Le Moal 2001, Koob and Le Moal 2005, Self and Nestler 1995). The next chapter will focus on the neurobiological and neurochemical mechanisms of the rewarding effects of alcohol, the stress systems involved in later stages of addiction, and overlapping pathways with systems that regulate natural rewards.

1.2.1 The mesolimbic dopamine system and alcohol addiction

The mesolimbic dopamine system mediates in part the rewarding properties of drugs of abuse. Alcohol can act on VTA interneurons to disinhibit dopaminergic neurons projecting from the VTA, located in the midbrain, which release DA into the NAc, and mediate drug-seeking, reinforcement, and reward learning (Di Chiara et al. 2004, Russo et al. 2010, Stuber et al. 2011). Self-administration of alcohol results in DA release in the NAc (Weiss et al. 1993), and intravenous alcohol administration increases firing of DA neurons in the VTA in a manner associated with DA release in the nucleus accumbens shell (NAcSh) (Kohl et al. 1998).

The action of alcohol on the mesolimbic dopamine system is to some extent similar to the mechanism of opioids (Johnson and North 1992), and it is likely that alcohol interacts with endogenous opioids in the VTA based on the fact that unselective opioid receptor antagonists decrease alcohol consumption (Herz 1997). Specifically, it is thought that alcohol intake results in release of endogenous opioids with μ -opioid activity, most likely β -endorphin, within the VTA; this in turn results in inhibition of the GABA-ergic interneurons that normally exert tonic inhibition of dopaminergic VTA cells. The net result is therefore ultimately a disinhibition of the DA cells, and increased DA release in their terminal areas in the NAc (Spanagel and Weiss 1999). It is important to note, however, that alcohol intake also results in endogenous opioid release in terminal areas of mesolimbic and mesocortical projections (Mitchell et al. 2012), and additional interactions could occur at these sites, as indicated in Figure 2.

Rewarding effects of drugs of abuse are mediated by D1 and D2 like DA receptors (D1R and D2R, respectively) (Rahman and McBride 2001). Psychoactive drugs, such as cocaine and amphetamine, regulate reward through different mechanisms and have stimulatory effect on

DA release or by inhibition of dopamine transporter (DAT) (Di Chiara and Imperato 1988, Koob 1992, Pontieri et al. 1995).

Finally, it should be recognized that DA effects may not be essential for alcohol reinforcement; several early studies indicated that near-complete lesions of the mesolimbic DA system only marginally or not at all influenced consumption of alcohol (Kiianmaa et al. 1979, Rassnick et al. 1993).

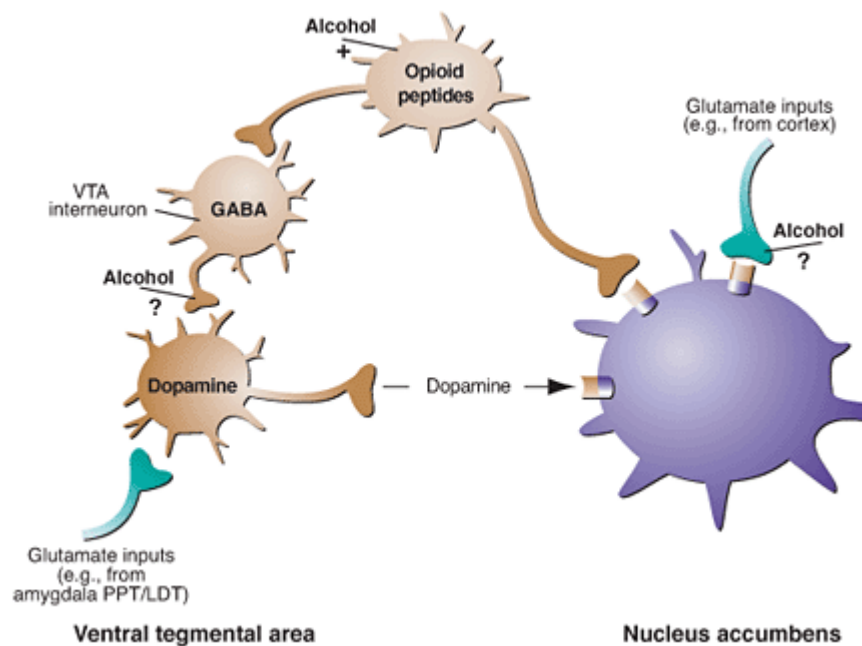


Figure 2. The effects of alcohol on dopamine release in the NAc. Alcohol affects GABA_A receptor function through a disinhibitory mechanism of GABAergic transmission in the VTA leading to increased release of DA in the NAc and activates processes regulating reward. At the same time, alcohol has an inhibitory function on release of glutamate from nerve terminals that act on neurons in both the VTA and the NAc. It should also be noted that several neuropeptide may interact with these pathways and regulate reward. Cartoon from (Gilpin and Koob 2008).

The accumbal neuronal population consists to 90-95% of GABAergic projection neurons, or medium spiny neurons (MSN) (Chang and Kitai 1985, Chang et al. 1982). These are output neurons, and can further be divided into two classes, defined by expression of D1R and D2R. The neurons expressing D1R contain specific neuropeptides, dynorphin and substance P (Brownstein et al. 1977). They project either back to the VTA or to the substantia nigra through the direct pathway. The D2R expressing neurons contain enkephalin and neurotensin, and project to limbic regions such as the ventral pallidum, constituting the indirect pathway

(Gerfen and Young III 1988, Gerfen et al. 1990). Of note, D1 receptors are Gs coupled, while the D2 subtype is Gi coupled. Because of this, and the additional inhibitory synapse within the indirect pathway, activation of D1 receptors of the direct pathway and the D2 receptors of the indirect pathway ultimately has the same effect, to increase GABA-ergic feedback onto the VTA.

Activation of the DA-system is associated with activation of dopamine and adenosine 3',5'-monophosphate-regulated phosphoprotein, 32 kDa (DARPP-32), an intracellular signaling lipoprotein, which is expressed in all dopaminergic neurons (Ouimet et al. 1984). It has been shown that DARPP-32 is a key molecule in regulation of the reward pathway and mediates the actions of drugs of abuse, including alcohol (Gould and Manji 2005, Svenningsson et al. 2005). However, it has become clear that DA release in the NAc is not exclusively regulating reward. Interactions with glutamatergic inputs from the medial prefrontal cortex (mPFC), amygdala and hippocampus also play a critical role in reward regulation (Pierce and Kumaresan 2006). Glutamate is the major excitatory neurotransmitter in the brain acting on several receptor subtypes, including N-methyl-D-aspartate (NMDA) receptors. Glutamate signaling is implicated in the acute reinforcing effects of alcohol, which inhibits glutamatergic activity. Acute alcohol exposure attenuates extracellular glutamate levels in the NAc (Carboni et al. 1993). Alcohol also affects glutamate transmission by acting on NMDA receptors (Lovinger et al. 1989) and metabotropic glutamate subtype 5 receptors (mGluR5) (Blednov and Harris 2008). NMDA receptors are thought to play an important role in alcoholism due to the fact that they are involved in neuroplasticity which contributes to hyperexcitability and craving during abstinence (Pulvirenti and Diana 2001).

1.2.2 Overlapping brain pathways regulate food and drug reward

Just like drugs of abuse, natural rewards such as palatable food involve activation of the mesolimbic DA system. Consumption of palatable food releases endogenous opioids in the brain and activates opioid receptors in the VTA to stimulate dopamine release in the NAc, in a manner similar to that of alcohol (Colantuoni et al. 2001, Di Chiara and Imperato 1988, Wise and Rompré 1989). This circuit is highly associated with drug abuse, but has also been shown to become activated by bingeing of palatable food (Zhang et al. 2003). In addition, sugar shares select properties with drugs of abuse, and can induce signs of addiction-like behaviors in preclinical models. Specifically, rats on sugar restriction learn to binge drink and escalate their intake over time, which are typical features associated with the behavioral

pathology of addiction (Avena et al. 2006, Avena et al. 2008). Compensatory down regulation of opioid and DA receptors has been demonstrated in rats after intermittent access to sugar, and rats also display somatic signs of withdrawal when sugar is removed (Colantuoni et al. 2002, Colantuoni et al. 2001).

In humans, substance abuse is often comorbid with eating disorders, suggesting that underlying mechanisms regulating these pathological conditions are mediated by overlapping neural pathways (Volkow et al. 2008, Volkow et al. 2012). The NAc is involved in regulation of food intake and receives innervation from the hypothalamus (Maldonado-Irizarry et al. 1995, Mogenson et al. 1983), which harbors a network of anorectic and orexigenic peptides that together regulate food homeostasis (Havel 2001, Hillebrand et al. 2002, Schwartz et al. 2000). Since alcohol and food intake are both consummatory behaviors, and since alcohol is also a caloric nutrient, it is not surprising that hypothalamic neuropeptides are being studied in the alcohol field as potential new therapeutic targets.

1.2.3 The hypothalamus as a crossroad of food and drugs

The hypothalamus is a brain region located in the mesencephalon, just underneath the thalamus and above the pituitary. It consists of several nuclei which mediate essential physiological functions, such as regulation of body temperature, circadian rhythm, body weight and hunger (Elmqvist et al. 2005, Morton et al. 2006, Saper et al. 2005). The primary function of the hypothalamus is to control physiological homeostasis, and to do so, the hypothalamus serves as a link between the nervous system and the endocrine system (Luiten et al. 1987). Information is being sent from the nervous system and in response production of neurohormones occurs which in turn regulates physiological functions centrally as well as in the periphery.

Recently it has been shown that the lateral hypothalamus (LH) sends strong innervation to the NAcSh (Brog et al. 1993, Stratford and Kelley 1999), suggesting an important role in mediating both food intake (and natural rewards) and reward from drugs of abuse.

1.2.4 Stress-systems

During the later stages of alcohol addiction, endogenous stress-systems are involved in mediating aversive feelings and negative emotional states associated with this phase of the addictive process. In addition to its role in regulating reward-related behaviors, the

hypothalamus is also highly implicated in stress-regulation through the hypothalamic-pituitary-adrenal (HPA) axis. The HPA-axis is activated by both physiological and psychological stressors (Pacák and Palkovits 2001). The paraventricular nucleus (PVN) of the hypothalamus is central in the regulation of the endocrine stress response system (Herman et al. 1996) and contains a high concentration of cell bodies that express corticotropin-releasing hormone (CRH) (Merchenthaler et al. 1982, Vale et al. 1981), a neuropeptide known to regulate stress responses. Activation of the anterior pituitary by CRH stimulates release of adrenocorticotropin hormone (ACTH), which in turn travels within the blood stream to the adrenal cortex and induces release of the effector hormones of the HPA axis, glucocorticoids (corticosterone in rodents and cortisol in humans).

However, CRH containing cell-bodies are also found in extra-hypothalamic circuits (Swanson et al. 1983), where CRH mediates behaviors such as anxiety and stress responses within the extended amygdala, a circuit that includes the bed nucleus of stria terminalis (BNST) and the central amygdala (CeA) (Davis 1998, Davis et al. 2009). Additional stress-related neuropeptides are likely to be involved in negatively reinforcing properties of alcohol, with dynorphin acting at κ -opioid receptors being a prime example [see e.g. (Kissler et al. 2014)].

1.3 THE HYPOTHALAMIC MCH-SYSTEM AND ALCOHOL ADDICTION

The hypothalamus produces multiple neuropeptides involved in regulation of food intake. These peptides may also regulate other physiological and motivational functions and have therefore been of great interest in the addiction field (Aston-Jones et al. 2009, Boutrel and de Lecea 2008, Di Leone et al. 2003, Kiefer and Wiedemann 2004). The LH consists of two distinct cell populations that produce orexigenic peptides, namely orexin and melanin concentrating hormone (MCH) (Elias et al. 1998). The work in this thesis focuses on the role of the MCH-1 receptor (MCH1-R) in alcohol addiction.

1.3.1 Melanin Concentrating Hormone (MCH)

In 1983 MCH was first discovered in chum salmon pituitaries, where it regulates the ability to change skin color of the fish in response to stress, a mechanism for protection from predators (Kawauchi et al. 1983). Almost a decade later, the cyclic peptide was found in rats and humans and is highly conserved amongst species (Presse et al. 1990). However, over the

course of evolution, the function of MCH has changed. In higher species, it regulates homeostasis and energy expenditure and is no longer associated with skin pigmentation (Nahon 2006, Shi 2004). The rat and human peptides are identical and consist of 19 amino acids (Vaughan et al. 1989). The gene encoding MCH in humans, *PMCH*, generates the precursor pro-MCH, which generates three mature peptides through differential processing that depends on the tissue where it is expressed, see Figure 3. The physiological functions of the two additional peptides produced, NGE (neuropeptide glycine-glutamic acid) and NEI (neuropeptide glutamic acid-isoleucineamide) (Nahon et al. 1989), remain unclear to date. MCH-containing cell bodies are found in the LH and can also be detected in the adjacent structure *zona inserta* (Bittencourt et al. 1992), regions known to control food intake. The MCH neurons project widely throughout the brain from the LH, including projections to limbic regions (Bittencourt et al. 1992). Two known receptors for MCH exist within the primate central nervous system, MCH1-R and Melanin Concentrating Hormone 2 receptor (MCH2-R) (Tan et al. 2002). The MCH2-R is not expressed in rodents.

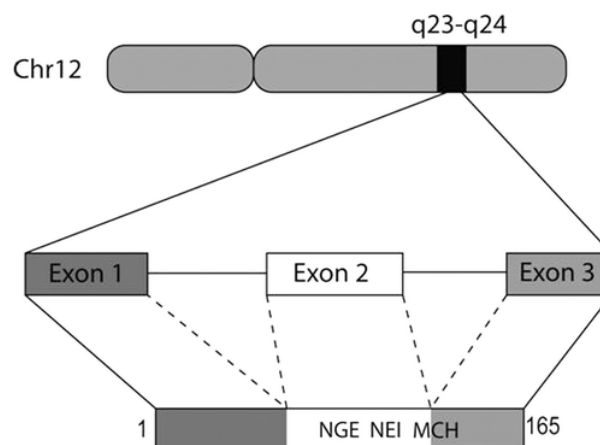


Figure 3. The genomic structure of the MCH gene. The MCH gene (in humans) is located on chromosome 12 (12q23–24) and consist of three exons and two introns. The peptides NGE, NEI, and MCH are all encoded in the second and third exons. Reproduced with permission from Endocrine Society (Pissios et al. 2006).

1.3.2 MCH-1 receptors and signaling pathways

The MCH1-R was an orphan receptor, SLC-1/GPR24, until 1999 when it was discovered to bind or become activated by MCH. Several research groups discovered the MCH1-R at the same time using reverse pharmacology (Bächner et al. 1999, Chambers et al. 1999, Lembo et al. 1999). In rats and humans, expression levels of the MCH1-R are highly enriched in the brain and much lower levels have been found in the periphery, in fat, liver, and heart tissue

(Kolakowski et al. 1996, Lembo et al. 1999, Saito et al. 1999). The receptor is expressed in many areas of the brain, with high density in limbic regions. The highest expression levels are found in the NAcSh and caudate putamen in rats and mice (Hervieu et al. 2000, Saito et al. 2001).

The MCH1-R is a G-protein coupled receptor (GPCR) and signals through Gi, Go and Gq (Figure 4). The most favored signaling pathway is via Gi, which inhibits cyclic adenosine monophosphate (cAMP) and leads to activation of protein kinase A (PKA). Activation of this pathway also involves induction of mitogen activated protein kinases (MAPK) signaling cascades. When the receptor couples to Go it also induces the MAPK pathway but through a different mechanism, which involves protein kinase C (PKC). The Gq pathway increases calcium levels and excitability of the cell. Calcium gets mobilized to the endoplasmic reticulum where it facilitates packaging of vesicles and exocytosis (Chung et al. 2009a, Hawes et al. 2000).

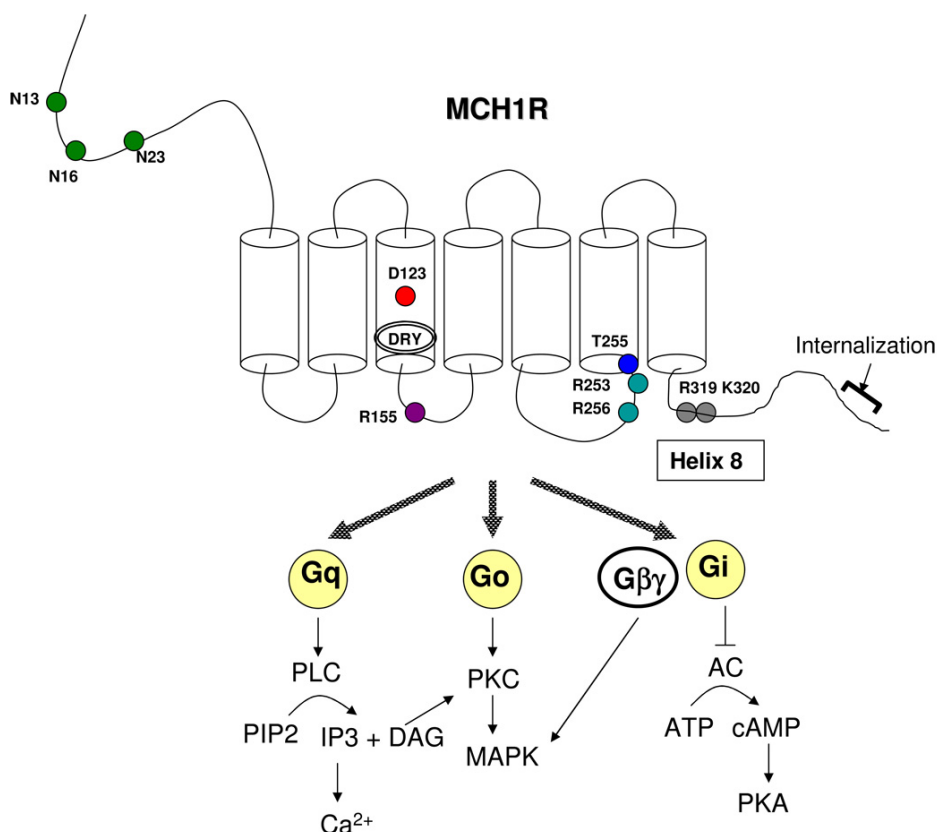


Figure 4. Intracellular signaling pathways of the MCH1-R. The MCH1-R couples to Gi, Go and Gq, to either inhibit the cAMP dependent pathway, activate MAPK signaling cascades or increase calcium levels in the cell. Reproduction with permission from Elsevier, (Chung et al. 2009a).

1.3.3 MCH-2 receptors

Shortly after the discovery of the MCH1-R, a human orphan GPCR, SLT, was identified as the MCH2-R based on the sequence homology to the former receptor (Mori et al. 2001). The MCH2-R shares 38% sequence homology with the MCH1-R and activate Gq signaling pathways resulting in mobilization of calcium in the cell (Hill et al. 2001, Sailer et al. 2001). Unlike the MCH1-R, this subtype is only expressed in higher species such as ferrets, dogs, rhesus macaques and humans (Tan et al. 2002), and little is known about its physiological role (Sailer et al. 2001). However, the distribution pattern of MCH2-R is similar to that of MCH1-R, and most likely it also mediates energy balance and homeostasis. It should be noted that MCH is the only ligand for MCH1-R and MCH2-R. The pro-MCH derived peptides NEI and NGE do not activate these receptors (Chambers et al. 1999, Sailer et al. 2001, Saito et al. 1999).

1.4 FUNCTIONS OF THE MCH/MCH1-R SYSTEM

Recently it has been discovered that MCH mediates numerous physiological functions in addition to regulation of energy homeostasis. For example, MCH has been shown to regulate stress responses and anxiety, behaviors which are closely related to substance use disorders. The following chapter will further discuss the MCH-system in regulation of food intake, emotionality, and drug addiction.

1.4.1 MCH in regulation of food intake

Energy balance is a complex mechanism regulated by multiple peripheral signals to the brain. Hormones such as ghrelin, leptin and insulin are acting on first order neurons in the arcuate nucleus of hypothalamus to control food intake and energy expenditure (Obici et al. 2002, Porte et al. 1998). The first order neurons contain either the orexigenic mediator neuropeptide Y (NPY) or the anorectic peptide proopiomelanocortin (POMC), and synapse onto secondary MCH neurons in the LH (Elias et al. 1998). Hunger signals inhibit POMC cells and instead activate NPY neurons, which in turn stimulate MCH-containing neurons and increase food intake. Satiety has the opposite effect on POMC and NPY neurons, and regulates MCH secretion in the opposite direction.

Studies report that central administration of MCH increases food intake in rodents both acutely and after chronic administration, with the latter being accompanied by increased body

weight (Della-Zuana et al. 2002, Qu et al. 1996). In line with these findings, studies in transgenic mice demonstrate that deletion of the *Pmch* gene results in a lean phenotype as a result of hypophagia (Shimada et al. 1998), while overexpression of MCH generates hyperphagic mice with increased body weight (Ludwig et al. 2001). Studies using global, constitutive knock-out (KO) mice or central administration of MCH affect the whole brain and may not mimic the endogenous role, which could potentially confound the results. However, studies investigating the effects of MCH1-R antagonists show that systemic administration successfully reduce food intake and body weight in normally fed and diet induced obese rats (Tavares et al. 2006), which strengthen previous findings and indicates that these compounds may be potential treatments for obesity. Further, studies suggest that mechanisms mediating the role of MCH in regulating food intake depend on hypothalamic- limbic circuits. Injections of MCH into several different nuclei of the hypothalamus have been shown to result in orexigenic effects (Abbott et al. 2003). Further, Georgescu et al. showed that food intake is mediated by MCH in the NAcSh. Food intake was increased after injection of MCH, and oppositely, the effect could be reversed by local administration of an MCH1-R antagonist in the NAcSh (Georgescu et al. 2005).

1.4.2 MCH in regulation of stress-responses and anxiety

In addition to its established role in feeding and energy balance, MCH also plays a role in other physiological functions. The MCH1-R is highly expressed in multiple nuclei of the hypothalamus, including the PVN, that also receive MCH projections, and some evidence suggests that MCH can stimulate the HPA-axis (Bittencourt et al. 1992, Herman et al. 1996). Studies show that intracerebroventricular (i.c.v) administration of MCH increased corticosterone levels in rats, an effect that was reduced by pretreatment with an anti-CRH antibody (Jezová et al. 1992). Moreover, an acute intra PVN injection of MCH increased ACTH and plasma corticosterone levels in rats, which suggests that MCH can directly activate the HPA-axis, at least to some extent through activation of CRH (Kennedy et al. 2003).

An abnormal function of the HPA-axis is often seen in major depression and stress related disorders including alcohol addiction (Costa et al. 1996, Pariante and Lightman 2008, Wand and Dobs 1991, Watson et al. 2004). Many limbic regions such as the hippocampus, prefrontal cortex, and amygdala, where the MCH1-R is highly expressed, also interact with stress systems to regulate anxiety-like behaviors. There are several studies suggesting a role

for MCH in anxiety- and depression-like behaviors in rodents, although these results are somewhat conflicting. Central injections of MCH have anxiolytic effects as shown in the Vogel's punished drinking test and elevated plus maze in rats (Kela et al. 2003, Monzon and De Barioglio 1999). Paradoxically, MCH1-R antagonists decrease anxiety and depression-like behaviors in rats in the forced swim stress and social interaction tests, models of depression- and anxiety-like behavior, respectively (Borowsky et al. 2002). Injections of an MCH1-R antagonist in the NAcSh have been shown to exert antidepressant like effect on forced swim stress and conversely, MCH increases anxiety in the same task, suggesting interactions with the mesolimbic dopamine system (Georgescu et al. 2005). Behavioral studies in mice indicate that the MCH-system has anxiogenic effects, but the exact role needs to be further investigated (Gehlert et al. 2009, Georgescu et al. 2005, Smith et al. 2005a). However, numerous small molecule antagonists have been developed after the discovery of the MCH1-R. The outcome on anxiety-like behaviors has been ranging from no effect to potently decreased anxiety (Basso et al. 2006, Borowsky et al. 2002, Chaki et al. 2003, Chaki et al. 2005, Chung et al. 2011). These inconsistencies may be explained by route of administration, doses used, or differences in central receptor occupancy. To date, it still remains unclear how MCH1-R antagonists exert their antidepressant-like effects, and which brain regions and neurocircuits mediate anxiety-like responses.

1.4.3 MCH and drug addiction

Based on the functions of the MCH-system reviewed above, the MCH1-R may offer a promising target for treatment of eating and addictive disorders. While MCH1-receptor antagonism has been extensively studied in animals and evaluated in clinical trials for obesity (Mancini and Halpern 2006), only a few studies have investigated a potential role for MCH in drug addiction.

A recent study from Chung et al., demonstrated that the MCH1-R plays a role in cocaine reward and addiction. Mice with a genetic deletion of the MCH1-R displayed decreased conditioned place preference (CPP) for cocaine, and blockade of MCH1-R decreased cocaine self-administration and cue-induced reinstatement. Further, the authors suggested that MCH can potentiate DA-signaling. Spike firing in the NAcSh was increased by MCH as well as levels of phosphorylated DARPP-32, indicating that MCH interacts with the mesolimbic dopamine system and regulates cocaine reward (Chung et al. 2009b). Another report failed to find effects on MCH1-R KO mice in cocaine or amphetamine induced CPP (Tyhon et al.

2008). The discrepancies between these studies may be explained by doses of drug used, background of the mouse strain and different CPP protocols.

Further, the MCH1-R has been evaluated with regard to alcohol related behaviors, once again yielding conflicting results. Central injection of MCH was reported to increase alcohol intake in rats, but consumption was not attenuated after administration of an MCH1-R antagonist (Duncan et al. 2006). Additionally, in MCH1-R KO mice, alcohol intake was increased compared to control mice, although at lower, not pharmacologically active alcohol concentrations (Duncan et al. 2007).

1.5 ANIMAL MODELS OF ALCOHOL ADDICTION

The complexity of alcohol addiction makes it challenging to model this condition in animals. Although there is no model that captures all features of alcohol addiction, animal models nevertheless serve as useful tools to better understand the behavioral and neural mechanisms behind key aspects of alcohol addiction, such as increased voluntary consumption (escalation), increased motivation to take alcohol, and relapse to alcohol seeking (reinstatement). Many studies possible to perform using rodents would not be ethically permissible or even possible to conduct in humans. Animal models are, thus, needed in order to provide a first screening procedure for new possible treatment targets in order to address the extensive unmet needs for pharmacological interventions for alcohol addiction that remain to date.

Animal models are generally divided into 3 groups which follow the progression to alcohol addiction. The models cover: binge/intoxication, withdrawal and negative affect, and anticipation and preoccupation. Binge /intoxication models include alcohol self-administration and conditioned place preference, measuring motivation to obtain, and the rewarding properties of alcohol. Withdrawal and negative affect includes models of anxiety-like behaviors and increased motivation to self-administer alcohol in a dependent state. Finally, models of anticipation and preoccupation involves cue-and stress-induced relapse (Koob and Volkow 2009, Sanchis-Segura and Spanagel 2006).

1.5.1 Ethics and animal experiments

The work presented in this thesis consists of animal experiments. All studies performed followed the respective guidelines for care and use of laboratory animals at National Institutes of Health, Karolinska Institutet, and Linköping University. Experiments were designed considering “the 3 Rs”, an ethical approach to treat animals in a humane way, that was first proposed by Russell and Burch (Russell et al. 1959). The 3 Rs stand for reduce, replace, and refine.

Reduction: decrease the number of animals in the study. A great example in this thesis is the use of the Latin square design when the animal receives several treatments and serves as its own control, which is also beneficial for the results of experiments.

Replace: the animal studies can eventually be replaced with other techniques in order to reach the same aim of the study. Sometimes techniques such as cell culture experiments or computer programs can replace animals.

Refinement: enhanced animal welfare, and the use of procedures that minimize eventual pain and stress for the animal.

2 AIM OF THE STUDY

The primary aim of this thesis was to evaluate the MCH1-R as a new potential treatment target for alcohol use disorders using a selective MCH1-R antagonist, GW803430.

Specific aims for paper I-IV:

- To evaluate effects of the MCH1-R antagonist, GW803430, on motivation to self-administer alcohol and relapse like behaviors (Paper I)
- To investigate the role of the MCH1-R in regulation of caloric intake and motivation to consume alcohol in states of escalated consumption (Paper II)
- To assess the effects of GW803430 on motivation to self-administer sweet solutions and on sucrose addiction like behaviors (Paper III)
- To investigate the involvement of the MCH1-R on alcohol reward in mice and assess signaling mechanisms downstream of MCH1-R induced by acute alcohol administration (Paper IV)

3 MATERIAL AND METHODS

First, this section describes the animals used in the studies and the MCH1-R antagonist, GW803430. Second, preclinical models and molecular techniques used will be covered in this chapter. Detailed descriptions of the methods can be found in the papers/manuscripts.

3.1 ANIMALS

In paper I -III, male Wistar rats were used, and were purchased from Charles River Laboratories (Wilmington, MA, USA). For experiments in paper II, performed in Sweden (Linköping University and Karolinska Institutet), male Wistar rats were purchased from Harlan (Horst, the Netherlands). Rats were kept on a 12h light/dark cycle and experiments were conducted during the dark phase.

In paper IV, mice with a genetic deletion of the MCH1-R were used. These were kindly provided by Dr. Donald R. Gehlert, Lilly Research Laboratories (Indianapolis, IN, USA). These mice were bred in our animal facility and generated by heterozygote breeding and genotyped as previously described (Chen et al. 2002). Mice were kept on a 12h light/dark cycle and experiments were conducted during the light phase. Mice were on a C57BL/6 background.

3.2 GW803430

The MCH1-R antagonist, GW803430, was synthesized and provided by Lilly Research Laboratories (Indianapolis, IN, USA). GW803430 is a small molecule, non-peptidergic compound which is orally available and penetrates the blood brain barrier (Gehlert et al. 2009). Studies show that GW803430 is selective for, and has high affinity for the MCH1-R (Hertzog et al. 2006). The doses used in this thesis were based on previous work (Gehlert et al. 2009). When administered to rats (0-30mg/kg, 1ml/kg) GW803430 was suspended in 10% Tween 80 and distilled water, and delivered as an acute intraperitoneal injection (i.p) 45 min before testing (paper I-III). In mouse experiments, GW803430 was suspended in 5% mannitol and distilled water and delivered by gavage (0-30mg/kg, 10ml/kg) 2 hours before testing.

3.3 BEHAVIORAL MODELS IN RATS

3.3.1 Operant self-administration and reinstatement paradigms

The self-administration paradigm is commonly used to measure drug intake, as well as reward and motivated behaviors (Cippitelli et al. 2007, McBride and Li 1998). Rats readily administer drugs of abuse, food and sweet solutions. In paper I, we evaluated effects of GW803430 on operant self-administration of alcohol. Rats were trained to self-administer alcohol using a saccharin fading procedure. Following training, baseline responding for 10% alcohol was established. During baseline, responding on the right lever (FR-1) was paired with a house light stimulus and a 5sec timeout was in effect. Responding on the left lever had no consequences. All sessions were 30 minutes long.

Next, cue-induced reinstatement was assessed as previously described (Cippitelli et al. 2005). Briefly, an olfactory cue (orange scent) predictive of alcohol was introduced during baseline sessions. Extinction followed, during which no alcohol cues or alcohol were available. On the test day, cues but not alcohol were reintroduced and the responding recorded. Further, stress-induced reinstatement of alcohol seeking was evaluated (Cippitelli et al. 2008). As described above, rats were trained to self-administer alcohol and went through extinction. On the test day, rats were exposed to an intermittent foot shock stressor for 15 min prior to the test session when responses were recorded.

In paper III, GW803430 was evaluated in self-administration of sweet caloric and non-caloric solutions using 10% sucrose and 0.06% saccharin. Reinforcers were paired with a house light stimulus, and there was a 20sec timeout period following responding. Finally, seeking and motivation to self-administer sucrose was assessed. Cue-induced reinstatement was performed as described above. Increased motivation to obtain sucrose was assessed under a progressive ratio schedule of reinforcement (Cippitelli et al. 2007). The number of lever presses required for sucrose delivery increased during the session and the last completed ratio was defined as the breakpoint. Self-administration and progressive ratio experiments were analyzed using repeated measures one-way analysis of variance (ANOVA) with treatment as the within subjects factor. Two separate analyses were conducted in the reinstatement experiments. First, responses on the last day of extinction were compared to vehicle treated rats following reintroduction of cues or stress exposure. Next, one-way repeated measures ANOVA followed with treatment as factor, using a within subjects design in the cue-induced reinstatement experiments and between subjects design in the foot-shock experiment.

3.3.2 Intermittent access

Repeated cycles of intermittent access to alcohol induce escalation of consumption in rats. In paper II, rats had access to 20% alcohol or water on alternating days, 3 days a week using the two bottle free choice paradigm. On all other days only water was accessible. No saccharin fading was necessary for this procedure (Simms et al. 2008, Wise 1973). After 3 months of intermittent drinking, the effect of GW803430 was assessed on alcohol and food consumption in Wistar rats. Alcohol or food consumption after treatment was compared by one-way repeated measures ANOVA using a within subjects design.

Intermittent access to sucrose similarly increases its consumption over the time and rats learn to binge during the first hour of access (Avena et al. 2006, Avena et al. 2008). In paper III, intermittent access to sucrose and food intake was examined after administration of GW803430. One group of rats had continuous access to 10% sucrose, food and water. The second group had restricted access to 10% sucrose and food for 12 hours per day (water *ad libitum*). Sucrose and food consumption was measured after 1, 12 and 24 hours over 24 days before rats were treated with GW803430. Sucrose and food consumption was analyzed by three-way repeated measures ANOVA, with group as between subject factor and time and treatment as within subject factors.

3.3.3 Intermittent vapor exposure followed by self-administration

Alcohol dependence can be induced by vapor exposure in rats (paper II). In this experiment, one group of rats was exposed to normal air and the second group was exposed to intermittent alcohol vapor for 7 weeks (14 hours/day and 5 days a week) (Tapocik et al. 2012). Blood samples were collected once a week, and analyzed for blood alcohol levels (BAL). During the last three weeks of exposure, rats were trained to self-administer 20% alcohol to reach a stable baseline. Sessions were run during the withdrawal phase. The effect of GW803430 on alcohol self-administration during acute withdrawal (approximately 6-7 hours after vapor was turned off) was assessed. Alcohol self-administration was analyzed by two-way repeated measures ANOVA, with group as between and treatment as within subjects factors.

3.3.4 Intermittent access to 20% alcohol followed by self-administration

Alcohol self-administration in protracted abstinence was assessed in paper II. First, rats were trained on alcohol self-administration as described in 3.3.1. Once self-administration was stable, rats had intermittent access to 20% alcohol for 3 months as described above. Rats were divided into high and low drinkers after alcohol consumption. During the last 5 weeks of intermittent access to alcohol, food intake was measured to assess the caloric contribution from alcohol. The effects on alcohol and food intake were evaluated in high versus low drinking rats and after treatment with GW803430.

Next, high drinking rats went through 3 weeks of abstinence followed by self-administration of 20% alcohol. Rats were treated with the antagonist once baseline was established.

3.3.5 Models for evaluation of behavioral specificity of effects after administration of GW803430

Effects of GW803430 were evaluated in a battery of control experiment including the loss of righting reflex (LORR), alcohol metabolism and elimination, locomotor activity and taste preference. In the LORR and alcohol elimination tests, rats were injected with a highly sedative dose of alcohol, 3.5 g/kg (Khisti et al. 2003). Sleeping time was recorded in the LORR test and regaining of the righting reflex was considered to be when the rat could turn around on four paws within a minute. In the alcohol elimination test, blood samples were collected at 60, 120 and 360 min for examination of BALs.

Locomotor activity was measured in the open field. On the first day, rats were allowed to habituate to the open field for 10 min to eliminate the effects of novelty on locomotor activity. On the second day drug effects on locomotor activity was measured.

In the taste preference test, consumption of a solution containing saccharin and quinine was measured (Goodwin and Amit 1998). Water intake and food consumption was also recorded. Results from LORR, locomotor activity and taste preference were analyzed by one-way ANOVA using a between subjects design with treatment as factor while alcohol elimination was analyzed by two-way ANOVA, with time and treatment as factors.

3.4 BEHAVIORAL MODELS IN MICE

3.4.1 Conditioned place preference (CPP)

Mice were first screened in a pretest session to establish that they did not have an initial side preference for either side of the box. The two compartments had different visual and tactile cues. Mice spending more than 60% of the time on one side during the pretest were removed from the experiment. Mice were then conditioned for four days with alcohol, 2g/kg or saline morning and afternoon in a counterbalanced unbiased design. On test day, the time mice spent on the side associated with alcohol was recorded (Sanudo-Pena et al. 1997). In paper IV, the CPP paradigm was used in two experiments. First, alcohol induced CPP was measured in WT and MCH1-R KO mice, and second, wildtype C57BL/6J mice were treated with GW803430 or vehicle. The effect of genetic deletion of the MCH1-R or pharmacological blockade by GW803430 was assessed and the results were analyzed by two-way ANOVA with session as within subject factor and genotype or treatment as between subject factors.

3.4.2 Conditioned place aversion (CPA) as a control for associative memory

The CPA paradigm was employed to measure memory acquisition in WT and MCH1-R KO mice. Just as described above for the CPP, the CPA test consists of pretest, conditioning and test day. However, in this test mice learn to associate aversion induced by lithium chloride with environmental cues. Mice were conditioned with lithium chloride or saline once daily in an unbiased design (Risinger and Cunningham 2000). Time spent on lithium chloride side was measured on the test day and the experiment was analyzed by two-way ANOVA with session as within subject factor and genotype as between subject factor.

3.5 EX VIVO STUDIES

3.5.1 Autoradiography

Receptor occupancy was assessed after administration of GW803430 as previously described (Gehlert et al. 2009). The doses and administration route used in paper I-III were examined. Rats were decapitated after i.p. administration of GW803430. Brains were extracted and stored at -70°C until further analysis. Sections of the NAc (core and shell), caudate and putamen were collected and mounted on slides. Briefly, the slides were incubated in buffer containing a labeled ligand of the MCH1-R, 30pM ¹²⁵I-S036057 (PerkinElmer Life and

Analytical Sciences, Waltham, MD, USA) for 90 min. To assess non-specific binding, 10 μ M of unlabeled (“cold”) GW803430 was added to the incubation media. Slides were washed in buffer and dipped in water and dried. For 3 days sections were exposed to phosphorimaging plates before analysis. Determination of ED₅₀ and ED₉₀ were accomplished using GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA).

3.5.2 Real time PCR

Differences in gene expression of MCH and MCH1-R in acute withdrawal from alcohol vapor exposure and following intermittent access were examined using real time PCR. As described in 3.3.3 and 3.3.4, brains from rats in acute withdrawal and following intermittent access were collected, snap frozen and stored in -90°C until analysis. The hypothalamus and amygdala were punched out according to the Paxinos and Watson atlas (Paxinos and Watson 2006). Tissue was extracted using the RNeasy® Micro Kit (cat. no. 74004, Qiagen®) following the manufacturer’s protocol. RNA was reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription kit (Life Technologies Europe, Bleiswijk, the Netherlands) according to the manufacturer’s instructions. The samples were run using the following cycles: 25°C for 10 min, 37°C for 60 min, 85°C for 5 s (Veriti® 96-well Thermal Cycler, Life Technologies Europe, Bleiswijk, the Netherlands). The cDNA was then diluted 1:10 in RNase free water and stored at a -80°C until assayed for gene expression.

Gene-expression quantification was run on the 7900HT FAST Real-Time PCR system (Life Technologies Europe, Bleiswijk, the Netherlands) using TaqMan® Fast universal PCR Master Mix (2X) No AmpErase® UNG according to the manufacturer’s instructions. The gene expression assays used were: MCH: Rn01503866_m1, MCH1-R: Rn00755896_m1, GAPDH: Rn01775763_g1 Ywhaz: Rn00755072_m1). Gene-expression of MCH and MCH1-R was normalized to the expression of housekeeping genes and calculated using the $\Delta\Delta C_t$ method. Differences in gene expression levels were compared by a one-way ANOVA.

3.5.3 Immunohistochemistry

Immunohistochemistry was used to measure p-DARPP-32 and p-ERK as downstream signaling targets of the MCH1-R (paper IV). WT and MCH1-R KO mice were administered either a dose of alcohol, 2g/kg, or saline 30min before transcardial perfusion (Björk et al. 2010). Brains were extracted, snap frozen and stored at -80°C until further analysis. Brains

were sectioned in 30 μ m slices and sections of the NAcSh and central nucleus of the amygdala (CeA) were collected according to the Paxinos and Watson atlas (Paxinos and Watson 2006).

Free-floating sections were washed with PBS and incubated in H₂O₂, followed by washes in PBS. Non-specific binding sites were blocked using bovine serum albumin (Sigma, MO, USA) before application of primary antibodies (anti-pDARPP32, sc-21601-R, Santa Cruz Biotechnology, USA and anti-pERK, cat #4370, Cell Signaling Technology, MA, USA). Sections were incubated at 4°C on a rotating shaker for at least 24 hours. Next, sections were washed in PBS and the secondary antibody (p-DARPP-32, cat #K4003, DAKO, CA, USA and p-ERK cat #PK-6101, Vector Laboratories Inc., Burlingame, CA, USA) was applied. Following incubation and washes, diaminobenzidine (DAB) chromagen was added for visible brown staining. Finally, sections were mounted on slides, dehydrated and cover-slipped before light microscopy analysis. Quantitative analysis was performed using Leica DM6000CS light microscope (Leica Microsystem) at 40X magnification and pictures were captured by an attached digital camera. Levels of p-DARPP-32 and p-ERK were analyzed by two-way ANOVA with genotype and treatment as factors in a between subjects design.

4 RESULTS AND DISCUSSION

4.1 SELF-ADMINISTRATION AND REINSTATEMENT OF ALCOHOL SEEKING IS SUPPRESSED BY MCH1-R ANTAGONISM IN WISTAR RATS (PAPER I)

Stress and anxiety-like behaviors, which are closely related to alcohol addiction, have previously been shown to be affected by the MCH/MCH1-R system (Borowsky et al. 2002, Gehlert et al. 2009, Gehlert et al. 2007, Smith et al. 2005a). However, there is a limited and conflicting literature on the role of MCH in alcohol addiction. It has been reported that i.c.v. administration of MCH increased alcohol consumption in rats, while blockade of MCH1 receptors failed to suppress drinking (Duncan et al. 2005, Duncan et al. 2006). Drinking studies in mice showed that genetic deletion of the receptor increased alcohol consumption, although this may be a consequence of developmental compensation, since MCH1-R KO mice are hyperphagic and have a lean phenotype (Duncan et al. 2007, Marsh et al. 2002). The purpose of this study was to examine the role of the MCH1-R in motivation to obtain alcohol and relapsing behaviors using the MCH1-R antagonist GW803430.

4.1.1 GW803430 potently decreases alcohol self-administration and eliminates cue-induced reinstatement

Blockade of MCH1-Rs potently and dose-dependently decreased alcohol self-administration (Figure 5A). These results are in contrast to previous findings by Duncan et al. who did not find any effect of an MCH1 receptor antagonist on home cage alcohol consumption (Duncan et al. 2006). Negative results are difficult to interpret in general; in this case, we note that the negative study used a peptidergic MCH1-R antagonist (“Compound B”) with unknown *in vivo* properties. Furthermore, our results were obtained in an operant self-administration model, which is generally thought to better reflect motivation to obtain alcohol compared to home cage drinking.

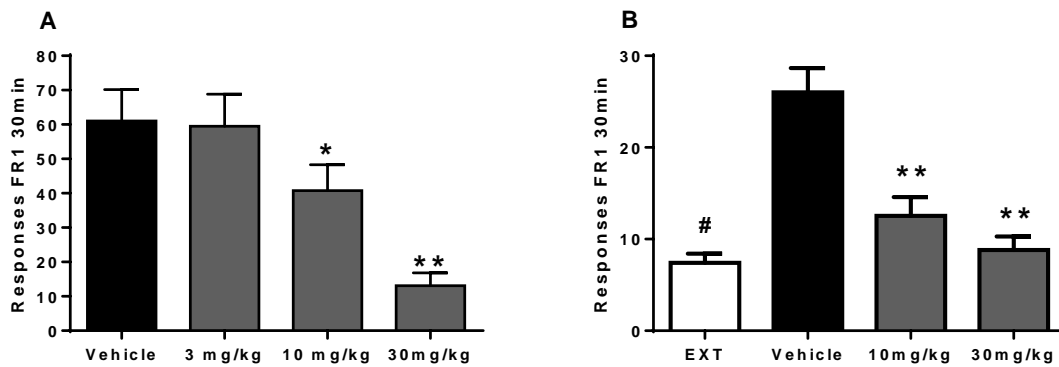


Figure 5. Administration of GW803430 attenuates alcohol self-administration and cue-induced reinstatement in rats. Graphs show total responding on the active lever under a fixed ratio 1 (FR1) schedule of reinforcement during 30 min sessions. Administration of GW803430 dose dependently decreased self-administration of 10% alcohol. Total responding was significantly reduced up to 80% at the highest dose, 30mg/kg (A). Presentation of cues previously paired with alcohol, induced relapse compared to the last day of extinction (EXT) while blockade of MCH1-Rs potently attenuated cue-induced reinstatement (B). * $P < 0.05$ and ** $P < 0.01$ compared to vehicle treated rats. # $P < 0.05$, total responding on active lever compared to last day of extinction.

Reinstatement of previously extinguished alcohol seeking behavior elicited by alcohol associated cues (Figure 5B), but not a stressor, was also significantly reduced by MCH1-R antagonism. Our findings are in agreement with a recent study suggesting that the MCH1-R regulates cocaine reward and seeking. Blockade of MCH1-Rs decreased cocaine self-administration and cue-induced reinstatement without affecting stress-induced relapse (Chung et al. 2009b). Moreover, yohimbine-induced relapse to high fat food was also unaffected by MCH1-R antagonism (Nair et al. 2009). Although cue- and stress-induced reinstatement to drug-seeking are subserved by brain pathways that ultimately converge, cue-induced reinstatement may primarily be associated with the mesolimbic DA-system while stress-induced reinstatement relies on CRH and noradrenaline signaling pathways (Kalivas and McFarland 2003, Shaham et al. 2003).

The lack of effect by MCH1-R blockade on stress-induced reinstatement serves as an excellent control for behavioral specificity. This result may be unexpected given prior reports on involvement of the MCH system in stress responses. It remains unclear, however, how MCH regulates stress sensitivity. Some evidence indicates that MCH mediates stress responses by stimulating the HPA-axis (Jezová et al. 1992, Kennedy et al. 2003). Central administration of MCH increases plasma levels of ACTH and corticosterone in mice, and this effect was prevented by pretreatment with GW803430 (Gehlert et al. 2009). In contrast,

other studies have shown an opposite effect of MCH on the HPA-axis (Bluet-Pajot et al. 1995, Ludwig et al. 1998). However, central administration of exogenous MCH will impact the whole brain, and concentrations used may not mimic physiological conditions. The model we used in this study, stress-induced reinstatement to alcohol-seeking, has been shown to depend on central, extrahypothalamic CRH systems, while experiments using adrenalectomized animals receiving constant corticosterone replacement have shown that corticosterone levels are not involved in regulating relapse (Le et al. 2000).

A battery of control experiments was conducted to evaluate the specificity of the effects observed after GW803430 administration on alcohol self-administration and cue-induced reinstatement. Neither LORR, alcohol elimination, locomotor activity, nor taste preference was affected by GW803430.

4.2 MELANIN-CONCENTRATING HORMONE AND ITS MCH1 RECEPTOR: RELATIONSHIP BETWEEN EFFECTS ON ALCOHOL AND CALORIC INTAKE (PAPER II)

As previously mentioned, while the role of the MCH/MCH1-R system in energy homeostasis is well established (Gehlert et al. 2009, Kela et al. 2003, Pissios et al. 2006), little is known about the involvement in alcohol reward and escalation of consumption (Duncan et al. 2006, Duncan et al. 2007, Morganstern et al. 2010). Alcohol is both a pharmacodynamically active substance with an ability to activate brain reward systems, and a caloric nutrient. Here, we therefore extended the findings in paper I by assessing the MCH1-R in regulation of caloric intake and motivation to consume alcohol in states of escalated consumption.

4.2.1 Intermittent access to 20% alcohol decreases food intake, and MCH1-R blockade preferentially reduces alcohol consumption in high drinking rats

Food and alcohol intake was measured in rats during intermittent access to 20% alcohol. Rats consuming high amounts of alcohol (>4g/kg/day) showed a reduction in food intake on days when alcohol was provided compared to days when only water was available. In contrast, low drinking rats (<2g/kg/day) did not differ in food intake across days. Although the high drinking group displayed a decrease in food intake, the contribution of calories obtained from alcohol resulted in a total calorie intake which was comparable to the low drinking group. Further, regression analysis showed a significant negative correlation between alcohol intake and food consumption, as shown in Figure 6. As shown previously, MCH1-R antagonism significantly decreased food intake (Borowsky et al. 2002, Gehlert et al. 2009, Kowalski and McBriar 2004, Kowalski et al. 2006) in both groups while only alcohol consumption was affected by treatment in rats with escalated alcohol intake. The findings indicate that MCH primarily regulates homeostatic caloric balance but also may regulate alcohol reward in states of escalated consumption. Some caution is, however, needed in making this conclusion. Besides from being rewarding alcohol also contains calories, which makes it difficult to fully separate the effects on caloric intake vs. reward. The fact that the low drinking group does not obtain enough calories from alcohol may be due to a floor effect.

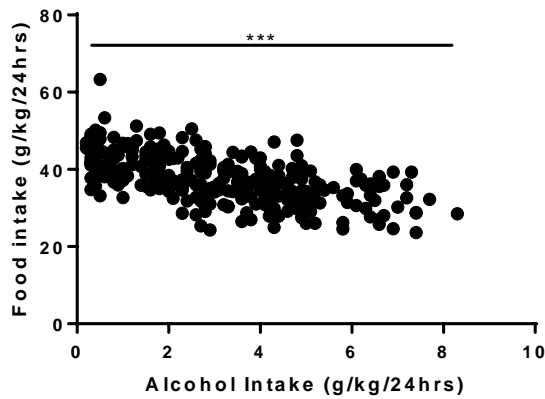


Figure 6. Correlation between food intake and alcohol consumption. A significant negative relationship between alcohol and food intake was observed during intermittent access to 20% alcohol. The graph shows food and alcohol consumption in both high and low drinking rats. Pearson $r = -0.6019$; $R^2=0.3623$; $p<0.001$.

4.2.2 MCH1-R antagonism in states of escalated alcohol consumption

The role of the MCH1-R was further assessed in animal models of escalated alcohol consumption. The effect of GW803430 on motivation to obtain alcohol was evaluated using operant self-administration in protracted abstinence following intermittent access, and during acute withdrawal from alcohol vapor. In protracted abstinence, GW803430 dose dependently decreased self-administration compared to controls (Figure 7). In contrast, no differences in self-administration in acute withdrawal were noticed between vapor and air exposed rats. Intermittent access to alcohol has previously been shown to generate high consuming animals (Simms et al. 2008, Steensland et al. 2007, Wise 1973), but the underlying mechanisms are not fully understood.

We demonstrated a potential role of the MCH/MCH1-R system in reward regulation through gene expression analysis following intermittent access. Specifically, MCH was down-regulated in the LH which harbors MCH positive cell-bodies, while an up-regulation of the MCH1-R was observed in the NAc which receives MCH projections from the LH. We hypothesize that these adaptations persist in protracted abstinence, which could explain the sensitivity to GW803430 on escalated self-administration during protracted abstinence. However, this needs to be further investigated. In addition, central injections or administration of MCH in the NAc or the PVN have been reported to increase alcohol consumption (Morganstern et al. 2010). That raises the possibility that alteration of circuits driving food and reward regulation underlie the escalation of alcohol intake.

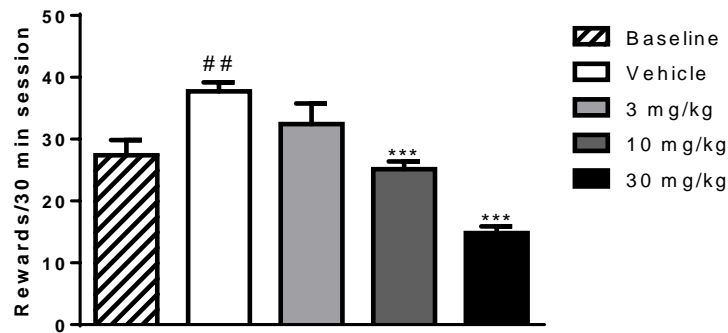


Figure 7. Regulation of escalated alcohol intake in protracted abstinence by the MCH-system. Escalation of alcohol self-administration was induced by intermittent access. Administration of GW803430 dose dependently attenuated alcohol self-administration in protracted abstinence. ### P<0.001 compared to baseline, ***P<0.001 compared to vehicle and control rats. * P<0.05, total responding on active lever compared to last day of extinction.

In contrast, we did not find evidence for a role of the MCH system in dependence-induced escalation during acute withdrawal. Escalation of alcohol consumption has previously been demonstrated after dependence induced through cycles of vapor exposure (O'Dell et al. 2004, Rimondini et al. 2002, Roberts et al. 2000). In acute withdrawal, animals show physical signs of withdrawal (Goldstein and Pal 1971, Roberts et al. 1996), promoting alcohol consumption driven by relief of these symptoms (Heilig and Koob 2007). It appears that the MCH-system is not involved in this state, supported by the lack of effect of the MCH1-R antagonist on alcohol self-administration during this stage, and in agreement with the notion that escalation of self-administration in acute withdrawal may involve stress-mediating pathways rather than reward regulation. Also in agreement with these observations, no changes in gene expression were observed of MCH or the MCH1-R in the hypothalamic - limbic circuit during acute withdrawal.

4.3 MCH1-R ANTAGONISM REDUCES APPETITE FOR CALORIES AND SUPPRESSES ADDICTIVE-LIKE BEHAVIORS (PAPER III)

Intake of palatable food as well as drugs of abuse activate overlapping neurobiological mechanisms (Avena et al. 2008, Volkow et al. 2008). Numerous studies have focused on the role of MCH in functions that regulate energy homeostasis (Borowsky et al. 2002, Gehlert et al. 2009, Kela et al. 2003, Pissios et al. 2006) and in regulation of intake of various types of rewarding feed (Barson et al. 2011). However, fewer studies have assessed the involvement of the MCH-system in motivation to obtain palatable food and sweet preference, or “sugar addiction”. Our previous studies (Paper I and II) suggested a combined effect of MCH1-R blockade on energy balance and alcohol reward, while the specificity of GW803430 on motivation to obtain calories from hedonic consumption had not yet been investigated.

4.3.1 MCH1-R antagonism decreases sucrose seeking and motivation

Motivation to obtain caloric and non-caloric sweet solutions was evaluated in rats after treatment with GW803430. Blockade of MCH1-Rs resulted in attenuated motivation to obtain sucrose, but not saccharin, in the self-administration paradigm. These observations strengthen previous findings in rats showing that the MCH-system mainly regulates caloric intake and is independent of the macronutrient source of the calories, and without having an effect on intake of the non-caloric sweetener saccharin (Barson et al. 2011, Duncan et al. 2005, Sakamaki et al. 2005). In contrast, genetic deletion of the MCH1-R in mice failed to affect consumption of sweet condensed milk (Gehlert et al. 2009). However, this may be explained by developmental compensation in constitutive MCH1-R KO mice since they exhibit a hyperphagic phenotype as well as increased metabolism (Marsh et al. 2002).

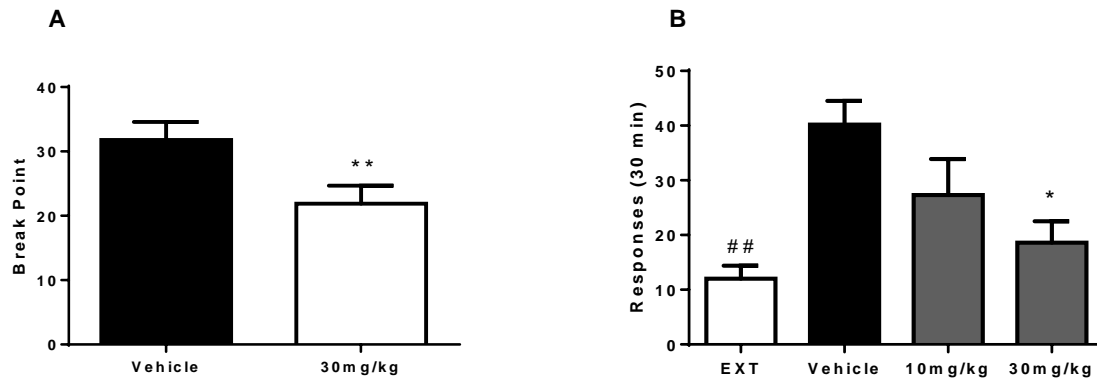


Figure 8. Motivation to obtain sucrose and sucrose-seeking. Rats were evaluated for enhanced motivation to obtain 10% sucrose under a progressive ratio schedule of reinforcement. The y-axis shows the breakpoint for sucrose, which was significantly decreased by 30mg/kg (A). Sucrose seeking was measured with the cue-induced reinstatement model. At the highest dose of 30mg/kg, a significant reduction in sucrose seeking was observed. ** $P < 0.001$ compared to vehicle and control rats. ## $P < 0.01$ compared to EXT, * $P < 0.01$ compared to vehicle. Mean values are represented (\pm S.E.M).

Models of addiction-like behavior were employed to evaluate MCH1-R antagonism on seeking and motivation to obtain sucrose. Administration of GW803430 decreased progressive ratio responding and cue-induced reinstatement (Figure 8A and B) suggesting that MCH1-R blockade primarily attenuates caloric intake but may also play a role in “sucrose addiction”. It also supports previous findings showing that mechanisms driving palatable food intake and drugs of abuse overlap, possibly by interactions with the dopamine system (Di Leone et al. 2003, Georgescu et al. 2005, Volkow et al. 2008).

4.4 THE MCH1-R MODULATES ALCOHOL-INDUCED REWARD AND DARPP-32 ACTIVATION (PAPER IV)

Previous findings indicate that MCH is involved in drug reward, and may interact with DA-signaling in the NAcSh (Chung et al. 2009b, Di Leone et al. 2003, Georgescu et al. 2005, Pissios et al. 2008). However, downstream mechanisms of the MCH1-R which mediate alcohol reward have not been investigated to date. Due to the fact that alcohol contains a significant amount of calories, it is hard to parse out whether MCH1-R blockade decreases the need for calories or attenuates the rewarding properties of alcohol, or both. Use of the CPP paradigm allowed us to assess the role of the MCH system in alcohol reward without intake of calories as a possible confounding factor.

4.4.1 The MCH1-R regulates alcohol reward

Alcohol induced CPP was first measured in WT and MCH1-R KO mice (Figure 9). In a subsequent experiment C57BL/6 mice were evaluated after treatment with either vehicle or GW803430. Genetic deletion as well as pharmacological blockade of the receptor resulted in decreased alcohol CPP. These findings support a recent study by Chung at al. showing that MCH1-R KO mice fail to express cocaine induced CPP (Chung et al. 2009b). In contrast, another group did not find differences in cocaine or amphetamine induced CPP between KO and control mice (Tyhon et al. 2008). Once again, negative results are difficult to interpret. The latter study used mice on a different genetic background and used lower doses of cocaine which may explain the discrepancy.

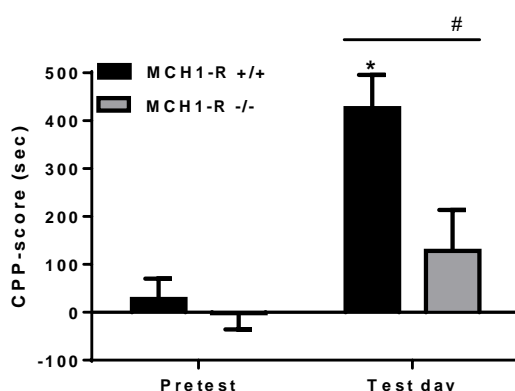


Figure 9. Alcohol induced CPP is attenuated in MCH1-R KO mice. Alcohol, 2g/kg, induced robust CPP in WT compared to pretest preference, while KO mice failed to display CPP. The CPP-score was calculated as time spent on alcohol-paired side minus time spent on the saline side. * $P < 0.01$ compared to KO mice. # $P < 0.001$, pretest compared to test day

Several studies in MCH1-R KO mice suggest that developmental compensations occur as a consequence of the constitutive gene deletion in these animals (Marsh et al. 2002, Smith et al. 2005b). For instance, they show hyperlocomotion, increased metabolism and decreased body weight. It has also been demonstrated that these KO mice drink more alcohol compared to control mice (Duncan et al. 2007), a finding likely related to their hyperphagic phenotype and the caloric content of alcohol. To further evaluate the behavioral specificity of the reward-related effects we found in these mice, a battery of control experiments was carried out. To control for differences in the ability to acquire associative memories, CPA was performed. Both genotypes displayed equal aversion induced by LiCl. Further, mice were tested in the LORR, alcohol elimination and locomotor activity without showing any significant differences in behavior.

4.4.2 Interactions between the MCH1-R and dopamine signaling

Activation of the MCH1-R induces several intracellular signaling cascades (Hawes et al. 2000). Two downstream targets, DARPP-32 (activated through Gi signaling) and ERK (activated through Gi/o signaling), have previously been associated with alcohol reward and linked to the NAcSh (Nestler 2002, Svenningsson et al. 2005, Thorsell et al. 2013). In this study, levels of alcohol induced DARPP-32 and ERK phosphorylation were evaluated in the NAcSh and CeA using immunohistochemistry. To match the CPP experiments, mice were administered an acute dose of alcohol, 2g/kg, or saline. Alcohol induced significantly higher levels of p-DARPP-32 in WT mice compared to KO mice and their respective saline controls. Levels of p-DARPP-32 did not differ between genotypes in the CeA. In contrast, p-ERK levels in the NAcSh, were significantly higher in both WT and KO mice after an acute administration of alcohol compared to saline treated mice.

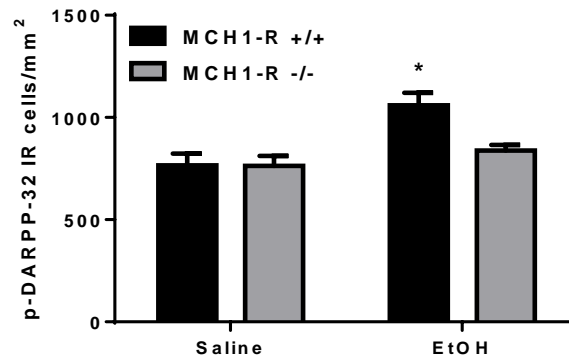


Figure 10. Acute alcohol administration increased levels of p-DARPP-32 in the NAcSh in WT but not KO mice. Levels of p-DARPP-32 were measured by immunohistochemistry in WT and KO mice after an acute dose of saline or alcohol, 2g/kg. Immunoreactive cells were counted under a 40X magnification. * $P < 0.01$ different from all other groups.

Our findings provide further support for the notion that MCH interacts with the dopamine system to regulate drug reward through actions in the NAcSh (Chung et al. 2009b, Hopf et al. 2013). MCH1-Rs are densely expressed in the NAcSh, on cells expressing both D1R and D2R (Georgescu et al. 2005). It presently remains unclear which of these populations is involved in reward-related effects of the MCH system.

5 SUMMARY

Recently, hypothalamic neuropeptide systems have been suggested to play a role in alcohol addiction. MCH is one of these peptides, and exerts its effects through the MCH1-R in rodents. It is well established that MCH is involved in regulation of energy homeostasis and additionally regulates anxiety-related behaviors and stress responses. However, the involvement of this system in alcohol-related behaviors and alcohol addiction has not been well understood.

Our findings demonstrated that MCH1-R blockade by the novel, peripherally available antagonist GW803430, dose-dependently decreased alcohol self-administration in rats and efficiently attenuated cue-induced reinstatement of alcohol seeking, a model of relapse. No effect on stress-induced reinstatement was observed, in agreement with previously published results using cocaine or food. The antagonist used is highly selective for the MCH1-R; a specificity of its effects on alcohol self-administration is supported by the observations that it did not affect stress-induced reinstatement, locomotor activity or taste preference.

The involvement of the MCH1-R had previously not been evaluated in states of escalated alcohol consumption. Rats consuming high amounts of alcohol during intermittent access showed a decrease in food intake when alcohol was provided, and both feed and alcohol consumption was decreased after treatment with GW803430. A potent decrease in alcohol self-administration following MCH1-R antagonist treatment was seen in protracted abstinence after intermittent access. In contrast, no effect was observed on escalated alcohol self-administration during acute withdrawal from vapor-induced physical dependence, which is thought to be primarily driven by stress-related mechanisms.

Treatment with GW803430 reduced sucrose, but not saccharin self-administration. In addition, GW803430 was also effective in attenuating motivation to obtain sucrose, which indicates a combined role in “homeostatic” as well as “hedonic” consumption of sucrose. To study the rewarding properties of alcohol without the possible confounding effects of calories, the CPP paradigm was used. In mice lacking MCH1-receptors, alcohol failed to induce CPP, a finding that was replicated in wildtype C57BL mice treated with the MCH1-R antagonist. The underlying mechanisms for the MCH1-R in regulating alcohol reward have not been investigated to date. Here, we identified p-DARPP-32 as a biomarker downstream of the MCH1-R associated with acute alcohol reward.

In summary, we present a consistent line of data suggesting that, in addition to regulating energy homeostasis, the MCH system in part mediates alcohol reward. It should be noted that

GW803430 has been discontinued from clinical development due to cardiac toxicity, but still remains a useful research tool because it is potent, selective, orally available and brain penetrant. As such, it has helped provide evidence that MCH1-R may offer a novel treatment target for alcohol addiction, in particular during its early stages when alcohol consumption is primarily driven by positively reinforcing, or “rewarding” properties of alcohol. Those findings could pave the way for other compounds, with better clinical safety and tolerability.

6 CONCLUDING REMARKS

Despite the fact that alcoholism has a high prevalence globally and significantly contributes to health care and financial burden, only a limited number of effective pharmacological treatments are available. This highlights the importance of finding new treatments with better efficacy than the medications that are currently available. Increased knowledge of the processes that are driving the underlying biological mechanisms of alcohol addiction is necessary in the search for novel medications. Hypothalamic neuropeptide systems involved in regulation of food intake have been extensively studied in the alcohol addiction field and may offer new possible treatment targets for alcoholism. Preclinical models cannot model all clinically relevant symptoms of alcohol use disorder in humans, but remain helpful as tools for target identification and validation. Molecular techniques such as expression studies and protein analysis can provide indications of brain mechanisms and neural circuits that are involved in regulating alcohol seeking and consumption. These tools will help us on the way, and hopefully one day we will reach the aim to find better treatments.

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8 REFERENCES

- Abbott CR, Kennedy AR, Wren AM, Rossi M, Murphy KG, Seal LJ, Todd JF, Ghatei MA, Small CJ, Bloom SR. 2003. Identification of hypothalamic nuclei involved in the orexigenic effect of melanin-concentrating hormone. *Endocrinology* 144: 3943-3949.
- Altman J, Everitt B, Robbins T, Glautier S, Markou A, Nutt D, Oretti R, Phillips G. 1996. The biological, social and clinical bases of drug addiction: commentary and debate. *Psychopharmacology* 125: 285-345.
- American Psychiatric Association. 1994. *Diagnostic and Statistical Manual of Mental Disorders*. Washington D.C: American Psychiatric Press.
- American Psychiatric Association. 2013. *Diagnostic and Statistical Manual of Mental Disorders: DSM 5*. Washington, D.C: American Psychiatric Association.
- Antai D, Lopez G, Antai J, Anthony D. 1993. Alcohol drinking patterns and differences in alcohol-related harm: A population-based study of the United States. *Biomed Research International* 2014: 853410.
- Aston-Jones G, Smith RJ, Moorman DE, Richardson KA. 2009. Role of lateral hypothalamic orexin neurons in reward processing and addiction. *Neuropharmacology* 56: 112-121.
- Avena NM, Rada P, Hoebel BG. 2006. Sugar bingeing in rats. *Current protocols in neuroscience*: 9.23 C. 21-29.23 C. 26.
- Avena NM, Rada P, Hoebel BG. 2008. Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neuroscience and Biobehavioral Reviews* 32: 20-39.
- Barson JR, Morganstern I, Leibowitz SF. 2011. Similarities in hypothalamic and mesocorticolimbic circuits regulating the overconsumption of food and alcohol. *Physiology & Behavior* 104: 128-137.
- Basso AM, Bratcher NA, Gallagher KB, Cowart MD, Zhao C, Sun M, Esbenshade TA, Brune ME, Fox GB, Schmidt M. 2006. Lack of efficacy of melanin-concentrating hormone-1 receptor antagonists in models of depression and anxiety. *European Journal of Pharmacology* 540: 115-120.
- Biggio G, Dazzi L, Biggio F, Mancuso L, Talani G, Busonero F, Mostallino MC, Sanna E, Follsea P. 2003. Molecular mechanisms of tolerance to and withdrawal of GABA(A) receptor modulators. *European Neuropsychopharmacology* 13: 411-423.
- Bittencourt JC, Presse F, Arias C, Peto C, Vaughan J, Nahon JL, Vale W, Sawchenko PE. 1992. The melanin-concentrating hormone system of the rat brain: an immuno- and hybridization histochemical characterization. *Journal of Comparative Neurology* 319: 218-245.
- Björk K, Terasmaa A, Sun H, Thorsell A, Sommer WH, Heilig M. 2010. Ethanol-induced activation of AKT and DARPP-32 in the mouse striatum mediated by opioid receptors. *Addiction Biology* 15: 299-303.
- Blednov YA, Harris R. 2008. Metabotropic glutamate receptor 5 (mGluR5) regulation of ethanol sedation, dependence and consumption: relationship to acamprosate actions. *International Journal of Neuropsychopharmacology* 11: 775-793.

- Bluet-Pajot MT, Presse F, Voko Z, Hoeger C, Mounier F, Epelbaum J, Nahon JL. 1995. Neuropeptide-E-1 Antagonizes the Action of Melanin-Concentrating Hormone on Stress-Induced Release of Adrenocorticotropin in the Rat. *Journal of neuroendocrinology* 7: 297-303.
- Borowsky B, Durkin MM, Ogozalek K, Marzabadi MR, DeLeon J, Heurich R, Lichtblau H, Shaposhnik Z, Daniewska I, Blackburn TP. 2002. Antidepressant, anxiolytic and anorectic effects of a melanin-concentrating hormone-1 receptor antagonist. *Nature Medicine* 8: 825-830.
- Boutrel B, de Lecea L. 2008. Addiction and arousal: the hypocretin connection. *Physiology & Behavior* 93: 947-951.
- Brodie MS, Pesold C, Appel SB. 1999. Ethanol directly excites dopaminergic ventral tegmental area reward neurons. *Alcoholism: Clinical and Experimental Research* 23: 1848-1852.
- Brog JS, Salyapongse A, Deutch AY, Zahm DS. 1993. The patterns of afferent innervation of the core and shell in the "Accumbens" part of the rat ventral striatum: Immunohistochemical detection of retrogradely transported fluoro-gold. *Journal of Comparative Neurology* 338: 255-278.
- Brown SA, Goldman MS, Inn A, Anderson LR. 1980. Expectations of reinforcement from alcohol: their domain and relation to drinking patterns. *Journal of Consulting and Clinical Psychology* 48: 419.
- Brownstein MJ, Mroz EA, Tappaz ML, Leeman SE. 1977. On the origin of substance P and glutamic acid decarboxylase (GAD) in the substantia nigra. *Brain Research* 135: 315-323.
- Bächner D, Kreienkamp H-J, Weise C, Buck F, Richter D. 1999. Identification of melanin concentrating hormone (MCH) as the natural ligand for the orphan somatostatin-like receptor 1 (SLC-1). *FEBS Letters* 457: 522-524.
- Carboni S, Isola R, Gessa G, Rossetti Z. 1993. Ethanol prevents the glutamate release induced by N-methyl-D-aspartate in the rat striatum. *Neuroscience Letters* 152: 133-136.
- Chaki S, Hirota S, Funakoshi T, Suzuki Y, Suetake S, Okubo T, Ishii T, Nakazato A, Okuyama S. 2003. Anxiolytic-Like and Antidepressant-Like Activities of MCL0129 (1-[(S)-2-(4-Fluorophenyl)-2-(4-isopropylpiperidin-1-yl) ethyl]-4-[4-(2-methoxynaphthalen-1-yl) butyl] piperazine), a Novel and Potent Nonpeptide Antagonist of the Melanocortin-4 Receptor. *Journal of Pharmacology and Experimental Therapeutics* 304: 818-826.
- Chaki S, Funakoshi T, Hirota-Okuno S, Nishiguchi M, Shimazaki T, Iijima M, Grottick AJ, Kanuma K, Omodera K, Sekiguchi Y. 2005. Anxiolytic-and antidepressant-like profile of ATC0065 and ATC0175: nonpeptidic and orally active melanin-concentrating hormone receptor 1 antagonists. *Journal of Pharmacology and Experimental Therapeutics* 313: 831-839.
- Chambers J, Ames RS, Bergsma D, Muir A, Fitzgerald LR, Hervieu G, Dytko GM, Foley JJ, Martin J, Liu W-S. 1999. Melanin-concentrating hormone is the cognate ligand for the orphan G-protein-coupled receptor SLC-1. *Nature* 400: 261-265.
- Chamorro AJ, Marcos M, Mirón-Canelo JA, Pastor I, González-Sarmiento R, Laso FJ. 2012. Association of μ -opioid receptor (OPRM1) gene polymorphism with response to naltrexone in alcohol dependence: a systematic review and meta-analysis. *Addiction Biology* 17: 505-512.

- Chang H, Kitai S. 1985. Projection neurons of the nucleus accumbens: an intracellular labeling study. *Brain Research* 347: 112-116.
- Chang H, Wilson C, Kitai S. 1982. A Golgi study of rat neostriatal neurons: light microscopic analysis. *Journal of Comparative Neurology* 208: 107-126.
- Chen Y, Hu C, Hsu C-K, Zhang Q, Bi C, Asnicar M, Hsiung HM, Fox N, Sliker LJ, Yang DD. 2002. Targeted disruption of the melanin-concentrating hormone receptor-1 results in hyperphagia and resistance to diet-induced obesity. *Endocrinology* 143: 2469-2477.
- Cherpitel CJ. 1993. Alcohol, Injury, and Risk-Taking Behavior: Data from a National Sample. *Alcoholism: Clinical and Experimental Research* 17: 762-766.
- Chung S, Saito Y, Civelli O. 2009a. MCH receptors/gene structure-in vivo expression. *Peptides* 30: 1985-1989.
- Chung S, Parks GS, Lee C, Civelli O. 2011. Recent updates on the melanin-concentrating hormone (MCH) and its receptor system: lessons from MCH1R antagonists. *Journal of Molecular Neuroscience* 43: 115-121.
- Chung S, Hopf FW, Nagasaki H, Li CY, Belluzzi JD, Bonci A, Civelli O. 2009b. The melanin-concentrating hormone system modulates cocaine reward. *Proceedings of the National Academy of Sciences* 106: 6772.
- Cippitelli A, Damadzic R, Singley E, Thorsell A, Ciccocioppo R, Eskay RL, Heilig M. 2012. Pharmacological blockade of corticotropin-releasing hormone receptor 1 (CRH1R) reduces voluntary consumption of high alcohol concentrations in non-dependent Wistar rats. *Pharmacology Biochemistry and Behavior* 100: 522-529.
- Cippitelli A, Bilbao A, Gorriti MA, Navarro M, Massi M, Piomelli D, Ciccocioppo R, De Fonseca FR. 2007. The anandamide transport inhibitor AM404 reduces ethanol self-administration. *European Journal of Neuroscience* 26: 476-486.
- Cippitelli A, Cannella N, Braconi S, Duranti A, Tontini A, Bilbao A, DeFonseca FR, Piomelli D, Ciccocioppo R. 2008. Increase of brain endocannabinoid anandamide levels by FAAH inhibition and alcohol abuse behaviours in the rat. *Psychopharmacology* 198: 449-460.
- Cippitelli A, Bilbao A, Hansson AC, Del Arco I, Sommer W, Heilig M, Massi M, Bermúdez-Silva FJ, Navarro M, Ciccocioppo R, De Fonseca FR. 2005. Cannabinoid CB1 receptor antagonism reduces conditioned reinstatement of ethanol-seeking behavior in rats. *European Journal of Neuroscience* 21: 2243-2251.
- Colantuoni C, Rada P, McCarthy J, Patten C, Avena NM, Chadeayne A, Hoebel BG. 2002. Evidence that intermittent, excessive sugar intake causes endogenous opioid dependence. *Obesity* 10: 478-488.
- Colantuoni C, Schwenker J, McCarthy J, Rada P, Ladenheim B, Cadet JL, Schwartz GJ, Moran TH, Hoebel BG. 2001. Excessive sugar intake alters binding to dopamine and mu-opioid receptors in the brain. *Neuroreport* 12: 3549-3552.
- Costa A, Bono G, Martignoni E, Merlo P, Sances G, Nappi G. 1996. An assessment of hypothalamo-pituitary-adrenal axis functioning in non-depressed, early abstinent alcoholics. *Psychoneuroendocrinology* 21: 263-275.
- Covarrubias MY, Khan RL, Vadigepalli R, Hoek JB, Schwaber JS. 2006. Chronic alcohol exposure alters transcription broadly in a key integrative brain nucleus for homeostasis: the nucleus tractus solitarius. *Physiological Genomics* 24: 45-58.

- Cowen MS, Lawrence AJ. 1999. The role of opioid-dopamine interactions in the induction and maintenance of ethanol consumption. *Progress in Neuro-psychopharmacology and Biological Psychiatry* 23: 1171-1212.
- Davis M. 1998. Are different parts of the extended amygdala involved in fear versus anxiety? *Biological Psychiatry* 44: 1239-1247.
- Davis M, Walker DL, Miles L, Grillon C. 2009. Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology* 35: 105-135.
- Della-Zuana O, Presse F, Ortola C, Duhault J, Nahon J, Levens N. 2002. Acute and chronic administration of melanin-concentrating hormone enhances food intake and body weight in Wistar and Sprague-Dawley rats. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity* 26: 1289-1295.
- Di Chiara G, Imperato A. 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proceedings of the National Academy of Sciences* 85: 5274-5278.
- Di Chiara G, Bassareo V, Fenu S, De Luca MA, Spina L, Cadoni C, Acquas E, Carboni E, Valentini V, Lecca D. 2004. Dopamine and drug addiction: the nucleus accumbens shell connection. *Neuropharmacology* 47: 227-241.
- Di Leone RJ, Georgescu D, Nestler EJ. 2003. Lateral hypothalamic neuropeptides in reward and drug addiction. *Life Sciences* 73: 759-768.
- Donovan DM, Marlatt GA. 1980. Assessment of Expectancies and Behaviors Associated with Alcohol Consumption; A Cognitive-Behavioral Approach. *Journal of Studies on Alcohol and Drugs* 41: 1153.
- Duncan EA, Proulx K, Woods SC. 2005. Central administration of melanin-concentrating hormone increases alcohol and sucrose/quinine intake in rats. *Alcoholism: Clinical and Experimental Research* 29: 958-964.
- Duncan EA, Rider TR, Jandacek RJ, Clegg DJ, Benoit SC, Tso P, Woods SC. 2006. The regulation of alcohol intake by melanin-concentrating hormone in rats. *Pharmacology Biochemistry and Behavior* 85: 728-735.
- Duncan EA, Sorrell JE, Adamantidis A, Rider T, Jandacek RJ, Seeley RJ, Lakaye B, Woods SC. 2007. Alcohol drinking in MCH receptor-1-deficient mice. *Alcoholism: Clinical and Experimental Research* 31: 1325-1337.
- Edwards G, Gross MM. 1976. Alcohol dependence: provisional description of a clinical syndrome. *British medical journal* 1: 1058.
- Elias CF, Saper CB, Maratos-Flier E, Tritos NA, Lee C, Kelly J, Tatro JB, Hoffman GE, Ollmann MM, Barsh GS. 1998. Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area. *Journal of Comparative Neurology* 402: 442-459.
- Elmquist JK, Coppari R, Balthasar N, Ichinose M, Lowell BB. 2005. Identifying hypothalamic pathways controlling food intake, body weight, and glucose homeostasis. *Journal of Comparative Neurology* 493: 63-71.
- Funk D, Marinelli PW, Le AD. 2006. Biological processes underlying co-use of alcohol and nicotine: neuronal mechanisms, cross-tolerance, and genetic factors. *Alcohol Research and Health* 29: 186-192.

- Gallegos RA, Lee R-S, Criado JR, Henriksen SJ, Steffensen SC. 1999. Adaptive responses of γ -aminobutyric acid neurons in the ventral tegmental area to chronic ethanol. *Journal of Pharmacology and Experimental Therapeutics* 291: 1045-1053.
- Garbutt JC, Greenblatt AM, West SL, Morgan LC, Kampov-Polevoy A, Jordan HS, Bobashev GV. 2014. Clinical and biological moderators of response to naltrexone in alcohol dependence: a systematic review of the evidence. *Addiction* 109: 1274-1284.
- Gehlert DR, Rasmussen K, Shaw J, Li X, Ardayfio P, Craft L, Coskun T, Zhang HY, Chen Y, Witkin JM. 2009. Preclinical Evaluation of Melanin-Concentrating Hormone Receptor 1 Antagonism for the Treatment of Obesity and Depression. *Journal of Pharmacology and Experimental Therapeutics* 329: 429-438.
- Gehlert DR, et al. 2007. 3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethyl-imidazo[1,2-b]pyridazine: a novel brain-penetrant, orally available corticotropin-releasing factor receptor 1 antagonist with efficacy in animal models of alcoholism. *Journal of Neuroscience* 27: 2718-2726.
- Georgescu D, Sears RM, Hommel JD, Barrot M, Bolanos CA, Marsh DJ, Bednarek MA, Bibb JA, Maratos-Flier E, Nestler EJ. 2005. The Hypothalamic Neuropeptide Melanin-Concentrating Hormone Acts in the Nucleus Accumbens to Modulate Feeding Behavior and Forced-Swim Performance. *Journal of Neuroscience* 25: 2933-2940.
- Gerfen CR, Young III S. 1988. Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. *Brain Research* 460: 161-167.
- Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma F, Sibley DR. 1990. D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 250: 1429-1432.
- Gilpin NW, Koob GF. 2008. Neurobiology of alcohol dependence: focus on motivational mechanisms. *Alcohol Research and Health* 31: 185-195.
- Goeders N. 2003. The impact of stress on addiction. *European Neuropsychopharmacology* 13: 435-441.
- Goldman D, Oroszi G, Ducci F. 2005. The genetics of addictions: uncovering the genes. *Nature Reviews Genetics* 6: 521-532.
- Goldstein DB, Pal N. 1971. Alcohol dependence produced in mice by inhalation of ethanol: grading the withdrawal reaction. *Science* 172: 288-290.
- Goodwin FL, Amit Z. 1998. Do Taste Factors Contribute to the Mediation of Ethanol Intake? Ethanol and Saccharin-Quinine Intake in Three Rat Strains. *Alcoholism: Clinical and Experimental Research* 22: 837-844.
- Gould TD, Manji HK. 2005. DARPP-32: A molecular switch at the nexus of reward pathway plasticity. *Proceedings of the National Academy of Sciences* 102: 253-254.
- Grobin AC, Matthews DB, Devaud LL, Morrow AL. 1998. The role of GABA_A receptors in the acute and chronic effects of ethanol. *Psychopharmacology* 139: 2-19.
- Grüsser SM, Wrase J, Klein S, Hermann D, Smolka MN, Ruf M, Weber-Fahr W, Flor H, Mann K, Braus DF. 2004. Cue-induced activation of the striatum and medial prefrontal cortex is associated with subsequent relapse in abstinent alcoholics. *Psychopharmacology* 175: 296-302.

- Harris BR, Prendergast MA, Gibson DA, Rogers DT, Blanchard JA, Holley RC, Fu MC, Hart SR, Pedigo NW, Littleton JM. 2002. Acamprosate Inhibits the Binding and Neurotoxic Effects of Trans-ACPD, Suggesting a Novel Site of Action at Metabotropic Glutamate Receptors. *Alcoholism: Clinical and Experimental Research* 26: 1779-1793.
- Havel PJ. 2001. Peripheral signals conveying metabolic information to the brain: short-term and long-term regulation of food intake and energy homeostasis. *Experimental Biology and Medicine* 226: 963-977.
- Hawes BE, Kil E, Green B, O'Neill K, Fried S, Graziano MP. 2000. The melanin-concentrating hormone receptor couples to multiple G proteins to activate diverse intracellular signaling pathways. *Endocrinology* 141: 4524-4532.
- Heilig M, Koob GF. 2007. A key role for corticotropin-releasing factor in alcohol dependence. *Trends in Neurosciences* 30: 399-406.
- Heilig M, Egli M, Crabbe JC, Becker HC. 2010. Acute withdrawal, protracted abstinence and negative affect in alcoholism: are they linked? *Addiction biology* 15: 169-184.
- Heilig M, Goldman D, Berrettini W, O'Brien CP. 2011. Pharmacogenetic approaches to the treatment of alcohol addiction. *Nature Reviews Neuroscience* 12: 670-684.
- Heinz A, Löber S, Georgi A, Wrase J, Hermann D, Rey E-R, Wellek S, Mann K. 2003. Reward craving and withdrawal relief craving: assessment of different motivational pathways to alcohol intake. *Alcohol and Alcoholism* 38: 35-39.
- Herman JP, Prewitt CM-F, Cullinan WE. 1996. Neuronal circuit regulation of the hypothalamo-pituitary-adrenocortical stress axis. *Critical Reviews in Neurobiology* 10: 371-394.
- Hertzog DL, Al-Barazanji KA, Bigham EC, Bishop MJ, Britt CS, Carlton DL, Cooper JP, Daniels AJ, Garrido DM, Goetz AS. 2006. The discovery and optimization of pyrimidinone-containing MCH R1 antagonists. *Bioorganic and Medicinal Chemistry Letters* 16: 4723-4727.
- Hervieu G, Cluderay J, Harrison D, Meakin J, Maycox P, Nasir S, Leslie R. 2000. The distribution of the mRNA and protein products of the melanin-concentrating hormone (MCH) receptor gene, *slc-1*, in the central nervous system of the rat. *European Journal of Neuroscience* 12: 1194-1216.
- Herz A. 1997. Endogenous opioid systems and alcohol addiction. *Psychopharmacology* 129: 99-111.
- Hill J, Duckworth M, Murdock P, Rennie G, Sabido-David C, Ames RS, Szekeres P, Wilson S, Bergsma DJ, Gloger IS. 2001. Molecular cloning and functional characterization of MCH2, a novel human MCH receptor. *Journal of Biological Chemistry* 276: 20125-20129.
- Hillebrand J, De Wied D, Adan R. 2002. Neuropeptides, food intake and body weight regulation: a hypothalamic focus. *Peptides* 23: 2283-2306.
- Holmes A, Spanagel R, Krystal JH. 2013. Glutamatergic targets for new alcohol medications. *Psychopharmacology* 229: 539-554.
- Hopf FW, Seif T, Chung S, Civelli O. 2013. MCH and apomorphine in combination enhance action potential firing of nucleus accumbens shell neurons in vitro. *PeerJ* 1: e61.

- Jezová D, Bartanusz V, Westergren I, Johansson BB, Rivier J, Vale W, Rivier C. 1992. Rat melanin-concentrating hormone stimulates adrenocorticotropin secretion: evidence for a site of action in brain regions protected by the blood-brain barrier. *Endocrinology* 130: 1024-1029.
- Johnson S, North R. 1992. Opioids excite dopamine neurons by hyperpolarization of local interneurons. *Journal of Neuroscience* 12: 483-488.
- Jørgensen CH, Pedersen B, Tønnesen H. 2011. The efficacy of disulfiram for the treatment of alcohol use disorder. *Alcoholism: Clinical and Experimental Research* 35: 1749-1758.
- Kalivas PW, McFarland K. 2003. Brain circuitry and the reinstatement of cocaine-seeking behavior. *Psychopharmacology* 168: 44-56.
- Kawauchi H, Kawazoe I, Tsubokawa M, Kishida M, Baker BI. 1983. Characterization of melanin-concentrating hormone in chum salmon pituitaries. *Nature* 305: 321-323.
- Kela J, Salmi P, Rimondini-Giorgini R, Heilig M, Wahlestedt C. 2003. Behavioural analysis of melanin-concentrating hormone in rats: evidence for orexigenic and anxiolytic properties. *Regulatory Peptides* 114: 109-114.
- Kennedy A, Todd J, Dhillo W, Seal L, Ghatei M, O'Toole C, Jones M, Witty D, Winborne K, Riley G. 2003. Effect of direct injection of melanin-concentrating hormone into the paraventricular nucleus: Further evidence for a stimulatory role in the adrenal axis via SLC-1. *Journal of Neuroendocrinology* 15: 268-272.
- Khisti RT, VanDoren MJ, O'Buckley T, Morrow AL. 2003. Neuroactive steroid 3 α -hydroxy-5 α -pregnan-20-one modulates ethanol-induced loss of righting reflex in rats. *Brain Research* 980: 255-265.
- Kiefer F, Wiedemann K. 2004. Neuroendocrine pathways of addictive behaviour. *Addiction Biology* 9: 205-212.
- Kiianmaa K, Andersson K, Fuxe K. 1979. On the role of ascending dopamine systems in the control of voluntary ethanol intake and ethanol intoxication. *Pharmacology Biochemistry and Behavior* 10: 603-608.
- Kissler JL, Sirohi S, Reis DJ, Jansen HT, Quock RM, Smith DG, Walker BM. 2014. The one-two punch of alcoholism: role of central amygdala dynorphins/kappa-opioid receptors. *Biological Psychiatry* 75: 774-782.
- Kitson TM. 1977. The Disulfiram—Ethanol Reaction; a Review. *Journal of Studies on Alcohol and Drugs* 38: 96-113
- Kohl R, Katner J, Chernet E, McBride W. 1998. Ethanol and negative feedback regulation of mesolimbic dopamine release in rats. *Psychopharmacology* 139: 79-85.
- Kolakowski LF, Jung BP, Nguyen T, Johnson MP, Lynch KR, Cheng R, Heng HH, George SR, O'Dowd BF. 1996. Characterization of a human gene related to genes encoding somatostatin receptors. *FEBS Letters* 398: 253-258.
- Koob GF. 1992. Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends in Pharmacological Sciences* 13: 177-184.
- Koob GF, Le Moal M. 2001. Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology* 24: 97-129.
- Koob GF, Le Moal M. 2005. Plasticity of reward neurocircuitry and the 'dark side' of drug addiction. *Nature Neuroscience* 8: 1442-1444.

- Koob GF, Volkow ND. 2009. Neurocircuitry of addiction. *Neuropsychopharmacology* 35: 217-238.
- Kowalski TJ, McBriar MD. 2004. Therapeutic potential of melanin-concentrating hormone-1 receptor antagonists for the treatment of obesity. *Expert Opinion on Investigational Drugs* 13: 1113-1122.
- Kowalski TJ, Spar BD, Weig B, Farley C, Cook J, Ghibaudi L, Fried S, O'Neill K, Del Vecchio RA, McBriar M. 2006. Effects of a selective melanin-concentrating hormone 1 receptor antagonist on food intake and energy homeostasis in diet-induced obese mice. *European Journal of Pharmacology* 535: 182-191.
- Kroslak T, LaForge KS, Gianotti RJ, Ho A, Nielsen DA, Kreek MJ. 2007. The single nucleotide polymorphism A118G alters functional properties of the human mu opioid receptor. *Journal of Neurochemistry* 103: 77-87.
- Le A, Harding S, Juzytsch W, Watchus J, Shalev U, Shaham Y. 2000. The role of corticotrophin-releasing factor in stress-induced relapse to alcohol-seeking behavior in rats. *Psychopharmacology* 150: 317-324.
- Lembo PM, Grazzini E, Cao J, Hubatsch DA, Pelletier M, Hoffert C, St-Onge S, Pou C, Labrecque J, Groblewski T. 1999. The receptor for the orexigenic peptide melanin-concentrating hormone is a G-protein-coupled receptor. *Nature Cell Biology* 1: 267-271.
- Lovinger DM, White G, Weight FF. 1989. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. *Science* 243: 1721-1724.
- Ludwig DS, Mountjoy KG, Tatro JB, Gillette JA, Frederich RC, Flier JS, Maratos-Flier E. 1998. Melanin-concentrating hormone: a functional melanocortin antagonist in the hypothalamus. *American Journal of Physiology-Endocrinology and Metabolism* 274: E627-E633.
- Ludwig DS, Tritos NA, Mastaitis JW, Kulkarni R, Kokkotou E, Elmquist J, Lowell B, Flier JS, Maratos-Flier E. 2001. Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance. *Journal of Clinical Investigation* 107: 379-386.
- Luiten P, Ter Horst G, Steffens A. 1987. The hypothalamus, intrinsic connections and outflow pathways to the endocrine system in relation to the control of feeding and metabolism. *Progress in Neurobiology* 28: 1-54.
- Maldonado-Irizarry CS, Swanson CJ, Kelley AE. 1995. Glutamate receptors in the nucleus accumbens shell control feeding behavior via the lateral hypothalamus. *The Journal of Neuroscience* 15: 6779-6788.
- Mancini MC, Halpern A. 2006. Investigational therapies in the treatment of obesity. *Expert Opinion on Investigational Drugs* 15: 897-915.
- Mann K, Schäfer DR, Längle G, Ackermann K, Croissant B. 2005. The long-term course of alcoholism, 5, 10 and 16 years after treatment. *Addiction* 100: 797-805.
- Marsh DJ, Weingarth DT, Novi DE, Chen HY, Trumbauer ME, Chen AS, Guan X-M, Jiang MM, Feng Y, Camacho RE. 2002. Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive, and hyperphagic and have altered metabolism. *Proceedings of the National Academy of Sciences* 99: 3240-3245.
- Mayo-Smith MF, Beecher LH, Fischer TL, Gorelick DA, Guillaume JL, Hill A, Jara G, Kasser C, Melbourne J. 2004. Management of alcohol withdrawal delirium: an evidence-based practice guideline. *Archives of Internal Medicine* 164: 1405-1412.

- McBride WJ, Li T-K. 1998. Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. *Critical Reviews in Neurobiology* 12: 339-369.
- McEwen BS. 2000. Allostasis and allostatic load: implications for neuropsychopharmacology. *Neuropsychopharmacology* 22: 108-124.
- Merchenthaler I, Vigh S, Petrusz P, Schally A. 1982. Immunocytochemical localization of corticotropin-releasing factor (CRF) in the rat brain. *American Journal of Anatomy* 165: 385-396.
- Mitchell JM, O'Neil JP, Janabi M, Marks SM, Jagust WJ, Fields HL. 2012. Alcohol consumption induces endogenous opioid release in the human orbitofrontal cortex and nucleus accumbens. *Science Translational Medicine* 4: 116ra116.
- Mogenson G, Swanson L, Wu M. 1983. Neural projections from nucleus accumbens to globus pallidus, substantia innominata, and lateral preoptic-lateral hypothalamic area: an anatomical and electrophysiological investigation in the rat. *Journal of Neuroscience* 3: 189-202.
- Monzon M, De Barioglio S. 1999. Response to novelty after icv injection of melanin-concentrating hormone (MCH) in rats. *Physiology & Behavior* 67: 813-817.
- Morganstern I, Chang G-Q, Chen Y-W, Barson J, Zhiyu Y, Hoebel B, Leibowitz S. 2010. Role of melanin-concentrating hormone in the control of ethanol consumption: region-specific effects revealed by expression and injection studies. *Physiology & behavior* 101: 428-437.
- Mori M, Harada M, Terao Y, Sugo T, Watanabe T, Shimomura Y, Abe M, Shintani Y, Onda H, Nishimura O. 2001. Cloning of a novel G protein-coupled receptor, SLT, a subtype of the melanin-concentrating hormone receptor. *Biochemical and biophysical research communications* 283: 1013-1018.
- Morton G, Cummings D, Baskin D, Barsh G, Schwartz M. 2006. Central nervous system control of food intake and body weight. *Nature* 443: 289-295.
- Nahon JL. 2006. The melanocortins and melanin-concentrating hormone in the central regulation of feeding behavior and energy homeostasis. *Comptes Rendus Biologies* 329: 623-638.
- Nahon JL, Presse F, Bittencourt J, Sawchenko P, Vale W. 1989. The Rat Melanin-Concentrating Hormone Messenger Ribonucleic Acid Encodes Multiple Putative Neuropeptides Coexpressed in the Dorsolateral Hypothalamus. *Endocrinology* 125: 2056-2065.
- Nair SG, Adams-Deutsch T, Pickens CL, Smith DG, Shaham Y. 2009. Effects of the MCH1 receptor antagonist SNAP 94847 on high-fat food-reinforced operant responding and reinstatement of food seeking in rats. *Psychopharmacology* 205: 129-140.
- Nestler EJ. 2002. Common molecular and cellular substrates of addiction and memory. *Neurobiology of Learning and Memory* 78: 637-647.
- Nestler EJ. 2005. Is there a common molecular pathway for addiction? *Nature Neuroscience* 8: 1445-1449.
- O'Dell LE, Roberts AJ, Smith RT, Koob GF. 2004. Enhanced Alcohol Self-Administration after Intermittent Versus Continuous Alcohol Vapor Exposure. *Alcoholism: Clinical and Experimental Research* 28: 1676-1682.

- O'Malley SS, Jaffe AJ, Rode S, Rounsaville BJ. 1996. Experience of a "slip" among alcoholics treated with naltrexone or placebo. *The American Journal of Psychiatry* 153: 136-143.
- Obici S, Zhang BB, Karkanas G, Rossetti L. 2002. Hypothalamic insulin signaling is required for inhibition of glucose production. *Nature Medicine* 8: 1376-1382.
- Ouimet C, Miller P, Hemmings H, Walaas SI, Greengard P. 1984. DARPP-32, a dopamine- and adenosine 3': 5'-monophosphate-regulated phosphoprotein enriched in dopamine-innervated brain regions. III. Immunocytochemical localization. *Journal of Neuroscience* 4: 111-124.
- Pacák K, Palkovits M. 2001. Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. *Endocrine Reviews* 22: 502-548.
- Paille FM, Guelfi JD, Perkins AC, Royer RJ, Steru L, Parot P. 1995. Double-blind randomized multicentre trial of acamprosate in maintaining abstinence from alcohol. *Alcohol and Alcoholism* 30: 239-247.
- Pariante CM, Lightman SL. 2008. The HPA axis in major depression: classical theories and new developments. *Trends in Neurosciences* 31: 464-468.
- Paxinos G, Watson C. 2006. *The rat brain in stereotaxic coordinates*. London: Academic Press.
- Peachey JE, Naranjo CA. 1983. The use of disulfiram and other alcohol-sensitizing drugs in the treatment of alcoholism. Pages 397-431. *Research advances in alcohol and drug problems*. New York, NY: Springer.
- Pellmar TC, Brandt Jr EN, Baird MA. 2002. Health and behavior: the interplay of biological, behavioral, and social influences: Summary of an Institute of Medicine report. *American Journal of Health Promotion* 16: 206-219.
- Pierce RC, Kumaresan V. 2006. The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? *Neuroscience & Biobehavioral Reviews* 30: 215-238.
- Pissios P, Bradley RL, Maratos-Flier E. 2006. Expanding the scales: the multiple roles of MCH in regulating energy balance and other biological functions. *Endocrine Reviews* 27: 606-620.
- Pissios P, Frank L, Kennedy AR, Porter DR, Marino FE, Liu F-F, Pothos EN, Maratos-Flier E. 2008. Dysregulation of the Mesolimbic Dopamine System and Reward in MCH^{-/-} Mice. *Biological Psychiatry* 64: 184-191.
- Pontieri F, Tanda G, Di Chiara G. 1995. Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens. *Proceedings of the National Academy of Sciences* 92: 12304-12308.
- Porte D, Seeley R, Woods S, Baskin D, Figlewicz D, Schwartz M. 1998. Obesity, Diabetes and the Central Nervous System. *Diabetologia* 41: 863-881.
- Presse F, Nahon J-L, Fischer WH, Vale W. 1990. Structure of the human melanin concentrating hormone mRNA. *Molecular Endocrinology* 4: 632-637.
- Pulvirenti L, Diana M. 2001. Drug dependence as a disorder of neural plasticity: focus on dopamine and glutamate. *Reviews in the Neurosciences* 12: 141-158.

- Qu D, Ludwig DS, Gammeltoft S, Piper M, Pelleymounter MA, Cullen MJ, Mathes WF, Przypek R, Kanarek R, Maratos-Flier E. 1996. A role for melanin-concentrating hormone in the central regulation of feeding behaviour. *Nature* 380: 243-247.
- Rahman S, McBride WJ. 2001. D1–D2 dopamine receptor interaction within the nucleus accumbens mediates long-loop negative feedback to the ventral tegmental area (VTA). *Journal of Neurochemistry* 77: 1248-1255.
- Rassnick S, Stinus L, Koob GF. 1993. The effects of 6-hydroxydopamine lesions of the nucleus accumbens and the mesolimbic dopamine system on oral self-administration of ethanol in the rat. *Brain Research* 623: 16-24.
- Rehm J, Mathers C, Popova S, Thavorncharoensap M, Teerawattananon Y, Patra J. 2009. Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. *The Lancet* 373: 2223-2233.
- Rimondini R, Arlinde C, Sommer W, Heilig M. 2002. Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. *The FASEB Journal* 16: 27-35.
- Risinger FO, Cunningham CL. 2000. DBA/2J mice develop stronger lithium chloride-induced conditioned taste and place aversions than C57BL/6J mice. *Pharmacology Biochemistry and Behavior* 67: 17-24.
- Roberts AJ, Cole M, Koob GF. 1996. Intra-amygdala muscimol decreases operant ethanol self-administration in dependent rats. *Alcoholism: Clinical and Experimental Research* 20: 1289-1298.
- Roberts AJ, Heyser CJ, Cole M, Griffin P, Koob GF. 2000. Excessive ethanol drinking following a history of dependence: animal model of allostasis. *Neuropsychopharmacology* 22: 581-594.
- Ronis MJ, Wands JR, Badger TM, De La Monte SM, Lang CH, Calissendorff J. 2007. Alcohol-Induced Disruption of Endocrine Signaling. *Alcoholism: Clinical and Experimental Research* 31: 1269-1285.
- Russell WMS, Burch RL, Hume CW. 1959. *The principles of humane experimental technique*. London: Methuen.
- Russo SJ, Dietz DM, Dumitriu D, Morrison JH, Malenka RC, Nestler EJ. 2010. The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. *Trends in Neurosciences* 33: 267-276.
- Sailer AW, Sano H, Zeng Z, McDonald TP, Pan J, Pong S-S, Feighner SD, Tan CP, Fukami T, Iwaasa H. 2001. Identification and characterization of a second melanin-concentrating hormone receptor, MCH-2R. *Proceedings of the National Academy of Sciences* 98: 7564-7569.
- Saito Y, Cheng M, Leslie FM, Civelli O. 2001. Expression of the melanin-concentrating hormone (MCH) receptor mRNA in the rat brain. *Journal of Comparative Neurology* 435: 26-40.
- Saito Y, Nothacker H-P, Wang Z, Lin SH, Leslie F, Civelli O. 1999. Molecular characterization of the melanin-concentrating-hormone receptor. *Nature* 400: 265-269.
- Sakamaki R, Uemoto M, Inui A, Asakawa A, Ueno N, Ishibashi C, Hirono S, Yukioka H, Kato A, Shinfuku N. 2005. Melanin-concentrating hormone enhances sucrose intake. *International Journal of Molecular Medicine* 15: 1033-1039.

- Sanchis-Segura C, Spanagel R. 2006. Behavioural assessment of drug reinforcement and addictive features in rodents: an overview. *Addiction Biology* 11: 2-38.
- Sanudo-Pena M, Tsou K, Delay ER, Hohman AG, Force M, Walker JM. 1997. Endogenous cannabinoids as an aversive or counter-rewarding system in the rat. *Neuroscience Letters* 223: 125-128.
- Saper CB, Scammell TE, Lu J. 2005. Hypothalamic regulation of sleep and circadian rhythms. *Nature* 437: 1257-1263.
- Schwartz MW, Woods SC, Porte D, Seeley RJ, Baskin DG. 2000. Central nervous system control of food intake. *Nature* 404: 661-671.
- Self DW, Nestler EJ. 1995. Molecular mechanisms of drug reinforcement and addiction. *Annual Review of Neuroscience* 18: 463-495.
- Shaham Y, Shalev U, Lu L, de Wit H, Stewart J. 2003. The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology* 168: 3-20.
- Shi Y. 2004. Beyond skin color: emerging roles of melanin-concentrating hormone in energy homeostasis and other physiological functions. *Peptides* 25: 1605-1611.
- Shimada M, Tritos NA, Lowell BB, Flier JS, Maratos-Flier E. 1998. Mice lacking melanin-concentrating hormone are hypophagic and lean. *Nature* 396: 670-674.
- Simms JA, Steensland P, Medina B, Abernathy KE, Chandler LJ, Wise R, Bartlett SE. 2008. Intermittent access to 20% ethanol induces high ethanol consumption in Long-Evans and Wistar rats. *Alcoholism: Clinical and Experimental Research* 32: 1816-1823.
- Sinha R, Li CS. 2007. Imaging stress-and cue-induced drug and alcohol craving: association with relapse and clinical implications. *Drug and Alcohol Review* 26: 25-31.
- Smith DG, Davis RJ, Rorick-Kehn L, Morin M, Witkin JM, McKinzie DL, Nomikos GG, Gehlert DR. 2005a. Melanin-concentrating hormone-1 receptor modulates neuroendocrine, behavioral, and corticolimbic neurochemical stress responses in mice. *Neuropsychopharmacology* 31: 1135-1145.
- Smith DG, Tzavara ET, Shaw J, Luecke S, Wade M, Davis R, Salhoff C, Nomikos GG, Gehlert DR. 2005b. Mesolimbic dopamine super-sensitivity in melanin-concentrating hormone-1 receptor-deficient mice. *Journal of Neuroscience* 25: 914-922.
- Solomon RL, Corbit JD. 1974. An opponent-process theory of motivation: I. Temporal dynamics of affect. *Psychological Review* 81: 119.
- Spanagel R. 2009. Alcoholism: a systems approach from molecular physiology to addictive behavior. *Physiological Reviews* 89: 649-705.
- Spanagel R, Weiss F. 1999. The dopamine hypothesis of reward: past and current status. *Trends in Neurosciences* 22: 521-527.
- Spanagel R, Vengeliene V, Jandeleit B, Fischer W-N, Grindstaff K, Zhang X, Gallop MA, Krstew EV, Lawrence AJ, Kiefer F. 2013. Acamprosate produces its anti-relapse effects via calcium. *Neuropsychopharmacology* 39: 783-791.
- Steensland P, Simms JA, Holgate J, Richards JK, Bartlett SE. 2007. Varenicline, an $\alpha 4\beta 2$ nicotinic acetylcholine receptor partial agonist, selectively decreases ethanol consumption and seeking. *Proceedings of the National Academy of Sciences* 104: 12518-12523.

- Stewart SH, Zvolensky MJ, Eifert GH. 2001. Negative-reinforcement drinking motives mediate the relation between anxiety sensitivity and increased drinking behavior. *Personality and Individual Differences* 31: 157-171.
- Stratford TR, Kelley AE. 1999. Evidence of a functional relationship between the nucleus accumbens shell and lateral hypothalamus subserving the control of feeding behavior. *Journal of Neuroscience* 19: 11040-11048.
- Stuber GD, Sparta DR, Stamatakis AM, van Leeuwen WA, Hardjoprajitno JE, Cho S, Tye KM, Kempadoo KA, Zhang F, Deisseroth K. 2011. Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. *Nature* 475: 377-380.
- Swanson LW, Sawchenko PE, Rivier J, Vale WW. 1983. Organization of Ovine Corticotropin-Releasing Factor Immunoreactive Cells and Fibers in the Rat Brain: An Immunohistochemical Study. *Neuroendocrinology* 36: 165-186.
- Svenningsson P, Nairn AC, Greengard P. 2005. DARPP-32 mediates the actions of multiple drugs of abuse. *The AAPS Journal* 7: E353-E360.
- Tan CP, Sano H, Iwaasa H, Pan J, Sailer AW, Hreniuk DL, Feighner SD, Palyha OC, Pong SS, Figueroa DJ. 2002. Melanin-Concentrating Hormone Receptor Subtypes 1 and 2: Species-Specific Gene Expression. *Genomics* 79: 785-792.
- Tapocik J, Solomon M, Flanigan M, Meinhardt M, Barbier E, Schank J, Schwandt M, Sommer W, Heilig M. 2012. Coordinated dysregulation of mRNAs and microRNAs in the rat medial prefrontal cortex following a history of alcohol dependence. *The Pharmacogenomics Journal* 13: 286-296.
- Tavares FX, Al-Barazanji KA, Bishop MJ, Britt CS, Carlton DL, Cooper JP, Feldman PL, Garrido DM, Goetz AS, Grizzle MK. 2006. 6-(4-Chlorophenyl)-3-substituted-thieno [3, 2-d] pyrimidin-4 (3 H)-one-Based Melanin-Concentrating Hormone Receptor 1 Antagonist. *Journal of Medicinal Chemistry* 49: 7108-7118.
- Thorsell A, Tapocik JD, Liu K, Zook M, Bell L, Flanigan M, Patnaik S, Marugan J, Damadzic R, Dehdashti SJ. 2013. A novel brain penetrant NPS receptor antagonist, NCGC00185684, blocks alcohol-induced ERK-phosphorylation in the central amygdala and decreases operant alcohol self-administration in rats. *Journal of Neuroscience* 33: 10132-10142.
- Tybon A, Lakaye B, Adamantidis A, Tirelli E. 2008. Amphetamine-and cocaine-induced conditioned place preference and concomitant psychomotor sensitization in mice with genetically inactivated melanin-concentrating hormone MCH(1) receptor. *European Journal of Pharmacology* 599: 72-80.
- Umhau JC, Momenan R, Schwandt ML, Singley E, Lifshitz M, Doty L, Adams LJ, Vengeliene V, Spanagel R, Zhang Y. 2010. Effect of acamprosate on magnetic resonance spectroscopy measures of central glutamate in detoxified alcohol-dependent individuals: a randomized controlled experimental medicine study. *Archives of General Psychiatry* 67: 1069-1077.
- Valdez GR, Koob GF. 2004. Allostasis and dysregulation of corticotropin-releasing factor and neuropeptide Y systems: implications for the development of alcoholism. *Pharmacology Biochemistry and Behavior* 79: 671-689.
- Vale W, Spiess J, Rivier C, Rivier J. 1981. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* 213: 1394-1397.

- Wand GS, Dobs AS. 1991. Alterations in the Hypothalamic-Pituitary-Adrenal Axis in Actively Drinking Alcoholics. *Journal of Clinical Endocrinology & Metabolism* 72: 1290-1295.
- Watson S, Gallagher P, Ritchie JC, Ferrier IN, Young AH. 2004. Hypothalamic-pituitary-adrenal axis function in patients with bipolar disorder. *The British Journal of Psychiatry* 184: 496-502.
- Vaughan J, Fischer W, Hoeger C, Rivier J, Vale W. 1989. Characterization of Melanin-Concentrating Hormone from Rat Hypothalamus. *Endocrinology* 125: 1660-1665.
- Weiss F, Lorang MT, Bloom FE, Koob GF. 1993. Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. *Journal of Pharmacology and Experimental Therapeutics* 267: 250-258.
- Victor M, Adams RD. 1952. The effect of alcohol on the nervous system. *Research Publications-Association for Research in Nervous and Mental Disease* 32: 526-573.
- Wise RA. 1973. Voluntary ethanol intake in rats following exposure to ethanol on various schedules. *Psychopharmacologia* 29: 203-210.
- Wise RA, Rompré P-P. 1989. Brain dopamine and reward. *Annual Review of Psychology* 40: 191-225.
- Volkow ND, Wang G-J, Fowler JS, Telang F. 2008. Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology. *Philosophical Transactions of the Royal Society B: Biological Sciences* 363: 3191-3200.
- Volkow ND, Wang G, Fowler J, Tomasi D, Baler R. 2012. Food and drug reward: overlapping circuits in human obesity and addiction. *Brain Imaging in Behavioral Neuroscience* 11:1-24.
- Volpicelli JR, Watson NT, King AC, Sherman CE, O'Brien CP. 1995. Effect of naltrexone on alcohol "high" in alcoholics. *American Journal of Psychiatry* 152: 613-615.
- Volpicelli JR, Balaraman G, Hahn J, Wallace H, Bux D. 1999. The role of uncontrollable trauma in the development of PTSD and alcohol addiction. *Alcohol Research and Health* 23: 256-262.
- World Health Organization. 2004. *Global status report on alcohol 2004*. Geneva: World Health Organization.
- Xiao C, Ye J-H. 2008. Ethanol dually modulates GABAergic synaptic transmission onto dopaminergic neurons in ventral tegmental area: role of μ -opioid receptors. *Neuroscience* 153: 240-248.
- Zhang M, Balmadrid C, Kelley AE. 2003. Nucleus accumbens opioid, GABAergic, and dopaminergic modulation of palatable food motivation: contrasting effects revealed by a progressive ratio study in the rat. *Behavioral Neuroscience* 117: 202-211.
- Zilberman M, Tavares H, El-Guebaly N. 2004. Gender Similarities and Differences: The Prevalence and Course of Alcohol and Other Substance—Related Disorders. *Journal of Addictive Diseases* 22: 61-74.
- Åmark C. 1951. A study in alcoholism; clinical, social-psychiatric and genetic investigations. *Acta Psychiatry Neurology Scandinavian Supplement* 70: 1-283.

