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Hypothalamic neuropeptide signaling in alcohol addiction

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Abstract

The hypothalamus is now known to regulate alcohol intake in addition to its established role in food intake, in part through neuromodulatory neurochemicals termed neuropeptides. Certain orexigenic neuropeptides act in the hypothalamus to promote alcohol drinking, although they affect different aspects of the drinking response. These neuropeptides, which include galanin, the endogenous opioid enkephalin, and orexin/hypocretin, appear to stimulate alcohol intake not only through mechanisms that promote food intake but also by enhancing reward and reinforcement from alcohol. Moreover, these neuropeptides participate in a positive feedback relationship with alcohol, whereby they are upregulated by alcohol intake to promote even further consumption. **They contrast** with other orexigenic neuropeptides, such as melanin-concentrating hormone and neuropeptide Y, which promote alcohol intake under limited circumstances, are not consistently stimulated by alcohol, and do not enhance reward. **They** also contrast with neuropeptides that can be anorexigenic, including the endogenous opioid dynorphin, corticotropin-releasing factor, **and melanocortins**, which act in the hypothalamus to inhibit alcohol drinking as well as reward and therefore counter the ingestive drive promoted by orexigenic neuropeptides. Thus, while multiple hypothalamic neuropeptides may work together to regulate different aspects of the alcohol drinking response, excessive signaling from orexigenic neuropeptides or inadequate signaling from anorexigenic neuropeptides can therefore allow alcohol drinking to become dysregulated.

Keywords

dynorphin; enkephalin; galanin; melanin-concentrating hormone; neuropeptide Y; orexin/hypocretin

1. Introduction

The hypothalamus has long been known to participate in ingestive behavior. Its role in feeding was first established by Hetherington and Ranson in 1940 (Hetherington and Ranson, 1940), when they showed that rats became obese after bilateral electrolytic lesions

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of the medial hypothalamic nuclei. In 1951, Anand and Brobeck then showed that rats after bilateral destruction of the lateral hypothalamus (LH) instead failed to eat and became emaciated, leading these researchers to designate the LH as a "feeding center" (Anand and Brobeck, 1951). It was not until 1970 that it was suggested, by Marfaing-Jallat, Larue, and Le Magnen (1970), that the hypothalamus also participated in alcohol drinking. These researchers demonstrated that rats with lesions of the medial hypothalamus exhibit hyperphagia not just toward food but also toward alcohol (Marfaing-Jallat et al., 1970). Conversely, electrical stimulation of the LH led rats to drink alcohol at intoxicating levels (Wayner et al., 1971). Alcohol itself is energy dense, containing 7 kcal/gram, as compared to the 4 kcal/gram of protein and carbohydrate and the 9 kcal/gram of fat. For this reason, alcohol can be considered to be a food as well as a drug. It is notable that the hypothalamus, in addition to controlling energy intake, also controls reward from ingestive behavior. Rats work to receive electrical stimulation of the LH ("self-stimulation"), and manipulation of the hypothalamic feeding sites correspondingly affects their rate of self-stimulation (Hoebel and Teitelbaum, 1962). Thus, the hypothalamic regulation of alcohol intake may be connected to both the calories it provides and the reward it induces.

The discovery of neuropeptides in the brain (de Wied, 1974) has led researchers to consider that these local neuromodulatory neurochemicals may contribute to the ability of the hypothalamus to influence alcohol intake. In fact, many hypothalamic neuropeptides that participate in food intake have also been found to participate in alcohol intake. Much of this work has focused on certain orexigenic neuropeptides traditionally associated with increased food intake, including galanin (GAL), enkephalin (ENK), orexin/hypocretin (OX), melanin-concentrating hormone (MCH), and neuropeptide Y (NPY), which generally are upregulated by alcohol intake and can also promote its intake. In addition, neuropeptides sometimes described as anorexigenic, those that reduce food intake, such as dynorphin (DYN), corticotropin-releasing factor (CRF), **and melanocortins (MCs)** are **often** also upregulated by alcohol drinking, but more often act to reduce this behavior, possibly due to the influence of these hypothalamic neuropeptides on processes of reward.

This review presents the current knowledge about the relationship of neuropeptides in the hypothalamus to alcohol drinking, including their role in reward, which may itself affect this behavior.

2. Neuropeptides with a positive feedback relationship to alcohol

Certain neuropeptides in the hypothalamus have a positive feedback relationship to alcohol, which stimulates the expression of these neuropeptides that in turn promote further intake. This relationship may exist due to the neuropeptides' commonly known roles in promoting food intake as well as reward associated with alcohol intake. These neuropeptides acting within a positive feedback circuit include GAL, ENK, and OX.

2.1. Galanin

The neuropeptide GAL is well-conserved amongst species, consisting of 29 amino acids in rats and pigs and 30 amino acids in humans (Bersani et al., 1991; Tatemoto et al., 1983; Vrontakis et al., 1987). Widely distributed throughout the brain, GAL within the

hypothalamus of the rat is dense in both cell bodies and fibers, being found in the paraventricular nucleus (PVN), arcuate nucleus (ARC), lateral hypothalamus (LH), dorsomedial nucleus (DMN), and periventricular area (Rokaeus et al., 1984). The peptide GAL can interact with any of three G protein-coupled receptors, GalR1, GalR2 or GalR3, all of which are expressed in the hypothalamus (Smith et al., 1997; Smith et al., 1998). While GalR1 and GalR3 are thought to be G_i/G_o-coupled receptors, GalR2 appears to be coupled to a G_q/G_{11}-mediated pathway (Smith et al., 1997; Smith et al., 1998). Therefore, the effects of GAL on alcohol intake may depend in part on the receptor with which it interacts.

Within the PVN in particular, GAL acts to promote alcohol drinking. Injection of GAL into the PVN of rats has been shown in a variety of paradigms to increase **moderate** alcohol drinking **(bringing blood alcohol levels to 30-60 mg/dl)** while a general GAL receptor antagonist decreases it (Chen et al., 2014a; Rada et al., 2004; Schneider et al., 2007). Notably, this stimulatory effect of GAL does not occur with injection in the LH (Schneider et al., 2007), paralleling the finding that hypothalamic GAL increases food intake only when injected into the PVN or adjacent periventricular region (Kyrkouli et al., 1990). The ability of GAL to promote excessive alcohol intake (bringing blood alcohol levels to > 60 mg/dl) may be due to actions at the GalR3, the only GAL receptor found to be associated with alcoholism in humans (Belfer et al., 2007). Peripheral injection of a GalR3 antagonist in alcohol-preferring rats reduces alcohol self-administration, breakpoint on a progressive ratio schedule, and cue-induced reinstatement of alcohol-seeking (Ash et al., 2014; Ash et al., 2011). Recent evidence in our laboratory using an episodic intake monitor has shown that GAL in the PVN acts by increasing the size but not duration of alcohol drinking bouts (Chen et al., 2014a). Thus, rats with elevated endogenous GAL appear to drink more alcohol because they consume it more quickly, similar to how hypothalamic GAL is believed to promote food intake (Koegler et al., 1999). This evidence suggests that GAL in the hypothalamus might increase the drinking of alcohol, in part, because of its similarity to food.

Additional evidence supports a role for endogenous GAL in promoting alcohol drinking. Outbred Sprague-Dawley rats predicted to drink higher levels of alcohol show elevated mRNA expression of GAL in the PVN (Barson et al., 2013). Notably, this was found in prone rats identified by two different predictor tests, both high novelty-induced locomotor activity possibly reflecting novelty-seeking and high fat-induced triglyceride levels possibly reflecting slower lipid metabolism (Barson et al., 2013). Moreover, mice overexpressing the GAL gene drink more alcohol and show a higher preference for alcohol than their wild-type peers (Karatayev et al., 2009), while those lacking the GAL gene drink less alcohol and show a lower preference for it (Karatayev et al., 2010).

Hypothalamic GAL may also promote alcohol drinking due to its apparent involvement in reward. Injection of GAL into the PVN has been found to increase extracellular levels of dopamine (DA) in the nucleus accumbens (NAc) while simultaneously lowering levels of acetylcholine (ACh) (Rada et al., 1998). Evidence shows that accumbal DA through the D1 receptor can promote alcohol-induced conditioned place preference (Young et al., 2014) as well as alcohol drinking (Bahi and Dreyer, 2012). In contrast, the cholinergic agonist nicotine reduces operant alcohol self-administration when injected into the NAc (Nadal et

al., 1998). Moreover, peripheral injection with a GalR3 antagonist decreases the reinforcing value of alcohol, as evidenced by its ability to reduce breakpoint on a progressive ratio schedule of responding for alcohol (Ash et al., 2014). Therefore, in addition to stimulating the intake of alcohol due to its similarity to food, hypothalamic GAL may promote it by making alcohol intake more rewarding.

Evidence supports a positive-feedback relationship of GAL to alcohol. Both experimenter-administered alcohol and voluntary drinking of alcohol **at moderate and excessive levels** lead to increased gene expression and peptide levels of GAL in the anterior PVN, DMN, and perifornical LH (Leibowitz et al., 2003). This positive relationship is evident even with brief alcohol exposure, with an increase in GAL mRNA levels in the PVN observed with as little as a single injection of alcohol (Chang et al., 2007). Therefore, hypothalamic GAL not only endogenously promotes alcohol drinking, but alcohol drinking in turn increases levels of hypothalamic GAL, completing the positive feedback circuit.

2.2. Enkephalin

The endogenous opioid pentapeptide ENK comes in two forms, met-ENK and leu-ENK, which contain methionine and leucine, respectively (Hughes et al., 1975b). Like GAL, ENK is found in numerous species, including rats, mice, pigs, cows, and humans (Gramsch et al., 1979; Hughes et al., 1975a). Also widely distributed through the brain, ENK within the rat hypothalamus has been described in cell bodies and fibers of the PVN, ARC, LH, DMN, and perifornical area (Simantov et al., 1977; Williams and Dockray, 1983). This neuropeptide is most potent at the delta opioid receptor (DOR) (Wuster et al., 1979), which is G_i/G_o-coupled (Belcheva et al., 1998; North et al., 1987) and inhibits the production of cAMP.

Hypothalamic ENK can promote **moderate** alcohol drinking through its actions in the PVN. With injection into the PVN, a DOR agonist stimulates alcohol drinking while a general opioid antagonist decreases it (Barson et al., 2010). This is in contrast to injections in the LH, where the ENK agonist decreases alcohol drinking, and the general opioid antagonist increases it (Chen et al., 2013b). These results suggest that endogenously active ENK has opposite effects in the PVN compared to the adjacent LH. Using an episodic intake monitor, our laboratory has shown that, while PVN ENK is similar to GAL in promoting alcohol drinking by increasing the size of alcohol drinking bouts, it differs from GAL in also increasing the duration of those bouts (Chen et al., 2014a), suggesting that rats with endogenously elevated ENK drink alcohol for longer periods of time before they achieve satiety. Once again, this parallels results with food, which show that ENK in the PVN increases food intake by prolonging eating sessions (McLean and Hoebel, 1983). Thus ENK in the hypothalamus, like GAL, might promote the drinking of alcohol due to its similarities with food.

Evidence with measurements of ENK gene expression suggests that, while endogenous ENK like GAL can promote alcohol drinking, it may not be necessary in the way that GAL appears to be. While rats prone to drinking higher levels of alcohol have elevated PVN ENK mRNA when identified by a measure of novelty-induced locomotor activity, they show no such effect when identified by their fat-induced triglyceride levels, a measure that predicted higher GAL expression (Barson et al., 2013). Further, mice lacking the ENK gene show no

difference from wild-type **controls** in either their alcohol intake or alcohol preference (Koenig and Olive, 2002), and no differences have been observed in hypothalamic ENK gene expression of the alcohol-preferring C57BL/6J compared to alcohol-avoiding DBA/2J mice (Ng et al., 1996). This indicates that ENK does not endogenously promote alcohol drinking in all individuals or under all conditions.

There is evidence that hypothalamic ENK, like GAL, may also promote alcohol drinking due to its involvement in reward. Injection of a DOR agonist into the PVN increases extracellular levels of DA in the NAc, although it leaves ACh unaffected (Rada et al., 2010). Further, rats will voluntarily self-administer a DOR agonist into the posterior LH, the same brain region where they press for electrical self-stimulation (Olds and Williams, 1980). These results support the idea that hypothalamic ENK promotes alcohol drinking in part due to its ability to increase positive reinforcement.

There is also clear evidence for a positive-feedback relationship between PVN ENK and alcohol. Both gene expression and peptide levels of ENK are increased in the PVN in response to **moderate and excessive** alcohol drinking (Chang et al., 2007; Oliva and Manzanares, 2007), and this effect occurs with as little as a single alcohol injection (Chang et al., 2007; de Gortari et al., 2000). Anatomical analysis has shown that the alcohol-induced transcriptional activation of ENK neurons specifically occurs in the medial parvocellular area of the PVN at all anterior-posterior levels (Chang et al., 2014). Therefore, just as with GAL, hypothalamic ENK not only endogenously promotes alcohol drinking, but the drinking of alcohol in turn increases the activity and levels of hypothalamic ENK, completing the positive feedback circuit.

2.3.Orexin

The orexigenic peptide, OX, is cleaved from a precursor neuropeptide into orexin A (OX-A) and orexin B (OX-B) (also called hypocretin 1 and hypocretin 2) (de Lecea et al., 1998; Sakurai et al., 1998). Cell bodies containing OX, unlike ENK and GAL, lie exclusively within the hypothalamus, spanning the DMN through the perifornical area and into the LH (de Lecea et al., 1998; Sakurai et al., 1998), allowing the effects found from changes in OX levels to be traced back to the actions of neurons within this prescribed hypothalamic region. The projections of these OX-containing neurons terminate in a vast array of brain areas, including the locus coeruleus, thalamus, NAc, ventral tegmental area, amygdala, and various nuclei of the hypothalamus (Peyron et al., 1998), such that OX has effects throughout the brain. There are two known receptors for OX, the orexin 1 receptor (OX1R) and orexin 2 receptor (OX2R), or the hypocretin 1 and 2 receptors. While OX1R binds to OXA with an affinity that is two to three orders of magnitude greater than for OX-B, OX2R binds to OX-A and OX-B with nearly equal affinity (Sakurai et al., 1998). Binding of **the OX receptors** activates G_q, $\mathbf{G_s}$, and $G_{i/o}$ subunits (Magga et al., 2006; Sakurai et al., 1998; Tang et al., 2008; van den Pol et al., 1998). Notably, the pattern of OX receptor expression is such that OX1R and OX2R tend to predominate in different subregions of the same nuclei. This can be seen in the hypothalamus, where OX1R is most dense in the anterior hypothalamic nucleus while sparse in the LH and absent from the ARC and PVN, and OX2R is most dense in the LH, ARC and PVN while sparse in the anterior hypothalamic nucleus (Marcus

et al., 2001). Therefore, the effects of OX on alcohol intake depend in part on both the receptor and the brain region with which it interacts.

In general, OX is found to promote alcohol drinking, but this effect does not occur in all brain areas. Within the hypothalamus, injection of OX-A into the PVN or LH of rats stimulates **moderate** alcohol drinking (Chen et al., 2014a; Schneider et al., 2007). However, outside the hypothalamus, OX promotes **excessive** alcohol drinking in the NAc core but not the shell (Brown et al., 2013; Schneider et al., 2007), the ventral tegmental area but not substantia nigra (Srinivasan et al., 2012), and the anterior but not posterior paraventricular thalamus (Barson et al., 2014). Tests in our lab using an episodic intake monitor reveal a clear difference between the effect of OX and those of GAL and ENK. Whereas the latter peptides stimulate alcohol drinking by increasing the size and duration of alcohol drinking bouts, OX in the hypothalamus stimulates alcohol drinking by increasing the number of alcohol drinking bouts (Chen et al., 2014a), suggesting that rats with elevated OX initiate more drinking responses. This parallels results with food, which show that OX promotes food intake by increasing meal frequency (Baird et al., 2009). Thus, this orexigenic hypothalamic neuropeptide may also promote the drinking of alcohol due to similarities with food intake.

Peripheral injections using antagonists have parsed out the mechanisms through which the two OX receptors differentially contribute to alcohol drinking. In rats and mice, antagonists of OX1R and OX2R both specifically decrease alcohol self-administration, rather than sucrose or saccharin self-administration (Anderson et al., 2014; Richards et al., 2008; Shoblock et al., 2011). This effect may be due, in part, to the ability of these antagonists to reduce the reinforcing value of alcohol, as shown by evidence that they both decrease the breakpoint on a progressive ratio schedule of responding for alcohol (Anderson et al., 2014; Jupp et al., 2011). Other evidence, however, **suggests** that only antagonists of OX1R reduce alcohol-seeking, as measured using **cue-induced** reinstatement (Brown et al., 2013; Lawrence et al., 2006; Martin-Fardon and Weiss, 2014). These studies support the idea that, while OX in general promotes alcohol drinking, only the OX1R affects the seeking of alcohol, while both OX1R and OX2R affect the motivation to work for it.

Further evidence supports a role for endogenous OX in promoting alcohol drinking in certain individuals and scenarios. Manipulations that make rats drink more alcohol, including injections of D1 and glutamatergic agonists into the LH, increase gene expression of OX in this brain region (Chen et al., 2013a; Chen et al., 2014b). Conversely, manipulations that make rats drink less alcohol, like LH injection of a D2 agonist or oral gavage of a triglyceride-lowering drug, decrease OX expression in the LH (Barson et al., 2009; Chen et al., 2014b). Moreover, scenarios that induce reinstatement of alcohol seeking have been found to stimulate c-Fos activity within OX neurons (Dayas et al., 2008; Millan et al., 2010). In addition, Sprague-Dawley rats predicted to drink higher levels of alcohol based on their novelty-induced locomotor activity show elevated OX mRNA levels in the perifornical LH (Barson et al., 2013). It is notable, however, that those predicted to drink based on their fat-induced triglyceride levels show decreased OX mRNA (Barson et al., 2013), and OX levels are reported to be similar in alcohol-preferring iP rats compared to non-preferring NP rats (Lawrence et al., 2006). Recent research offers a possible explanation

of this apparent discrepancy in the role of endogenous OX. While OX on its own is not necessary for renewal of alcohol seeking, it may work in concert with other neuropeptides to induce this behavior (Prasad and McNally, 2014). Specifically, an OX antisense vivo morpholino microinjected into the LH diminished renewal of alcohol seeking when injected at a level that reduced both OX and MCH protein but not at a level that reduced only OX protein (Prasad and McNally, 2014). While hypothalamic OX is therefore capable of promoting alcohol drinking, it may not be sufficient for the execution of this behavior.

In line with the idea that activation of the OX receptors promotes different aspects of alcohol drinking, they also appear to have different relationships **to** processes of reward. Within the hypothalamus, OX neurons are responsive to conditions associated with reward. These neurons show c-Fos activation in response to conditioned contextual cues associated with sexual behavior (Di Sebastiano et al., 2011), a natural reward. Moreover, lesions of OX neurons reduce the conditioned place preference for a sexual behavior-paired chamber (Di Sebastiano et al., 2011). These effects of OX may be due specifically to actions at the OX2R rather than the OX1R. While peripheral injection of an OX2R antagonist reduces the acquisition, expression, and reinstatement of alcohol conditioned place preference (Shoblock et al., 2011), injection of an OX1R antagonist does not affect the acquisition and only weakly reduces the expression of alcohol conditioned place preference (Voorhees and Cunningham, 2011). Therefore, while OX can promote alcohol drinking through its actions at either of its receptors, its effects via the OX2R may be due to the ability of this receptor to promote reward from alcohol.

Evidence also supports a positive-feedback relationship between hypothalamic OX and alcohol similar to that observed with ENK and GAL. Most studies report that alcohol increases gene expression of OX within the LH. This has been observed in rats both with chronic drinking and with acute alcohol injection **at moderate more than at excessive levels** (Barson et al., 2014; Lawrence et al., 2006; Morganstern et al., 2010a). Additionally, chronic alcohol injection in mice leads to a small, albeit non-significant increase in c-Fos activity in OX neurons (Macedo et al., 2013). Overall, the results indicate that OX in the hypothalamus can help to promote alcohol drinking, while alcohol drinking in turn increases levels of endogenous OX.

3. Neuropeptides with a negative feedback relationship to alcohol

Other hypothalamic neuropeptides show a negative feedback relationship **to** alcohol, such that **they** counter a**n alcohol** drinking response and themselves are either stimulated or suppressed by drinking. This **influence on alcohol drinking** may occur due to the ability of these neuropeptides to reduce both food intake and reward. These neuropeptides include DYN, CRF, **and MCs**.

3.1. Dynorphin

The DYN precursor neuropeptide is cleaved into multiple active peptides, DYN-A, DYN-B, and the neoendorphins (Day et al., 1998). Found throughout the brain, DYN in the rat is most densely expressed within the hypothalamus, being located in cell bodies within the PVN, ARC, LH, ventromedial hypothalamus, and DMN and in fibers of the anterior

hypothalamic nucleus, ventromedial hypothalamus, and DMN, among others (Vincent et al., 1982). Unlike the other neuropeptides under discussion here, DYN in the LH is highly co-expressed with the neuropeptide OX, with over 90% of DYN neurons in this region also containing OX and *vice versa* (Chou et al., 2001) and the two neuropeptides found packaged within the same synaptic vesicles in the hypothalamus (Muschamp et al., 2014). The DYN peptides interact preferentially with the kappa opioid receptor (KOR) (Garzon et al., 1983), which is expressed in the same hypothalamic nuclei as DYN cell bodies (Mansour et al., 1987). As with other opioid receptors, the KOR is coupled with a G_i/G_o-mediated pathway (Belcheva et al., 1998).

Unlike the orexigenic neuropeptides discussed above, DYN in the hypothalamus inhibits alcohol drinking. This has been observed with injection of a KOR agonist into the PVN or LH, which suppresses **moderate** alcohol drinking in rats (Barson et al., 2010; Chen et al., 2013b). This neuropeptide in certain brain regions has also been observed to decrease food intake (Carr et al., 1989; Leighton et al., 1988). It is likely that the ability of DYN to reduce alcohol drinking is also due to dysphoria, as injections of KOR agonists into the LH induce conditioned place aversion (Bals-Kubik et al., 1993).

As with the orexigenic neuropeptides, however, DYN is stimulated by alcohol intake. Alcohol drinking **at moderate levels** leads to elevated DYN gene expression and DYN-A peptide levels in the PVN (Chang et al., 2010; Chang et al., 2007) and also to elevated DYN-B peptide levels in the hypothalamus overall (Gustafsson et al., 2007; Palm et al., 2012). The increase in DYN mRNA levels has been observed with as little as a single alcohol injection (Chang et al., 2007). Thus, with negative feedback regulation, the rise in DYN levels following alcohol intake may have the function of curbing subsequent alcohol drinking and counteracting the drive for further intake induced by simultaneously-released OX.

3.2.Corticotropin-releasing factor

The neuropeptide CRF, also called corticotropin-releasing hormone, is a 41 amino acid peptide (Vale et al., 1981) that is well-conserved amongst rats, sheep, and humans (Jingami et al., 1985). While CRF-containing cell bodies are dense in the extended amygdala, they are found in the hypothalamus predominantly in the PVN but also in the LH (Pilcher and Joseph, 1984; Young et al., 1986). The peptide displays a 10- to 40-fold higher affinity for the CRF1 than the CRF2 receptor, but both receptors are primarily coupled to a G_s-mediated pathway (Hillhouse et al., 2002). The two receptors have been detected in the PVN, ARC, LH, and DMN, and the CRF2 receptor is additionally located in the ventromedial nucleus (Van Pett et al., 2000). The CRF peptide is therefore both released from the hypothalamus and has neuronally stimulatory actions throughout hypothalamic nuclei.

In general, CRF like DYN shows a negative feedback relationship **to** alcohol. Although injection of CRF directly into the PVN has no effect on **excessive** alcohol drinking in alcohol-preferring P rats (Knapp et al., 2011), injection into the lateral ventricles is found to reduce **moderate and excessive** drinking in outbred Wistar rats (Thorsell et al., 2005). In line with its inhibition of alcohol drinking, CRF also decreases food intake when injected into the PVN (Krahn et al., 1988). In addition, levels of CRF are reduced in the

hypothalamus of alcohol-dependent rats at the onset of withdrawal (Zorrilla et al., 2001), when animals presumably crave alcohol, further supporting the idea that hypothalamic CRF reduces the drive for alcohol drinking. These levels begin to normalize within one week of withdrawal (Lee et al., 2001; Silva et al., 2002; Zorrilla et al., 2001). Conversely, both acute alcohol injection and chronic alcohol drinking **at moderate and excessive levels** lead to elevated CRF gene expression, specifically in the parvocellular region of the PVN (Ogilvie et al., 1998; Oliva and Manzanares, 2007; Rivier and Lee, 1996), and CRF peptide is released from the hypothalamus *in vitro* in response to alcohol treatment (de Waele and Gianoulakis, 1993). These results support the idea that the rise in DYN levels in the hypothalamus following alcohol intake acts to curb subsequent alcohol drinking.

Similar to DYN, the reduced alcohol drinking in response to CRF may be due to its ability to induce aversion. Ventricular injection of a CRF agonist dose-dependently elevates intracranial self-stimulation threshold, indicating that it reduces brain stimulation reward (Macey et al., 2000). Therefore CRF, in mediating some of the aversive aspects of alcohol drinking, serves to inhibit subsequent alcohol drinking.

3.3.Melanocortins

The MC peptides, which are all derived from the large precursor polypeptide pro-opiomelanocortin (POMC), include α-melanoctye-stimulating hormone (MSH), β-MSH, γ-MSH, and adrenocorticotropic hormone. Cell bodies containing MCs are located primarily in the hypothalamus, specifically in the ARC (Dube et al., 1978), but have also been described in the intermediate lobe of pituitary (Dube et al., 1978) and nucleus tractus solitarius (Joseph et al., 1983). The projections of these MC-containing neurons terminate in all areas of the hypothalamus as well as in an array of other brain areas, such as the amygdala, periaqueductal gray, and septum, such that the MCs can act throughout the brain (Guy et al., 1980; Jacobowitz and O'Donohue, 1978; O'Donohue et al., 1979). There are five known receptors for the MCs, with the primary MC receptors in the brain being the MC3 receptor (MC3R) and MC4 receptor (MC4R), which are G_s-coupled (Mountjoy et al., 1992). These two receptors are anatomically and pharmacologically distinct, with MC3R found throughout the hypothalamus and limbic system (Roselli-Rehfuss et al., 1993) and bound most potently by γ-MSH (Roselli-Rehfuss et al., 1993) and with MC4R found in nearly every region of the brain (Mountjoy et al., 1994) and bound minimally by γ-MSH but equally by α-MSH, β-MSH, and adrenocorticotropic hormone (Gantz et al., 1993). The MC peptides are therefore similar to OX in being largely transcriptionally restricted to the hypothalamus but having neuroexcitatory effects throughout the brain.

What is notable about the MCs is that they show a double negative feedback loop in relation to alcohol, generally decreasing alcohol drinking and also being endogenously reduced by alcohol. Injection of MCR agonists into the NAc, ventral tegmental area, or amygdala reduces moderate and excessive alcohol drinking as well as food intake (Lerma-Cabrera et al., 2012; York et al., 2011). Whereas this effect has not been found in the LH (Lerma-Cabrera et al., 2012), further tests with alcohol drinking may reveal that it does occur in nuclei of the medial hypothalamus, such as the PVN and DMN, where MC agonists have been shown to inhibit feeding behavior more powerfully than in the LH (Kim et al., 2000).

The suppression of alcohol intake caused by MCs appears to be due more to central MC4R than to MC3R. Ventricular injection of the nonselective MCR agonist melanotan-II, while significantly reducing excessive alcohol drinking in MC3R knockout mice, fails to produce this effect in MC4R knockout mice (Navarro et al., 2011; Olney et al., 2014).

Studies of endogenous MCs provide evidence supporting their role in alcohol drinking, although the direction of this effect may differ between hypothalamic and extra-hypothalamic brain regions. Alcohol-preferring AA rats have higher POMC gene expression in the ARC than alcohol-avoiding ANA rats (Lindblom et al., 2002), and alcohol-preferring C57BL/6J mice have higher peptide levels of α-MSH than 129/SvJ mice in several regions of the hypothalamus (Cubero et al., 2010). This points to a potential protective effect of endogenous MCs within the hypothalamus against excessive alcohol drinking. In contrast, in areas outside the hypothalamus such as the medial amygdala, C57BL/6J mice are found to have lower levels of α-MSH (Cubero et al., 2010). Although MC4R knockout mice show no difference in alcohol drinking compared to their wild-type littermates (Navarro et al., 2011), this may be due to the differences between the hypothalamic and extra-hypothalamic MC systems, with the hypothalamic MCs elevated in alcohol preferring animals but the limbic MCs reduced.

The ability of MCs to inhibit alcohol drinking may be due to their ability to suppress reward or reinforcement from this behavior. Ventricular injection of α-MSH reduces the anxiolytic effect of alcohol drinking as demonstrated on an elevated plus maze (Kokare et al., 2006). In contrast, an MC4R antagonist enhances this anxiolytic effect and reduces the anxiety induced by alcohol withdrawal (Kokare et al., 2006). Moreover, an MC4R agonist in the NAc reduces hedonic reactions to alcohol while increasing aversive ones (Lerma-Cabrera et al., 2013). Therefore MCs, like CRF, may enhance the aversive aspects of alcohol drinking while simultaneously reducing the rewarding aspects.

Unlike the other anorexigenic neuropeptides described, endogenous MCs are generally inhibited by alcohol. Gene expression and peptide levels of POMC in the ARC are decreased after long-term high-level exposure to alcohol via a vapor chamber or an alcohol-containing diet (Navarro et al., 2013; Scanlon et al., 1992). Similarly, peptide levels of α-MSH after long-term consumption of an alcohol-containing diet are decreased in the ARC and may also be reduced in the LH and certain limbic regions, including the amygdala and substantia nigra (Navarro et al., 2008; Rainero et al., 1990). In rats, this effect can occur with as little as a single injection of alcohol (Kokare et al., 2008). Thus, with a double negative feedback loop, the reduction in MC levels following alcohol intake may in fact be permissive of subsequent alcohol drinking by reducing the aversive aspects of this intake.

4. Neuropeptides with complex relationships to alcohol

There are additional orexigenic hypothalamic neuropeptides that have been suggested to play a role in alcohol drinking, but their relationship **to** this behavior appears to be more complex than that of the other orexigenic neuropeptides. These neuropeptides include MCH and NPY.

4.1.Melanin-concentrating hormone

The 19-amino acid MCH (Kawauchi et al., 1983) is transcribed in neurons distinct from but adjacent to those containing OX, lying predominantly in the LH but also in the perifornical area and subzona incerta (Skofitsch et al., 1985). Like OX, effects found from changes in MCH levels can therefore be traced back to the actions of neurons within the hypothalamus. Projections from MCH-containing neurons terminate in many of the same areas as OX, including the locus coeruleus, thalamus, NAc, ventral tegmental area, amygdala, and nuclei of the hypothalamus (Bittencourt et al., 1992). Rodents have been found to have only one type of MCH receptor, MCHR1, but humans, rhesus monkeys, and dogs also have a functional MCH receptor 2 (Tan et al., 2002). Within the hypothalamus, the MCHR1 has been described in the PVN, ARC, LH, DMN, ventromedial nucleus, and periventricular area (Hervieu et al., 2000). Binding to MCHR1 activates both $G_{i/o}$ and G_q subunits (Hawes et al., 2000), but the predominant result of receptor binding is neuronal inhibition (Chambers et al., 1999; Lembo et al., 1999), suggesting that MCH acts through different mechanisms from OX.

While studies using agonist injections support the ability of MCH to promote alcohol drinking, those using antagonists and genetic manipulations reveal the complexity of this relationship. Injection of MCH into the PVN, adjacent third ventricle, or NAc has been shown to promote **moderate** alcohol drinking and stimulate operant responding for alcohol as well as sucrose (Duncan et al., 2005; Duncan et al., 2006; Morganstern et al., 2010b), just as these injections increase the intake of laboratory chow (Georgescu et al., 2005; Guesdon et al., 2009; Rossi et al., 1999). However, injection of an MCHR1 antagonist into the third ventricle failed to affect alcohol drinking or block MCH-induced drinking (Duncan et al., 2006), and MCHR1 knockout paradoxically led male mice to consume more alcohol than wild-type mice (Duncan et al., 2007). Evidence supporting a role for endogenous MCH in stimulating alcohol intake comes from a study showing peripheral administration of an MCHR1 antagonist to suppress alcohol self-administration and cue-induced reinstatement of alcohol-seeking (Cippitelli et al., 2010). Moreover, a recent study using antisense vivo morpholinos that reduce MCH and OX protein levels reported that this reduced the renewal of extinguished alcohol seeking (Prasad and McNally, 2014), lending further support to the idea that MCH does endogenously promote alcohol intake.

Gene expression studies of rats likely to drink alcohol, however, suggest that endogenous MCH on its own might not be necessary for alcohol drinking in all individuals. Our laboratory has shown that outbred Sprague-Dawley rats predicted to drink higher levels of alcohol due to their novelty-induced locomotor activity and fat-induced triglyceride levels show no difference in levels of MCH mRNA from non-prone rats (Barson et al., 2013). Moreover, manipulations that make rats drink more alcohol, including injections of D1 and glutamatergic agonists into the LH, fail to significantly affect gene expression of MCH in this brain region (Chen et al., 2013a; Chen et al., 2014b). Thus, it is possible that MCH endogenously promotes alcohol intake only in limited scenarios.

In contrast to the reward-promoting effects of GAL, ENK, and OX, the ability of MCH to promote alcohol drinking may be due to its induction of dysphoria. Mice lacking the

MCHR1 show greater evoked DA release from the NAc shell (Pissios et al., 2008), suggesting that activation of the MCHR1 normally inhibits these levels. Moreover, hypothalamic MCH gene expression positively correlates with aversive responses, including conditioned taste aversion (Mitra et al., 2012) and immobility in a forced swim test (Garcia-Fuster et al., 2012). Thus, alcohol drinking induced by elevated levels of MCH may represent an attempt to alleviate an unpleasant state.

Hypothalamic MCH also shows a mixed response to alcohol intake. Acute injection **of moderate to excessive levels of alcohol** dose-dependently increases gene expression of MCH, but low-**to-moderate** level**s of** chronic drinking in outbred rats dose-dependently decreases it (Morganstern et al., 2010b). These results indicate that MCH may in certain circumstances exhibit a positive-feedback relationship to alcohol, but overall has a complex relationship that remains to be fully characterized.

4.2. Neuropeptide Y

Consisting of 36 amino acids (Tatemoto et al., 1982), NPY is transcribed widely in the brain, in cells within the cortex, caudate and putamen, NAc, amygdala, periaqueductal gray, hippocampus, locus coeruleus, as well as the hypothalamic PVN and ARC (Allen et al., 1983). Within the hypothalamus, NPY terminals and peptide levels have been detected in the PVN, perifornical area, ventromedial nucleus, and DMN (Akabayashi et al., 1994; Allen et al., 1983). Five NPY receptor subtypes have been identified in mammals, four of which are functional in humans (Michel et al., 1998). In the rat hypothalamus, NPY receptors are found in most nuclei (Martel et al., 1990), with the Y1 receptor located within the PVN, ARC, and DMN (Kopp et al., 2002) and the Y2 receptor complementary to this, in the ARC, LH, perifornical area, and ventromedial nucleus (Stanic et al., 2006). In general, binding to NPY receptors activates G_i and G_o subunits (Limbird and Taylor, 1998), inhibiting the activation of adenylyl cyclase.

There is clear evidence that injection of NPY into regions outside of the hypothalamus, representing extra-hypothalamic NPY, significantly inhibit**s excessive** alcohol drinking (Sparrow et al., 2012), but NPY injection within the hypothalamus can promote alcohol intake under limited conditions. With injection in the PVN, NPY in rats with a long history of alcohol drinking stimulates **moderate or excessive** alcohol drinking and self-administration, and an antagonist reduces it (Gilpin et al., 2004; Kelley et al., 2001). This NPY-induced increase in drinking, however, is not seen in rats with a shorter history or lower levels of drinking (Kelley et al., 2001; Lucas and McMillen, 2004). This stands in contrast to the ability of hypothalamic NPY to robustly promote food intake (Stanley et al., 1985; Stanley and Leibowitz, 1984). Thus, hypothalamic injection of NPY may promote the intake of alcohol under specific conditions, but this may not be strongly related to its effect on feeding behavior.

Evidence with NPY gene expression also suggests that endogenous hypothalamic NPY is not necessary for alcohol intake. With a few exceptions (Hwang et al., 1999; Spence et al., 2005), the majority of studies of rats and mice prone to drinking alcohol have reported no differences in NPY gene expression and peptide levels from non-prone animals. Outbred Sprague-Dawley rats predicted to drink higher levels of alcohol due to their novelty-induced

locomotor activity and fat-induced triglyceride levels show no difference in levels of NPY mRNA in the ARC (Barson et al., 2013), and alcohol-preferring AA rats show no difference from alcohol-nonpreferring ANA or outbred Wistar rats in their NPY gene expression in the ARC or PVN (Caberlotto et al., 2001). In addition, alcohol-preferring P rats and C57BL/6J mice show no difference in their NPY peptide levels in the hypothalamus as a whole (Ehlers et al., 1998; Hayes et al., 2005). In line with the idea that hypothalamic NPY has limited influence on alcohol intake, PVN injection of NPY does not affect levels of DA or ACh in the NAc (Rada et al., 1998).

Hypothalamic NPY also shows minimal responsiveness to alcohol intake. Gene expression of NPY is not affected by one week of alcohol injections (Leibowitz et al., 2003), and hypothalamic NPY peptide levels are unchanged by several weeks of alcohol diet or exposure to alcohol vapor (Ehlers et al., 1998; Roy and Pandey, 2002). Instead, NPY peptide levels are found to be altered by withdrawal of this **excessive** alcohol intake and exposure, reduced after 24 hours but elevated after one month of withdrawal (Ehlers et al., 1998; Roy and Pandey, 2002). These results indicate that, while NPY may be related to alcohol intake, this relationship is more nuanced than those with other hypothalamic neuropeptides.

5. Conclusions

A large number of neuropeptides in the hypothalamus, which likely evolved to control food intake, have now been shown also to influence alcohol intake. In general, orexigenic neuropeptides, particularly GAL, ENK, and OX, act in the hypothalamus to promote alcohol drinking, although they clearly affect different aspects of this behavior. In addition to the similarity between alcohol and food, alcohol drinking may be enhanced by these neuropeptides because of their similar enhancement of reward and reinforcement. These neuropeptides also demonstrate a positive feedback relationship **to** alcohol, being upregulated by alcohol intake to promote even further consumption. While the orexigenic neuropeptides MCH and NPY can also promote alcohol drinking, this effect is seen under more limited circumstances, these neuropeptides are unrelated to or actually decrease reward, and their gene expression and peptide levels show a complex response to alcohol intake. In contrast, DYN, CRF **and MCs**, often described as anorexigenic neuropeptides, act to counter the ingestive drive promoted by orexigenic neuropeptides, suppressing alcohol drinking even as their levels are **sometimes** increased by this behavior. This may be related to the ability of these anorexigenic neuropeptides to promote aversion and dysphoria. Thus, alcohol drinking, by stimulating the activity and release of multiple neuropeptides, may be appropriately regulated by anorexigenic neuropeptides or can become excessive in response to high levels of orexigenic neuropeptides.

The hypothalamic neuropeptides are likely to interact in multiple and complex ways in the process of modulating alcohol drinking. For example, OX may initiate alcohol drinking in part by increasing the activity of MCH-containing neurons in the LH, of ENK-containing neurons in the PVN, or of NPY-containing neurons in the ARC, with OX receptors found to exist on each of these neuronal populations (Backberg et al., 2002; Barson et al., 2011). In response to this stimulation, MCH may then promote further alcohol drinking by inhibiting DYN or CRF in the PVN, or it may act to terminate alcohol drinking by inhibiting NPY in

the ARC (Chee et al., 2013), diverse actions that may explain its complex relationship with alcohol intake. In addition, the neuropeptides are often co-expressed within the same neurons, and they can be co-released to affect their targets in different ways. Not only is DYN co-localized with OX in neurons of the LH (Chou et al., 2001), but it is also co-expressed with NPY in the ARC (Lin et al., 2006). In the PVN, ENK is co-localized with orexigenic GAL (Barson et al., 2011) and also with anorexigenic CRF (Sawchenko and Swanson, 1985).

Although nearly all of the neuropeptides **discussed** are transcribed in multiple hypothalamic nuclei, **it is notable that** their major effects **on** alcohol drinking seem to occur in the PVN, a brain region that integrates neuroendocrine, autonomic, as well as limbic functions (Swanson and Sawchenko, 1980; Wittmann et al., 2009). The control of alcohol drinking by neuropeptides within the hypothalamus **may** therefore involve the coordination of caloric need **with** reward regulation, encompassing multiple features of alcohol addiction.

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Abbreviations

ACh	acetylcholine
ARC	arcuate nucleus of the hypothalamus
CRF	corticotropin-releasing factor
DA	dopamine
DMN	dorsomedial nucleus
DOR	delta opioid receptor
DYN	dynorphin
ENK	enkephalin
GAL	galanin
KOR	kappa opioid receptor
LH	lateral hypothalamus
MC	melanocortin
MC3R	melanocortin 3 receptor
MC4R	melanocortin 4 receptor
MCH	melanin-concentrating hormone
MSH	melanocyte-stimulating hormone
NAc	nucleus accumbens

NPY	neuropeptide Y
OX	orexin/hypocretin
OX1R	orexin 1 receptor
OX2R	orexin 2 receptor
POMC	pro-opiomelanocortin
PVN	paraventricular nucleus of the hypothalamus.

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Highlights

- Certain orexigenic hypothalamic neuropeptides promote alcohol drinking and reward
- These same neuropeptides participate in a positive feedback relationship to alcohol
- Anorexigenic hypothalamic neuropeptides inhibit alcohol drinking and reward
- These neuropeptides act in a negative feedback relationship to alcohol
- Hypothalamic neuropeptides regulating food intake also control alcohol intake