Abstract

Consummatory behavior is driven by both caloric and emotional need, and a wide variety of animal models have been useful in research on the systems that drive consumption of food and drugs. Models have included selective breeding for a specific trait, manipulation of gene expression, forced or voluntary exposure to a substance, and identification of biomarkers that predict which animals are prone to overconsuming specific substances. This research has elucidated numerous brain areas and neurochemicals that drive consummatory behavior. Although energy homeostasis is primarily mediated by the hypothalamus, reinforcement is more strongly mediated by nuclei outside the hypothalamus, in mesocorticolimbic regions. Orexigenic neurochemicals that control food intake can provide a general signal for promoting caloric intake or a more specific signal for stimulating consumption of a particular macronutrient, fat, carbohydrate, or protein. The neurochemicals involved in controlling fat ingestion—galanin, enkephalin, orexin, melanin-concentrating hormone, and the endocannabinoids—show positive feedback with this macronutrient, as these peptides both increase fat intake and are further stimulated by its intake. This positive association offers some explanation for why foods high in fat are so often overconsumed. Consumption of ethanol, a drug of abuse that also contains calories, is similarly driven by the neurochemical systems involved in fat intake, according to evidence that closely relates fat and ethanol consumption. Further understanding of the systems involved in consummatory behavior will enable the development of effective therapies for the treatment of both overeating and drug abuse.

Key Words: ethanol; fat; hypothalamus; mesocorticolimbic; orexigenic; peptide; rodent model

Introduction

Since the 1980s, the prevalence of obesity in the United States has more than doubled—from 14% to 31% (Carroll et al. 2008; Flegal et al. 2002). Scientists and medical providers have attributed this staggering change to the combination of sedentary behavior and unhealthy diet, including, specifically, an increase in both fat and carbohydrate intake (Wright et al. 2004).

Food intake is driven not only by caloric need but also by emotional need, particularly the desire for reward. This drive made sense evolutionarily as the most palatable and rewarding foods tend to be the most calorically dense, allowing for energy storage in an environment where food supply is uncertain. The reward obtained by eating these foods increases the likelihood that they will be consumed again. Whereas historically this reward system was effectively balanced by limited exposure, calorically dense foods are now readily available and hence overconsumed.

Neurochemical signaling that controls caloric intake may be categorized as orexigenic or anorexigenic, and overconsumption can be due to excessive orexigenic signaling or insufficient anorexigenic signaling. Notably, just as certain neurochemicals appear to stimulate the consumption of specific foods, they may also stimulate the consumption of specific drugs in certain environments, with their roles possibly changing as the consumer progresses from casual use to addiction.

In this review we discuss neurobiological mechanisms that control food intake, particularly the excessive consumption of foods rich in fat or carbohydrates, and how these systems may be coopted to control the consumption of drugs of abuse. The review is not intended to be exhaustive but rather to cover what we consider the most important facts for understanding how chemical systems in the brain can drive overeating or drug abuse. To this end, we focus specifically on orexigenic peptides, although many anorexigenic peptides also play an important role in these processes. We examine alcohol as our example of a drug of abuse, keeping in mind that findings with alcohol may not always generalize to other drugs.

We begin with a brief explanation of the animal models used to obtain this information, and then describe a number of brain regions involved in food and drug intake. Next we discuss food intake regulation. Finally, we examine how a number of neurochemicals may also regulate alcohol intake.
Animal Models of Consummatory Behavior

Animal models are useful for examining the neurobiological mechanisms that mediate consummatory behavior. These models most often involve the use of rats or mice that prefer palatable, high-energy diets or ethanol solution and have been selectively bred, genetically altered, or trained over time to consume these substances. Each model has unique advantages as well as disadvantages, depending on the question(s) under investigation. The main difference between models used to study palatable diet and ethanol consumption is that outbred animals, which are easily trained to consume a palatable diet, do not consume large amounts of ethanol. Therefore, the more popular models used in ethanol research require the occurrence of some form of natural or genetic selection. In this section we introduce some of the rodent models used in studies (described in later sections) that examine the involvement of hypothalamic and mesocorticolimbic neurochemical systems in controlling the consumption of food and ethanol.

Natural Preference/Selective Breeding

Inherent behavioral traits observed in mice and the selective breeding of both mice and rats have yielded a number of popular models of both palatable food and ethanol overconsumption. These models mostly stem from a serendipitous discovery in which certain animals from a particular line or strain show distinct behavioral patterns, such as enhanced food or ethanol preference or consumption, and are either directly used or bred for multiple generations until a stable and recurring phenotype is established.

Selective breeding has yielded several important models of palatable diet overconsumption. For example, Brattleboro rats, originally bred from a diabetic rat line, show a high preference for a lipid diet, consuming fat as 45% of their daily caloric intake when given a choice of three macronutrients (Laycock 1976; Odorizzi et al. 1999). Subjects bred from Sprague-Dawley (SD) rats show a high or low preference for a saccharin solution and are addiction-prone and addiction-resistant, respectively (Carroll et al. 2008). Other models, which are more often used in obesity research and show an increased affinity for dietary fat, include diet-induced obese rats, which are derived from SD rats and consume 25% more high-energy diet compared with diet-resistant rats (Levin et al. 1997), and Zucker obese (fa/fa) rats, which consume 45% more standard chow than their lean counterparts (Cleary et al. 1980; Zucker and Zucker 1961). With regard to ethanol consumption, the preferring C57BL/6J and the avoiding DBA/2J mice are the best characterized and perhaps the most commonly used models of high versus low ethanol consumption and preference (Belknap et al. 1997; Short et al. 2006). The C57BL/6J mice have a natural preference for ethanol, and investigations in these animals have significantly advanced understanding of the genetics of alcoholism.

Several well-established rat lines are also used to study ethanol drinking behavior. Most investigations on the neurochemical and behavioral mechanisms of ethanol consumption have involved selectively bred rats such as the alcohol-prefering (P) and nonpreferring (NP) lines originally bred by researchers at Indiana University from Wistar rats (Li et al. 1993). Other rat lines that have been successfully used to study ethanol drinking behavior are the ALKO alcohol (AA) and nonalcohol (ANA) rats derived from various rat lines (Somm er et al. 2006), the high and low alcohol-drinking (HAD and LAD, respectively) rats (Li et al. 1993), and the Sardinian preferring (sP) and nonpreferring (sNP) rats (Colombo 1997). Generally, after ethanol training, the preferring rats consume between 4 and 8 g/kg of ethanol per day, resulting in blood ethanol content (BEC) ranging from 20 to 200 mg% (Bell et al. 2006; Li et al. 1993; Middaugh et al. 2003), which is associated with legal intoxication in humans. Upon withdrawal, preferring rats trained in an ethanol choice paradigm and C57BL/6 mice trained on an ethanol-containing liquid diet show slight signs of dependence (Rabin et al. 1980; Waller et al. 1982).

Collectively, these rodent models are important for investigating the brain neurochemical systems involved in the overconsumption of various nutrients and ethanol, as well as the accompanying behaviors that may contribute to such abnormal patterns of consumption, as we discuss below.

Transgenes

Aside from selectively breeding animals, investigators can genetically manipulate mice to lack or overexpress a particular gene and can even site-specifically induce the mutation in particular tissues or brain regions. Such models enhance understanding of the function of a known gene or corresponding protein of interest in various behaviors, including those related to food and ethanol consumption.

Transgenic animals used in the study of consummatory behavior often show similar patterns of consumption when given access to a palatable diet or ethanol solution. In studies of fat consumption, these models focus on manipulating peptide systems that control energy balance via the hypothalamus, as discussed in detail in subsequent sections. For ethanol studies, one popular transgenic model is the dopamine D1 receptor knockout mouse, which shows reduced ethanol reward as well as ethanol self-administration (Cunningham et al. 2000; Phillips et al. 1998). Models used to investigate both fat and ethanol consumption include mice that lack or overexpress the galanin (GAL) gene, which is

1Abbreviations that appear ≥3x throughout this article: AgRP, agouti-related protein; AMY, amygdala; ARC, arcuate nucleus; BEC, blood ethanol content; CeA, central nucleus of the amygdala; DA, dopamine; ENK, enkephalin; GABA, γ-aminobutyric acid; GAL, galanin; GHRF, growth hormone–releasing factor; GLUT, glutamate; LH, lateral hypothalamus; MCH, melanin-concentrating hormone; mPFC, medial prefrontal cortex; mRNA, messenger RNA; NAc, nucleus accumbens; NPY, neuropeptide Y;OX, orexin; PFLH, perifornical lateral hypothalamus; PVN, paraventricular nucleus of the hypothalamus; VTA, ventral tegmental area; WT, wild-type
closely related to fat ingestion and energy balance in addition to ethanol consumption as described below. Mice lacking the mu opioid receptor (MOR), which is known to mediate the hedonic aspects of food and drug consumption (Pecina and Berridge 2005; Zhang and Kelley 2002), show reduced reinforcement from sucrose and chow (Papaleo et al. 2007) and consume less ethanol solution compared with their wild-type (WT) counterparts (Becker et al. 2002).

These models have gained increasing recognition in the field of consummatory behavior, as it is now understood that the genes involved in the consumption of one substance (e.g., fat) may also function in controlling the consumption of other substances (e.g., ethanol).

**Forced Exposure**

Most studies investigating the effects of fat on various behavioral or neurochemical parameters supply a palatable diet as the only source of calories (Chang et al. 2007b, 2010b), because animals strongly prefer a diet rich in fat and/or sucrose over a standard chow diet. This approach facilitates the exact monitoring of how many calories an animal consumes and how many of them come from fat. The experimenter can also manipulate the source of fat (Dziedzic et al. 2007; Wang et al. 2002) and examine the neurobiological consequences of various fatty acids. Other studies have exposed rats to fat via a lipid emulsion injected into the peritoneal cavity or via intubation with a feeding needle (Carrillo et al. 2004; Liu et al. 2004a). Such a procedure is most often used to study the acute effects of dietary fat and under conditions that require tight control over the number of calories provided.

In studies with ethanol, investigators use several methods that involve forced consumption to reach steady and pharmacologically relevant BEC values. The simplest such paradigms are intubation of ethanol with a feeding needle or injection of ethanol into the peritoneal cavity. These methods, usually performed in rats, enable experimenters to control the amount of ethanol and length of exposure, as described in studies examining ethanol’s acute or chronic regulation of certain neurochemical systems (Morganstern et al. 2010a,b). Rats can also be compelled to consume ethanol as part of a liquid diet that usually contains 36% of total calories from ethanol. Over several weeks, animals consume about 13 g/kg of ethanol each day, with BEC values ranging from 20 to 240 mg% depending on the time of measurement (Lumeng and Li 1986). In a similar manner but with chow also available, ethanol intake in rats can be forced by supply- ing it as the only source of liquid in varying concentrations that range from 3% to 30% (Lograno et al. 1993; Lucchi et al. 1988; Pietzak et al. 1988; Schramm-Sapyta et al. 2008). This method is often used to examine the long-term consequences of ethanol drinking on various behavioral or neurochemical measures.

Forced consumption can also serve as a useful technique to elicit later free-choice ethanol intake. In an early study, rats forced to consume a 20% ethanol solution intermittently (every other day) for 30 days continued to consume more ethanol and showed a slight preference for ethanol when switched to a free-choice paradigm with water also available (Wise 1973). This model, which has been used repeatedly and improved upon in alcohol research, has led to a new model of ethanol dependence. The new model, which uses chronic intermittent alcohol vapor exposure for 10 weeks (95% ethanol evaporated and delivered at 22–27 mg/mL for 14 hours), results in BECs of 150–200 mg% and leads to increased ethanol self-administration after withdrawal from the vapor chambers (Gilpin et al. 2008b; O’Dell et al. 2004). Although technically challenging, the model elicits clear signs of physical and psychological dependence (Gilpin et al. 2008b), suggesting that it may be a very useful tool with which to examine the neurochemical mechanisms of ethanol dependence.

**Restricted Access**

An additional paradigm used to induce binge-type behavior with palatable diets is the restricted or intermittent access model. Because rodents exhibit a natural preference for sugar, presentation of a sucrose solution for 12 hours per day can lead to binge-type behavior as well as sugar dependence in experimental rats (Avena et al. 2005; Colantuoni et al. 2002). In a similar paradigm but with vegetable shortening available for 2 hours per day, rats show very distinct binge eating and consume in 2 hours as many calories as they would normally eat over 24 hours with ad libitum access (Corwin 2004; Corwin and Wojnicki 2006). Others, who have reported similar binge eating with 2-hour access to a sweet fat–rich diet (Bernet et al. 2008) or 3-hour access to the chocolate-flavored nutrition drink Ensure (Kelley et al. 2003), have noted significant changes in various neurochemical systems as a result of such excess consumption. These studies underscore the utility of such a model in identifying the pathways that may be disturbed and contribute to binge eating of palatable diets rich in sugar and/or fat.

The restricted access paradigm can also be applied to ethanol, where it leads to a binge-type consumption pattern that resembles the clinical condition of binge drinking. One specific example of this model involves intermittent access to an escalating concentration of ethanol (2%, 4%, 7%, 9%) during the animals’ 12-hour dark period, which leads to greater intake values (2.5 g/kg/day) and BEC levels (39 mg/dL) compared to rats with 24-hour access to a similar concentration of ethanol (Avena et al. 2004; Carrillo et al. 2004; Chang et al. 2007a). Shorter periods of access are needed, however, to increase intake to levels that cause binge-type behavior. With a 30-minute access paradigm, C57BL/6J mice have been shown to consume approximately 2 to 2.5 g/kg of ethanol, which significantly elevates BEC levels and produces motor impairments (Cronise et al. 2005). Even greater ethanol consumption is obtained in this mouse strain with limited access to ethanol (20%) for 4 hours each day, starting 3 hours into the dark cycle. This model, known as
“drinking in the dark” (DID), can drive mice to consume as much as 7 g/kg of ethanol in the 4-hour access period (Hendrickson et al. 2009; Rhodes et al. 2005).

Overall, these studies suggest that animals will overconsume ethanol when it is presented during scheduled sessions, and this model can be helpful for examining mechanisms of binge-drinking behavior, which is characterized by intermittent excessive consumption.

Voluntary Access

Perhaps the most natural model used to examine consummatory behavior in rodents involves their voluntary access to nutrients or drugs such as ethanol. Although the intake levels using this method are considerably lower than those obtained with forced or restricted access, voluntary consumption presents a useful model for investigating natural patterns of consumption and their underlying neurochemical or behavioral mechanisms. This method has been used to understand the consumption of diets with varying levels of either fats or proteins (Kim et al. 1991), various sources of fats (Nakashima 2008; Orosc 2002), and different fatty acid composition (Rice et al. 2000).

Voluntary access presents a particular challenge with ethanol, however, as rodents often avoid it because of its bitter taste. One popular technique used to elicit voluntary drinking, “sucrose fading,” involves initially mixing ethanol with sucrose for training purposes and then slowly reducing the sucrose concentration until animals show stable drinking (Samson 1986). Another technique is to start animals on a very low ethanol concentration and then slowly increase it every few days until the desired concentration is reached (Karabayev et al. 2010a; Morganstern et al. 2010a; Sandbak and Murison 1996). Both of these methods give the animal a choice between an ethanol solution and water. Similar paradigms are used in feeding studies, with animals given a daily choice among several diets composed of varying macronutrients, such as fat, carbohydrate, or protein (Jean et al. 2002; Shor-Posner et al. 1994).

These voluntary access paradigms have enabled scientists to examine common behaviors and neurochemical systems regulating the natural choice of one substance over another.

Prediction Models

“Prediction” models are used to assess the innate characteristics of outbred animals that will go on to consume higher amounts of food or ethanol. These models have provided information about various behavioral or biological markers, making it possible to predict a phenotype before significant substance exposure and also to identify neurochemical changes that may be causally related to an overconsumption phenotype rather than a consequence of the substance exposure.

For palatable foods, initial intake of or natural preference for fat when first introduced to rats is positively related to changes in caloric intake, weight gain, and body fat accrual that occur weeks or months later (Chang et al. 2010b; Morganstern et al. 2010b; Shor-Posner et al. 1994). Furthermore, single measures of locomotor activity and of circulating triglyceride levels after a fat-rich meal are closely related to and even predict caloric intake of chronically available high-fat diet in SD rats (Karabayev et al. 2009b; Morganstern et al. 2010c). Most interesting is the finding that these measures of activity and triglycerides predict long-term patterns of ethanol drinking and lever pressing for ethanol (Karabayev et al. 2010a; Nadal et al. 2002), and the consumption of a sweet saccharin solution or a low 2% ethanol solution for just a few days can similarly predict long-term patterns of ethanol self-administration (Karabayev et al. 2010a; Koros et al. 1998). Conversely, alcohol-prefering P rats and several other rat lines and mouse strains (e.g., C57/BL) also show a strong preference for sweet taste (Overstreet et al. 1993; Sinclair et al. 1992), suggesting that sweet preference not only predicts ethanol intake but may also share common neurobiological mechanisms with the drive to consume this substance.

By identifying predictive factors, researchers can better understand what brain or behavioral abnormalities exist before substance exposure and may be involved in driving an animal to consume excess fat or ethanol.

Neural Circuitry Involved in Consummatory Behavior

Food and ethanol consumption not only provide energy and nutrition but also are rewarding. Energy homeostasis, the process of maintaining energy balance, is primarily mediated by nuclei in the hypothalamus, whereas emotion and reinforcement are more strongly mediated by nuclei outside the hypothalamus, in mesocorticolimbic regions. Figure 1 provides an overview of the principal nuclei involved in consummatory behavior and the connections between them, along with some of the neurochemicals produced in each of these nuclei. We provide details in this and subsequent sections.

Hypothalamic Areas

Arcuate Nucleus

The arcuate nucleus (ARC) contains “first-order” neurons involved in feeding: those that transcribe neuropeptide Y (NPY), agouti-related protein (AgRP), two orexigenic peptides that are largely coexpressed, and growth hormone–releasing factor (GHRF), and ghrelin. All of these peptide-expressing neurons, which lie adjacent to the median eminence and lack protection by the blood brain barrier, detect changes in peripheral signals, such as glucose and lipids. Thus, cells of the ARC recognize alterations in homeostatic parameters and transmit their information to “second-order” neurons in more dorsal regions of the hypothalamus, such as the paraventricular nucleus (PVN), perifornical lateral hypothalamus (PFLH) (Beaulieu et al. 1996). The ARC neurons also send projections outside the hypothalamus to...
emotion-regulating areas such as the amygdala (AMY\textsuperscript{1}) and arousal-regulating areas such as the midline thalamic nuclei (Kampe et al. 2009), and these two areas in turn send feedback to the ARC (Magoul et al. 1993) to modulate the central appetite signaling.

**Paraventricular Nucleus**

Second-order neurons in the PVN include those that transcribe the orexigenic peptides, GAL and enkephalin (ENK\textsuperscript{1}). Like the ARC, this region is involved in maintaining energy balance, but it also plays a role in reward functions. In addition to major input from the ARC, the PVN receives projections from the PFLH and extrahypothalamic areas such as the AMY and thalamus (Csaki et al. 2000). The efferent projections of the PVN remain predominantly in the hypothalamus, including the ARC (Magoul et al. 1993) and PFLH (Barone et al. 1981), although some PVN neurons send output to the ventral tegmental area (VTA\textsuperscript{1}) (Rodaros et al. 2007), a mesocorticolimbic structure.

**Periformal Lateral Hypothalamus**

Another hypothalamic nucleus with second-order neurons is the PFLH, which contains the peptides orexin (OX\textsuperscript{1}), also known as hypocretin, and melanin-concentrating hormone (MCH\textsuperscript{1}), which are transcribed almost exclusively within this nucleus. The PFLH has large reciprocal connections with mesocorticolimbic nuclei that provide an interface between the homeostatic and reward systems of the brain. In addition to afferents from the ARC and PVN, the PFLH receives input from and sends efferent projections to the VTA, AMY, nucleus accumbens (NAc\textsuperscript{1}), and medial prefrontal cortex (mPFC\textsuperscript{1}) (Barone et al. 1981; Cheng et al. 2003; Kita and Oomura 1981; Phillipson and Griffiths 1985; Silverman et al. 1981).

**Extrahypothalamic Areas**

**Ventral Tegmental Area**

Outside the hypothalamus, the VTA is a major source of the reinforcement-regulating neurotransmitter dopamine (DA\textsuperscript{1}) (Dahlstrom and Fuxe 1964) and also contains \(\gamma\)-aminobutyric acid (GABA\textsuperscript{1}) interneurons and projection neurons (Van Bockstaele and Pickel 1995). This nucleus receives a number of inputs from hypothalamic and extrahypothalamic regions, including the PVN (Rodaros et al. 2007), PFLH
(Fadel and Deutch 2002), NAc (Kalivas et al. 1993), AMY (Wallace et al. 1992), and mPFC (Sesack and Pickel 1992). In turn, it predominantly sends DA-containing projections back to the majority of these regions (Barone et al. 1981; Oades and Halliday 1987; Swanson 1982) to provide signals related to motivated behavior.

Nucleus Accumbens

The NAc processes reward signals from ingested food and drugs and programs action and motivation to obtain food and drugs. It receives major OX and MCH projections from the PFLH (Fadel and Deutch 2002; Georgescu et al. 2005), DA from the VTA (Swanson 1982), and glutamate (GLUT1) from the AMY and mPFC (Christie et al. 1985; Kelley et al. 1982), and sends output projections back to these regions (Barone et al. 1981; Kalivas et al. 1993; Kim et al. 2004). While the medium spiny output neurons of the NAc all contain GABA, specific efferents also colocalize with orexigenic peptides such as ENK (Meredith 1999).

Amygdala

The AMY plays a major role in processing emotion, particularly anxiety, as well as associative conditioning for pairing food and drug reward with action required to obtain the substance. Like the NAc, the AMY receives OX from the PFLH (Baldo et al. 2003), DA from the VTA (Oades and Halliday 1987), and GLUT from the mPFC (McDonald et al. 1996). It then sends GABA or GLUT outputs to the PVN (Csaki et al. 2000), PFLH (Barone et al. 1981), VTA (Wallace et al. 1992), NAc (Phillipson and Griffiths 1985), and mPFC (Bacon et al. 1996).

Prefrontal Cortex

The prefrontal cortex (PFC), particularly the mPFC, is responsible for processes of executive function, including attention and decision making needed to obtain food or drugs. In addition to major inputs from the VTA, which largely contain DA (Swanson 1982), the mPFC receives afferents from the hypothalamus and AMY (Hoover and Vertes 2007). It then sends GLUT efferents back to the VTA (Sesack and Pickel 1992), NAc (Phillipson and Griffiths 1985), PFLH (Kita and Oomura 1981), and AMY (Brinley-Reed et al. 1995) to inhibit behaviors inappropriate for goal-directed action such as obtaining food or drugs.

Neurochemicals Involved in Food and Macronutrient Intake

The regulation of food intake is coordinated by a large array of neurochemicals throughout the brain. Although anorexigenic signals that inhibit feeding behavior can be disturbed and lead to overeating, our focus here is on orexigenic signals, which initiate or prolong eating. Whereas some of these neurochemicals may provide a general signal for promoting caloric intake, there are others, particularly in the hypothalamus, that provide a more specific signal for stimulating consumption of a particular macronutrient: fat, carbohydrate, or protein. Figure 1 provides an overview of the different areas where these neurochemicals are transcribed.

Stimulation of Food Intake

The neurotransmitters DA, GABA, and GLUT, which are produced and released in multiple nuclei of the brain, appear to provide general signals to modulate food intake. A brief description of each signaling process appears below.

Dopamine

The role of the catecholamine neurotransmitter DA in overeating depends on the area of the brain examined. Injection of low doses of DA or dopaminergic agonists in the NAc can increase food intake (Evans and Vaccarino 1986), whereas in the hypothalamus DA decreases feeding (Yang et al. 1997). The orexigenic effects of DA injections have been shown for both fat (Rao et al. 2008a) and carbohydrates, particularly sucrose (Hodge et al. 1994). Levels of endogenous DA are also higher in the NAc after consumption of fat or sugar (Hajnal et al. 2004; Rada et al. 2010). These two pieces of evidence together suggest that an increase in DA may drive excessive intake of foods high in fat and carbohydrates.

Other evidence indicates that low basal levels of DA in mesocorticlimb regions may drive intake of these rewarding foods. Rats prone to overeating and becoming obese on a fat-rich diet exhibit reduced levels and evoked release of DA in both the NAc and mPFC (Geiger et al. 2008; Rada et al. 2010), and mice lacking the DA receptors D2 or D3, and hence having reduced DA function, consume more calories than WT mice with intact DA systems (Garcia-Tornadu et al. 2009, 2010; McQuade et al. 2004).

Clinical studies also support the role of low DA in driving food intake, as antipsychotic medications that block dopaminergic activity cause patients to overeat and become obese (Blouin et al. 2008; Kluge et al. 2007). Although this pharmacological effect could also occur through the blockade of serotonin or histamine receptors (Theisen et al. 2007; Ujike et al. 2008), the involvement of D2 receptors is supported by evidence that humans with specific variant alleles for these receptors exhibit greater weight gain on antipsychotic medication (Hong et al. 2010; Muller et al. 2010). Furthermore, other studies of humans with alleles for the D2 receptor and the DA transporter show a positive correlation with overeating and binge eating of foods high in fat and sucrose (Eny et al. 2009; Shinohara et al. 2004). Thus it is possible that subjects with low endogenous DA in the NAc compensate by overconsuming fat or carbohydrates to raise their DA levels to sufficiently high levels to be rewarding.
GABA

The inhibitory amino acid neurotransmitter GABA may drive eating behavior through actions in the PVN and NAc, although it does not show a close association with any particular macronutrient.

Injection of GABA agonists into the PVN or NAc causes rats to increase their intake of standard laboratory chow (Stratford and Kelley 1997; Tsuji and Bray 1991), whereas injection into the AMY or lateral hypothalamus (LH1) decreases intake (Minano et al. 1992; Turenius et al. 2009). When the diets are chronically available, peripheral injection of GABA increases consumption of fat but not sucrose (Berner et al. 2009), and injection directly into the NAc can stimulate intake of sucrose (Stratford and Wirtshafter 2010). However, when a fat-rich diet is available for only a brief period of 1 to 2 hours a day, binge eating of this diet is suppressed by peripheral GABA administration (Berner et al. 2009; Wojnicki et al. 2006), suggesting that GABA functions differently in binge eating than it does in general eating.

Studies also show that GABA levels increase in the hypothalamus in response to food intake and that this effect occurs with fat and carbohydrate as well as protein (Fisler et al. 1989; White et al. 2003; Zhu et al. 2010), indicating that this neurotransmitter may not be associated with any specific macronutrient. Studies in humans also support a role for GABA in overeating, with specific variant alleles of GAD2, a gene responsible for GABA production, linked with disinhibited eating and increased concentration of lipids or carbohydrates in the diet (Boutin et al. 2003; Choquette et al. 2009).

Glutamate

The excitatory amino acid neurotransmitter GLUT plays an opposite role to GABA in many cases, acting through the LH (rather than the PVN) to nonspecifically drive food intake. Although agonists of GLUT stimulate food intake when injected into the LH (Stanley et al. 1993), they reduce it when injected in the NAc (Stratford et al. 1998). Also in the NAc, GLUT agonists decrease sucrose intake (Stratford et al. 1998) but increase fat intake (Will et al. 2006), confirming that these effects, as with GABA, are not macronutrient specific.

In the LH, where GLUT injection stimulates feeding, this amino acid may show a positive feedback association with food consumption, as GLUT release in this region is stimulated by the ingestion of fat, carbohydrate, and protein (Thongkhao-on et al. 2008; White et al. 2003). Similarly, although GLUT in the NAc suppresses food intake, consumption of a sweet/fat diet reduces endogenous GLUT release in this region (Saulskaya and Mikhailova 2002), which would disinhibit further intake of rewarding foods. Mice with low GLUT function, due to deletion of the metabotropic GLUT receptor 5 subtype, consume less chow than their WT littermates (Bradbury et al. 2005), supporting the idea that high GLUT function in certain brain areas can drive food intake. As with GABA, GLUT can clearly promote overconsumption, through region- but not macronutrient-specific effects.

Stimulation of Fat Intake

Unlike carbohydrate or protein intake, which is more tightly regulated through negative feedback systems, the intake of fat shows additional elements of positive feedback, with the ingestion of a fat-rich food source often stimulating the desire for more fat. In both human and rodent studies, preloads containing fat result in larger subsequent meals and shorter latencies to those meals compared to preloads with carbohydrate or protein (Marmonier et al. 2000; Warwick et al. 2003). Fat intake also results in greater orexigenic and less anorexigenic signaling in both the circulation and the brain (Beck et al. 1990; Sanchez et al. 2010).

From an evolutionary point of view, this attenuated satiety from fat makes sense, as fatty foods are energy dense and have traditionally been difficult to procure. But in today’s developed societies, with abundant high-fat food sources, this quality can lead to overeating and a host of attendant health complications.

The neuropeptides GAL, ENK, OX, and MCH and the bioactive lipids endocannabinoids, all of which are produced in the hypothalamus, appear to have a special role in the positive feedback regulation of fat intake.

Galanin

The peptide GAL, which is widely expressed in the brain, including in the hypothalamus, appears to show a special association with fat.

Injections of GAL in the PVN or AMY are orexigenic and stimulate chow intake in rats maintained on a chow diet (Kyrkouli et al. 1990). Although GAL injections do not consistently alter the preference for pure fat over carbohydrate or protein (Smith et al. 1997; Tempel et al. 1988), the feeding response is nonetheless stronger and more prolonged in subjects maintained on a high-fat compared with a low-fat diet (Nagase et al. 2002). Studies with genetically modified mice also support this association: animals that overexpress the GAL gene consume more fat than WT mice, despite similar intake of standard chow (Hohmann et al. 2003; Karatayev et al. 2009a); conversely, GAL knockout mice show decreased fat intake and preference (Adams et al. 2008; Karatayev et al. 2010b).

Gene expression and peptide production of GAL in the PVN are stimulated by consumption of dietary fat but not by carbohydrate or protein (Akabayashi et al. 1994), demonstrating a positive association between GAL and fat intake. Furthermore, GAL expression and levels are elevated in situations where fat intake is preferred, such as in female rats at puberty onset and during proestrus (Leibowitz et al. 1998, 2003) and in Brattleboro rats, which have a high preference for fat (Beck and Max 2007). Circulating levels of GAL may reflect recent consumption of dietary fat, as obese menopausal women have higher serum levels of GAL (Milewicz et al. 2000) and recovered anorexic women have reduced levels in their cerebrospinal fluid (Frank et al. 2001).
Despite its association with fat intake, GAL may not be responsible for the development of obesity. The only study in humans to examine GAL alleles with regard to body weight found no difference between obese and healthy young adults (Schauble et al. 2005), and a study in rats found that chronic ventricular injection of GAL does not increase body weight (Smith et al. 1994). Thus, although it may not be responsible for body weight per se, GAL appears to drive consumption of diets high in fat content.

**Enkephalin**

The opioid peptide ENK is expressed in multiple mesocorticolimbic nuclei and, acting in the hypothalamus, is positively related to fat consumption.

Chow intake is stimulated by injection of ENK analogues in the PVN or PFLH (Stanley et al. 1988), VTA or NAc (Mucha and Iversen 1986), central nucleus of the amygdala (CeA) (Gosnell 1988), and even the mPFC (Mena et al. 1989, 1996). Cells that express ENK are largely concentrated in the lateral area of the PFLH (Wortley et al. 2003). In certain circumstances, ENK may also be positively related to sucrose, as binge access to this nutrient compared with standard chow stimulates hypothalamic ENK messenger RNA (mRNA1) expression (Olszewski et al. 2009).

The expression of ENK in the hypothalamus is positively related to fat intake (Chang et al. 2007b), but restricted access to a sweet/fat diet reduces or has little effect on ENK expression in the NAc (Kelley et al. 2003). Like GAL, ENK expression and levels are elevated in the PVN of female rats at puberty onset, when preference for fat increases (Leibowitz et al. 2009), and in the PVN, NAc, and CeA of outbred rats prone to overconsuming a high-fat diet (Chang et al. 2010b). The possibility that ENK is responsible for driving fat intake is supported by studies in mice that lack the ENK gene, as these mice are less willing to work to obtain a fat pellet reward as compared with WT mice (Hayward et al. 2002).

Unfortunately, no studies in humans exist to corroborate these rodent findings on ENK and fat.

**Orexin**

In addition to its well-known role in regulating sleep/wake states (Sakurai et al. 2010), OX plays a major role in the intake of rewarding food, particularly fat.

Injection of OX into the PVN, PFLH, or NAc (but not the CeA) stimulates chow intake (Dube et al. 1999; Thorpe and Kotz 2005). Similarly, injection of OX adjacent to the hypothalamus, in the third ventricle, stimulates rats to consume a high-fat diet in preference to a carbohydrate diet (Clegg et al. 2002), although it can also increase sucrose intake when that is the only food available (Baird et al. 2009). Furthermore, peripheral injection of OX antagonists suppresses fat but not sucrose intake (Richards et al. 2008; White et al. 2005). These latter effects have been observed using specific OX-1 receptor antagonists, although the recent commercial availability of OX-2 antagonists will likely soon result in information about this receptor and fat intake.

One unique property of OX is that cells expressing this neuronally excitatory peptide are confined almost entirely to the PFLH (de Lecea et al. 1998; Sakurai et al. 1998). Consumption of a high-fat diet compared with a moderate or low-fat diet for up to 2 weeks stimulates expression and levels of OX in the PFLH (Gaysinskaya et al. 2007; Wortley et al. 2003) and rats prone to overeating fat show elevated OX expression (Morganstern et al. 2010c), confirming the positive association between OX and dietary fat. This effect is site specific, occurring in the perifornical rather than the lateral area of the PFLH (Wortley et al. 2003). In certain circumstances, OX may also be positively related to sucrose, as binge access to this nutrient compared with standard chow stimulates hypothalamic OX messenger RNA (mRNA1) expression (Olszewski et al. 2009).

Some studies indicate, however, that this peptide may actually be inversely related to weight gain. In selectively bred obesity-prone rats OX mRNA is lower or no different from that in obesity-resistant rats (Levin et al. 2005; Teske et al. 2006), a finding that may have more to do with the ability of OX to increase energy expenditure than an inability to stimulate food intake (Funato et al. 2009). It is possible that OX ultimately stimulates intake of fatty foods by coordinating arousal with energy homeostasis (Sakurai et al. 2010). Although OX knockout mice compared with WT mice show little change in chow intake (Kaur et al. 2008), they are less able to acquire the behavioral output necessary to procure food (Akiyama et al. 2004; Sharf et al. 2010).

Together, this evidence supports a role for OX in promoting caloric intake and having a close association specifically with a high-energy, fat-rich diet.

**Melanin-Concentrating Hormone**

Cells that express MCH are largely concentrated in the PFLH (Skofitsch et al. 1985) and are thought to play a reciprocal role with OX in the sleep/wake cycle (Adamantidis and de Lecea 2008).

Injection of MCH or an MCH agonist into the PVN or NAc increases food intake (Georgescu et al. 2005; Rossi et al. 1999), and injection into the cerebral ventricles stimulates intake of a high-fat diet more strongly than it does intake of standard chow (Gomori et al. 2003). This effect may be due to a desire for rewarding foods in general, because ventricular injection of MCH also stimulates sucrose intake (Duncan et al. 2005).

The expression of MCH appears to be more responsive to fat content than to general reward. Hypothalamic MCH expression and peptide levels are stimulated by the consumption of a high-fat diet but not by saccharin (Elliott et al. 2004; Furudono et al. 2006), and are similarly elevated in the PFLH of rats, maintained on laboratory chow, that are prone to overconsuming a high-fat diet (Morganstern et al. 2010c). Studies using transgenic mouse strains also support the existence of a
special association between MCH and fat: although mice lacking the MCH gene consume less chow (Shimada et al. 1998), those that overexpress MCH consume greater quantities of a high-fat diet than WT mice (Ludwig et al. 2001).

Limited studies also suggest a role for MCH in human obesity. They report that certain populations with early-onset obesity are more likely to carry variations of the MCH 1 receptor than normal weight controls (Gibson et al. 2004; Wernter et al. 2005).

Collectively, these studies demonstrate a positive feedback association between MCH and the intake of fat.

**Cannabinoids**

The endogenously expressed endocannabinoids include N-arachidonoylthanolamine (anandamide) and 2-arachidonoxylglycerol (2-AG), which are present in multiple areas of the brain, including the hypothalamus.

Injection of endocannabinoids or the cannabinoid agonist delta-9-tetrahydrocannabinol (THC) into the PVN or NAc stimulates food intake (Mahler et al. 2007; Verty et al. 2005). Studies with peripheral injections suggest that, although cannabinoids play a role in carbohydrate intake, they are more tightly linked with dietary fat. For example, THC stimulates the intake of a high-fat or sweet-fat diet, but its effects are stronger for the pure fat diet (Koch 2001), and an antagonist decreases drinking of a lipid emulsion more than it does a sucrose solution (Thornton-Jones et al. 2007). Levels of 2-AG and binding to the cannabinoid CB1 receptor in the hypothalamus are also increased by a high-fat compared with a low-fat diet (Higuchi et al. 2011; South and Huang 2008), while CB1 receptor expression is upregulated by sucrose intake (Lindqvist et al. 2008), suggesting that sucrose decreases endocannabinoid levels in this brain region. In addition, like GAL and ENK, levels of anandamide in the hypothalamus are markedly elevated in female rats immediately after puberty onset (Wenger et al. 2002), when preference for fat is highest. Studies with knockout mice further support a strong role for endocannabinoids in controlling fat intake as well as a role in carbohydrate intake. Mice lacking the central CB1 receptor compared with WT mice exhibit a delayed onset of preference for high-fat compared with standard chow (Ravinet Trillou et al. 2004) and consume less of a sucrose solution (Poncet et al. 2003).

Studies in humans support these animal findings: specific alleles for the CB1 receptor are more common in men and women with higher levels of body fat (Jaeger et al. 2008; Russo et al. 2007).

**Stimulation of Carbohydrate Intake**

Compared with fat intake, carbohydrate intake is more tightly regulated by negative feedback systems. Fasting leads to the desire for carbohydrates, but its consumption more readily satiates this desire (Marmonier et al. 2000; Warwick et al. 2003). The neuropeptides NPY and AgRP, which are both expressed and synthesized in the ARC, appear to have a special role in the regulation of carbohydrate intake.

**Neuropeptide Y**

Neuropeptide Y is a highly orexigenic peptide that is produced in many areas of the brain but exerts its effects on feeding predominantly in the hypothalamus.

Injections of NPY in the ARC, PVN, or PFLH potently stimulate feeding but they are ineffective in the NAc or AMY (Bouali et al. 1995; Brown et al. 2000; Stanley et al. 1985a). These hypothalamic injections lead rats to increase carbohydrate intake in favor of fat (Brown et al. 2000; Stanley et al. 1985b). Expression and levels of NPY in the hypothalamus are increased by food deprivation, particularly immediately before scheduled feeding (Kalra et al. 1991; Marks et al. 1992), confirming the important role of NPY in driving food intake.

Carbohydrates have a stimulatory effect on NPY expression, although the effect may vary depending on the time of examination. One week of carbohydrate diet consumption results in elevated ARC NPY expression compared with high-fat or standard chow diets (Giraudo et al. 1994; Wang et al. 1998). A similar increase occurs a few hours after injection of glucose, although the reverse effect is evident immediately after the injection, supporting a negative feedback regulation of carbohydrate intake (Chang et al. 2005).

In further support of the link between NPY and food overconsumption, ob/ob mice that are prone to obesity when fed a high-energy diet have higher NPY levels than obesity-resistant rats even when both set of rats are maintained on a chow diet (Levin 1999). Moreover, NPY knockout mice consume less chow diet and binge less on a highly palatable diet than WT mice (Bannon et al. 2000; Sindelar et al. 2005), whereas NPY-overexpressing mice consume greater quantities of a sucrose diet (Kaga et al. 2001).

The role of NPY in energy balance is also supported by research in humans, as specific alleles for NPY and the NPY receptor Y1 have been linked to increased body mass index (Bray et al. 2000; Lavebratt et al. 2006).

**Agouti-Related Protein**

The peptide AgRP is related to both carbohydrate and fat, and its macronutrient specificity is less well defined than that of NPY. Largely coexpressed with NPY, it is produced exclusively in the ARC and acts by blocking receptors for the anorexigenic melanocortin peptides.

Injection of AgRP into specific regions of the hypothalamus, the PVN or dorsomedial nucleus (DMN), or the CeA potently stimulates food intake, whereas injection into the ARC or PFLH is ineffective (Kim et al. 2000; Wirth and Giraudo 2000). Injection of this peptide into the DMN...
preferentially stimulates intake of a sucrose diet (Wirth and Giraud 2001), while injection into the third ventricle stimulates intake of diets higher in fat content (Hagan et al. 2001).

Much like NPY, AgRP expression is increased by both food deprivation (Palou et al. 2009) and the injection of glucose (Chang et al. 2005); although it is suppressed immediately after glucose administration, its mRNA expression increases several hours later (Chang et al. 2005), indicating that, like NPY, it shows elements of negative feedback by carbohydrate. A general role for AgRP in overconsumption is strongly supported by studies in mice with altered AgRP expression. Targeted ablation of AgRP leads animals to die from starvation within 1 week (Bewick et al. 2005; Luquet et al. 2005), and transgenic overexpression of this peptide causes animals to overeat and develop severe obesity (Graham et al. 1997; Ollmann et al. 1997).

The role of AgRP in driving overconsumption in humans is supported by studies showing increased plasma levels of AgRP during fasting (Shen et al. 2002) and altered macronutrient intake and body weight in populations with specific AgRP alleles (Kalnina et al. 2009; Loos et al. 2005).

Stimulation of Protein Intake

Protein is not the macronutrient of choice for overeating because it is more satiating than fat or carbohydrate (Marmonier et al. 2000), but two hypothalamic peptides facilitate protein intake: GHRF and ghrelin.

Growth Hormone–Releasing Factor

The peptide GHRF, which stimulates the release of growth hormone into the circulation, is transcribed in cells in and around the hypothalamus (including the ARC) and shows a positive feedback association with protein intake.

Injection of GHRF into the suprachiasmatic nucleus-medial preoptic area or ventromedial hypothalamic nucleus, but not the PVN or PFLH, stimulates food intake (Dickson and Vaccarino 1990; Tanaka et al. 1991) and, specifically, intake of protein more than carbohydrate (Dickson and Vaccarino 1994). Lateral hypothalamic injection of an antibody against GHRF suppresses consumption of protein but not carbohydrate or fat (Rains et al. 2001), confirming the selective role of GHRF in protein intake.

As with the regulation of fat-related peptides by dietary fat, hypothalamic expression of GHRF is stimulated by dietary protein but not by fat or carbohydrate (Bruno et al. 1991). In contrast to the carbohydrate-related peptides, hypothalamic expression of GHRF is suppressed by food deprivation (Bruno et al. 1990; White et al. 1990). Although foods high in protein are not typically overconsumed, studies in mice with altered GHRF confirm the importance of this peptide in obesity. Transgenic mice that overexpress GHRF have significantly greater amounts of abdominal fat (Cai and Hyde 1999) and knockout mice have less than WT mice (Fintini et al. 2005).

One study in humans also supports a role for GHRF in obesity, as subjects heterozygous for a GHRF receptor allele weigh less than those homozygous for the common form of the allele (Pereira et al. 2007). Although high levels of protein in the diet may help prevent overeating and the development of obesity, GHRF appears to promote both protein intake and body fat accrual.

Ghrelin

Another peptide that stimulates the release of growth hormone, ghrelin, is expressed predominantly in the stomach but is also found in a population of cells in the ARC.

Ghrelin stimulates food intake after injection in a variety of brain areas—the PVN, PFLH, and ARC (Currie et al. 2005; Szentirmai et al. 2007) as well as the VTA, dorsal raphe nucleus, and even the hippocampus (Abizaid et al. 2006; Carlini et al. 2004). Although this peptide shows an association with all three macronutrients, it may have a particularly strong association with protein. Injection of ghrelin has been demonstrated to stimulate intake of the preferred diet, whether high or low in fat (Liu et al. 2004b), but it has also been shown to stimulate intake of chow over sucrose (Bomberg et al. 2007), suggesting that factors other than palatability are important.

Ingestion of any of the three macronutrients can reduce ghrelin peptide levels, although the most prolonged suppression occurs with protein (Koliaki et al. 2010). Unlike GHRF, fasting potently increases plasma ghrelin levels (Bagnasco et al. 2002), and fat-preferring rats have higher levels of plasma ghrelin (Beck and Max 2007), demonstrating that ghrelin shares some similarities with the fat- and carbohydrate-related peptides.

This peptide may not be as important as others in food consumption, despite its ability to stimulate intake, as ghrelin knockout mice show no difference from WT mice in consumption of chow or a high-fat diet (Sun et al. 2003, 2008). Furthermore, mice that overexpress ghrelin show no difference in chow intake (Reed et al. 2008) and actually consume less of a high-fat diet (Gardiner et al. 2010).

Studies in humans partially support the rodent work, as injection of the carbohydrate glucose has been found to decrease plasma ghrelin levels (Shiiya et al. 2002), and ghrelin alleles have been linked to early-onset or severe obesity (Hinney et al. 2002; Miraglia del Giudice et al. 2004). Thus, although ghrelin appears to have a more complex connection with feeding, it is similar to GHRF in showing a stronger association with protein.

Neurochemicals Involved in Ethanol Intake

The neurochemical systems involved in ethanol consumption are very similar to those that control food ingestion, particularly those related to dietary fat. Although DA, GABA, GLUT, and ghrelin appear to play a more general role in the regulation of food and ethanol consumption, the peptides...
GAL, ENK, OX, and MCH and the bioactive lipids endocannabinoids all have a stronger and more specific association with fat and ethanol. Furthermore, ethanol consumption shows a weaker link with peptides related to carbohydrate or protein consumption, such as NPY, AgRP, and GHRF.

Drawing on the discussions in the preceding section, we describe evidence that supports the idea that ethanol and fat intake are mediated by common neurochemical mechanisms.

**Dopamine**

The mesolimbic DA system, originating in the VTA and projecting to the NAc, has long been implicated in ethanol reinforcement and consumption. Acute exposure to ethanol potently stimulates extracellular DA levels in this system, and this stimulation is known to directly influence the rewarding aspects of ethanol consumption (Di Chiara and Imperato 1988; Piepponen et al. 2002; Yan et al. 2005). Dopamine levels also increase in the hypothalamus after systemic exposure to ethanol (Seilicovich et al. 1985).

Levels of DA are closely associated with ethanol preference and consumption in several animal models. Like rats prone to overeating fat, those selectively bred to prefer ethanol, but not exposed to it, exhibit reduced baseline levels of DA in the NAc (Bustamante et al. 2008) and mPFC (Engleman et al. 2006). However, when allowed to consume ethanol, they show an exaggerated release of DA in these areas as well as an increase in DA neuronal firing in the VTA (Bustamante et al. 2008; Katner and Weiss 2001; Morzorati et al. 2010; Tuomainen et al. 2003).

These studies, which suggest that a disruption of DA balance in mesolimbic and possibly hypothalamic regions can lead to enhanced ethanol consumption, are further strengthened by reports demonstrating an attenuation of ethanol intake with systemic administration (Ingman et al. 2006; Samson and Chappell 2003) or direct VTA injection (Eiler and June 2007; Samson and Chappell 1999) of agents that block DA. With the evidence described above suggesting that mesolimbic DA can also influence fat consumption (Rao et al. 2008a; Wong et al. 2009), it is likely that dopaminergic signaling, possibly by increasing reinforcement, may similarly drive the consumption of both ethanol and fat.

**GABA**

The inhibitory amino acid GABA controls ethanol consumption, particularly in mesolimbic or hypothalamic regions where GABA influences the release of DA or the actions of certain peptide systems, respectively.

Initial studies in rats and mice that assessed the central effects of ethanol exposure indicated that ethanol itself might potentiate GABAergic neurotransmission by modulating the activity and density of GABA-benzodiazepine (BDZ) receptor sites (Ticku et al. 1983). With significant advances in alcohol research since then, the effect of ethanol on GABA neurotransmission has been shown to be more complex than originally thought and largely dependent on the route of administration or brain region of interest. Voluntary consumption of ethanol increases extracellular levels of GABA (Szumlinski et al. 2007), whereas forced acute oral or systemic administration has little effect (Cowen et al. 1998; Dahchour et al. 1994; Heidbreder and De Witte 1993; Kemppainen et al. 2010). In vivo studies with ethanol-induced GABA release in regions outside the DA mesolimbic circuit are lacking, but in vitro application of ethanol enhances GABA release in the hypothalamus (Seilicovich et al. 1988).

Receptor changes also occur with oral ethanol intake in preferring rats—there is a specific reduction in GABA-A, subunit mRNA in the cerebral cortex (Devaud et al. 1995) and in GABA-BDZ receptor densities in the NAc and PFC (Thielen et al. 1997)—possibly leading to ethanol tolerance associated with chronic ethanol exposure. Research shows that a disruption in GABA neurotransmission promotes ethanol consumption. For example, studies in alcohol-prefering rats have indicated that a reduction in GABA release in the VTA (Melis et al. 2009) and in GABA$_A$ receptor expression in the NAc (Chen et al. 1998) may drive animals to consume more ethanol.

An important role for GABA in ethanol consumption is further supported by studies demonstrating reduced ethanol consumption or operant responding with administration of specific ionotropic GABA$_A$ antagonists or metabotropic GABA$_B$ agonists that reduce GABA activity and/or release, either centrally—into the NAc (Hodge et al. 1995), VTA (Moore and Boehm 2009), or CeA (Foster et al. 2004)—or systemically (Quintanilla et al. 2008; Walker and Koob 2007). Additionally, BDZ inverse agonists such as RO19-4603 or high-affinity antagonists such as ZK 93426 or CGS8216 have been shown to reduce ethanol responding in preferring rats (June et al. 1998a,b), suggesting that the GABA-BDZ receptor complex can mediate the rewarding effects of ethanol.

Although active blockade of GABA neurotransmission may reduce the rewarding effects of ethanol and thereby decrease consumption preclinically, in the clinic, agents that potentiate the actions of GABA (e.g., gabapentin) are used to reduce the anxiety associated with abstinence from ethanol (Brower et al. 2008; Furieri and Nakamura-Palacios 2007).

Together with findings that suggest a role for GABA in driving consumption of fat and other nutrients, these studies demonstrate that this inhibitory amino acid may function, although not exclusively, to enhance consumption of both fatty foods and ethanol.

**Glutamate**

The excitatory amino acid GLUT influences ethanol operant responding and consumption by interacting directly with DA neurons in the mesolimbic reward system.

Similar to the findings with GABA, GLUT neurotransmission is differentially affected by ethanol depending on the route of administration. Although no changes in GLUT release
have been observed in the VTA with acute ethanol injection (Kemppainen et al. 2010), GLUT neurotransmission is enhanced when ethanol is applied to isolated VTA neurons (Deng et al. 2009). In the NAc, a dose-dependent effect has been observed: the release of GLUT rises at a lower dose of systemic ethanol but is reduced or unaltered at a higher dose (Dahchour et al. 1994; Moghaddam and Boliniao 1994). Extracellular GLUT levels in the hypothalamus are also enhanced with ethanol perfusion (Noto et al. 1984). Together, these results indicate that GLUT may mediate the effects of ethanol on local circuits in the VTA, NAc, and hypothalamus.

A limited number of studies have examined the status of the GLUT system in alcohol-prefering animals, but they seem to indicate that preferring rats exposed to ethanol exhibit enhanced expression of metabotropic GLUT receptors in the NAc and CeA (Obara et al. 2009) and increased GLUT terminal density in the NAc shell (Zhou et al. 2006). Studies with specific GLUT antagonists have also suggested that systemic or local blockade of GLUT neurotransmission in the NAc leads to a pronounced reduction in ethanol self-administration in rats (Besheer et al. 2010; Rassnick et al. 1992).

These results are similar to the clinical outcome of increased abstinence and reduced relapse observed in alcoholics after treatment with the GLUT antagonist acamprosate (Tempesta et al. 2000; Whitworth et al. 1996).

The fact that GLUT neurotransmission plays a significant, albeit nonspecific, role in fat ingestion suggests that the consumption of fat and ethanol may be similarly controlled by the signaling cascades of this excitatory amino acid.

Galanin

The peptide GAL, which is specifically involved in fat consumption, is also closely associated with ethanol drinking.

In animals trained to voluntarily consume ethanol, central injection of GAL into the third ventricle or PVN leads to an increase in ethanol intake, which does not occur with injections of this peptide into the LH or NAc (Lewis et al. 2004; Rada et al. 2004; Schneider et al. 2007). The opposite effect of reduced ethanol consumption is seen with injection of the GAL antagonist M40 into the PVN or third ventricle, further supporting an important role for this peptide in the hypothalamus with respect to ethanol drinking behavior (Lewis et al. 2004; Rada et al. 2004).

At the same time, the consumption of ethanol stimulates the expression of GAL in certain hypothalamic nuclei, such as the PVN and DMN (Leibowitz et al. 2003), and of the GAL receptor 1 in the whole hypothalamus (Pickering et al. 2007), suggesting that ethanol itself, by enhancing GAL neurotransmission, can further increase ethanol drinking behavior. Although the status of GAL has not been explored in animals that prefer ethanol, transgenic mice that lack the gene encoding GAL show reduced ethanol consumption (Karatayev et al. 2010b), and those overexpressing this peptide consume more ethanol compared with WT animals (Karatayev et al. 2009a).

Clinical investigations corroborate the important role of GAL in ethanol consumption, showing that mutation of the GAL gene or a GAL receptor gene is associated with alcoholism in certain ethnic populations (Belfer et al. 2006, 2007).

Enkephalin

The opioid ENK plays a critical role in ethanol consumption in addition to its role in controlling fat ingestion. The injection of ENK analogues into the PVN or NAc results in enhanced ethanol drinking (Barson et al. 2009, 2010), whereas the injection of an opioid antagonist into the PVN, NAc, or AMY reduces such drinking (Barson et al. 2009, 2010; Heyser et al. 1999).

Ethanol alters the expression of endogenous ENK, but its effects appear to depend on the region and length of exposure. Acute systemic administration increases ENK expression in the PVN (Chang et al. 2007a), CeA (Criado and Morales 2000), and PFC (Mendez and Morales-Mulia 2006) as well as the NAc (Mendez and Morales-Mulia 2006). Whereas chronic voluntary consumption of ethanol also stimulates ENK expression in some of these same regions (Chang et al. 2007a; Cowen and Lawrence 2001), the effects in the NAc vary markedly depending on the dose and route of administration. In rats, forced administration has no effect on ENK expression in the NAc (Lindholm et al. 2000; Mathieu-Kia and Besson 1998), voluntary consumption of less than 2 g/kg/day leads to an increase in expression (Chang et al. 2010a), and consumption of 3 to 6 g/kg/day reduces ENK expression (Cowen and Lawrence 2001; Oliva and Manzanares 2007). In ethanol-prefering rats or mice compared with their nonpreferring counterparts, expression of ENK generally appears to be reduced in the hypothalamus (Blum et al. 1987), NAc (Cowen et al. 1998; Nylander et al. 1994), AMY, and VTA (Jamensky and Gionoulakis 1999).

Although these studies strongly suggest that ENK not only is stimulated in most brain regions by ethanol consumption but also functions to promote further drinking, animals that lack the ENK gene do not show disturbances in ethanol responding or preference (Hayward et al. 2004; Koenig and Olive 2002). However, mice that lack the MOR, through which ENK can function, do exhibit a reduction in ethanol reinforcement (Hall et al. 2001).

Recent clinical studies support the idea that disturbances in MOR expression may lead to abnormal patterns of ethanol consumption, as they show a close association between a variant allele of MOR-1 and alcoholism (Nishizawa et al. 2006; Town et al. 1999) as well as resistance to treatment with the general opioid antagonist naltrexone (Gelernter et al. 2007).

Orexin

In addition to its effects on arousal and the consumption of fat, the peptide OX plays an important role in ethanol drinking. It most likely influences ethanol consumption via hypothalamic circuits, since injection of OX into the PVN
and LH, but not the NAc, specifically enhances ethanol intake (Schneider et al. 2007). It also appears to mediate ethanol preference, as both OX-1 and -2 receptor antagonists, when systemically delivered to rats trained to self-administer ethanol, can significantly attenuate ethanol preference and responding for ethanol as well as other drugs of abuse (Lawrence et al. 2006; LeSage et al. 2010; Moorman and Aston-Jones 2009; Shoblock et al. 2011). The regulation of OX expression appears to depend on the length of administration—OX mRNA is increased by acute oral gavage of ethanol but reduced by chronic consumption (Morganstern et al. 2010a).

Interestingly, both expression and injection studies have revealed a functional difference between OX neurons in the more medial areas around the fornix and those in lateral areas of the LH: the latter, but not the former, are involved in the consumption of ethanol and other commonly abused substances (Aston-Jones 2005; Harris and Aston-Jones 2006; Morganstern et al. 2010b).

Clinical support is not available to support a role for OX in ethanol drinking behavior, but studies have shown that narcoleptic patients, who have extremely low OX levels (Nishino et al. 2000), rarely develop addiction to the drug methamphetamine, which is used in narcolepsy treatment (Guilleminault et al. 1974; Parkes et al. 1975).

**Melanin-Concentrating Hormone**

The peptide MCH regulates ethanol consumption, in addition to feeding and fat ingestion, via both hypothalamic and mesolimbic circuits.

Central injection of MCH into the third ventricle, PVN, or NAc selectively enhances ethanol intake in rats trained to drink ethanol in a two-bottle choice paradigm (Duncan et al. 2005; Morganstern et al. 2010b) or to press a lever for ethanol (Duncan et al. 2006). In contrast, injection into surrounding regions such as the LH or zona incerta (ZI) has no effect (Morganstern et al. 2010b). Furthermore, an MCH antagonist has been shown to be effective in reducing ethanol self-administration when injected systemically (Cippitelli et al. 2010), suggesting that complex neuronal networks surrounding the ventricle may limit the therapeutic effectiveness of such antagonists.

The consumption of ethanol also alters MCH peptide expression, depending largely on the length of exposure and region analyzed. Although acute ethanol exposure enhances MCH mRNA expression in the LH, it reduces it in the adjacent ZI, which is more closely related to locomotor activity than ethanol reward (Morganstern et al. 2010b). In contrast, chronic consumption of ethanol leads to significantly reduced levels of MCH mRNA in the entire LH and ZI region (Morganstern et al. 2010b), much as it does with OX in the PFLH.

The status of MCH has not yet been evaluated in alcohol-preferring animals, but one study demonstrates that mice lacking the gene encoding the MCH receptor 1 actually consume more ethanol solution than WT mice (Duncan et al. 2007). Although this finding appears at first glance to contradict a stimulatory role for MCH in ethanol consumption, the increase in ethanol consumption in these mutant animals may be a nonspecific result of a hyperaroused state produced by knockout of the MCH receptor, through which MCH is known to inhibit OX and thus arousal (Rao et al. 2008b).

To our knowledge, no studies in humans have yet examined the role of MCH in clinical alcoholism, which requires the availability of well-characterized and effective MCH antagonists.

**Cannabinoids**

In addition to their role in fat consumption, the cannabinoids have received recent attention in relation to ethanol consumption, with a specific role in stimulating ethanol drinking and self-administration behaviors.

In animal studies, peripheral injections of various CB1 agonists lead to enhanced ethanol drinking or relapse to drinking (Alen et al. 2009; Colombo et al. 2002). More specifically, the reward-related VTA may be a prime locus for these effects as the injection of a CB1 agonist into this region potently stimulates ethanol binge drinking (Linsenbardt and Boehm 2009). On the other hand, systemically or centrally treating alcohol-preferring animals with a CB1 antagonist significantly reduces ethanol intake (Serra et al. 2001).

Ethanol itself has an inhibitory effect on the endogenous cannabinoid anandamide as well as on CB1 levels. Animals that receive an acute injection or have access to ethanol show a marked reduction in levels of anandamide in the hypothalamus, NAc, and AMY (Ferrer et al. 2007; Rubio et al. 2007) as well as in CB1 levels in the AMY and PFC (Rubio et al. 2009).

Other support for an important role of cannabinoids in ethanol consumption comes from studies in transgenic mice with a deletion in the CB1 gene. These animals show reduced ethanol consumption and preference compared with their WT counterparts (Lallemand and de Witte 2005; Naassila et al. 2004; Wang et al. 2003). Furthermore, in both rat and mouse models with a high preference for ethanol, the density of CB1 receptors in brain regions such as the hypothalamus, NAc, AMY, and PFC is lower, suggesting that the animals may have enhanced endogenous cannabinoid levels in brain regions associated with consummatory behavior, reward, anxiety, and cognition (Hansson et al. 2007; Hungund and Basavarajappa 2000; Ortiz et al. 2004).

Clinical studies support the involvement of cannabinoids in alcoholism, with the identification of a mutant CB1 allele in alcoholics undergoing severe withdrawal or in patients who show high brain activation from alcohol use (Hutchison et al. 2008; Schmidt et al. 2002).

**Neuropeptide Y**

Neuropeptide Y, which has a specific function in stimulating carbohydrate consumption, also plays a role in ethanol intake. But in contrast to the neurochemicals positively related to fat
intake, NPY appears to be inversely related to the consumption of ethanol.

In alcohol-prefering rats, injection of NPY into the third ventricle, PVN, or CeA significantly reduces ethanol intake (Badia-Elder et al. 2001; Gilpin et al. 2008a; Lucas and McMillen 2004), whereas injections into similar brain regions of nonpreferring animals have little effect on drinking behavior (Badia-Elder et al. 2001; Katner and Weiss 2001; Lucas and McMillen 2004). Paradoxically, central injection of specific NPY antagonists similarly reduces ethanol intake, suggesting that this peptide’s regulation of ethanol consumption is more complex than originally believed (Schroeder et al. 2003; Sparta et al. 2004).

Ethanol ingestion itself can affect NPY expression, with studies showing that both acute and chronic intake of ethanol reduces the mRNA and peptide levels of NPY specifically in the ARC (Kinoshita et al. 2000; Leibowitz et al. 2003; Roy and Pandey 2002). Studies with mutant mice further support the inhibitory role of this peptide in ethanol consumption: mice lacking the NPY gene showed enhanced drinking behavior, and those overexpressing it reduced intake (Thiele et al. 1998). The NPY Y1 receptor may be specifically involved in such behaviors, inasmuch as deletion of the gene encoding this receptor yields a high ethanol–consuming phenotype (Thiele et al. 2002). However, deletion of the NPY Y1 gene has the opposite effect and results in reduced drinking, suggesting opposite regulation of ethanol ingestion by these two NPY receptor subtypes (Thiele et al. 2004).

The literature on NPY expression in alcohol-prefering animals is mixed and largely depends on the line of rats used as well as their access to ethanol. When ethanol is available, NPY immunoreactivity in various hypothalamic regions is greater in alcohol-prefering P rats compared with control alcohol-nonpreferring (NP) rats (Hwang et al. 1999), but reduced in these regions in high versus low alcohol-drinking rats (Hwang et al. 1999) and in mesolimbic regions of the ethanol-prefering C57BL/6J mice (Hayes et al. 2005). In contrast, studies have reported no changes in mRNA expression of NPY in any of these brain regions in alcohol-prefering animals at baseline (Caberlotto et al. 2001; Kinoshita et al. 2004).

Clinical studies support a role for NPY in ethanol use, with a variant allele of this peptide identified in heavy versus moderate drinkers (Kauhanen et al. 2000) and in alcoholics compared with control subjects (Lappalainen et al. 2002; Mottagui-Tabar et al. 2005).

Agouti-Related Protein

The peptide AgRP, which enhances both fat and carbohydrate consumption, also affects ethanol intake. Although the literature concerning AgRP regulation of ethanol consumption is limited, several studies suggest a positive association between this peptide and ethanol intake.

Ventricular administration of AgRP stimulates ethanol intake in C57BL/6J preferring mice (Navarro et al. 2005) but not in nonpreferring strains (Navarro et al. 2003; Polidori et al. 2006). Furthermore, acute administration of ethanol in C57BL/6J mice, but not nonpreferring mice, results in enhanced peptide levels of AgRP in the ARC (Cubero et al. 2010), although chronic consumption of an ethanol diet has little effect on this peptide (Navarro et al. 2008).

No clinical studies exist to support a role for this peptide in alcohol use, but mutant mice with a genetic deletion of AgRP exhibit reduced lever pressing for ethanol as well as ethanol binge drinking in a limited access paradigm (Navarro et al. 2009).

These few studies suggest that AgRP may have some function in controlling ethanol intake, although other peptide systems such as those specific for fat may have a more prominent role.

Growth Hormone–Releasing Factor

Little is known about the function of GHRF in ethanol consumption. An early report noted a decrease in GHRF mRNA in the hypothalamus of animals that consume an ethanol diet, and this decrease may have been the cause of the reduced weight gain also observed in these animals (Soszynski and Frohman 1992). In other studies, acute or chronic ethanol exposure has consistently led to reduced testosterone and growth hormone levels, although these changes were independent of altered GHRF levels in the hypothalamus (Steiner et al. 1997; Tentler et al. 1997). Together, these few studies indicate that GHRF, a peptide more closely related to the consumption of protein than fat (Dickson and Vaccarino 1994; Rains et al. 2001), has relatively little function in ethanol consumption.

Ghrelin

Ghrelin has been implicated in the consumption of foods rich in protein, carbohydrates, and fat, with a slightly greater role in protein ingestion. Investigators have also studied the role of this peptide in ethanol consumption and found that central administration of ghrelin enhances ethanol consumption in a site-specific manner.

Injection of ghrelin into the third ventricle or reward-related tegmental areas such as the VTA and lateral tegmental area can enhance ethanol intake, whereas in the hypothalamus it has little effect (Jerlhag et al. 2009; Schneider et al. 2007). The ethanol intake–promoting effects of ghrelin may be attributed in part to enhanced activity of the reward pathway, because tegmental injection of the peptide increases DA levels in the NAc (Jerlhag et al. 2007). In contrast, peripheral administration of ghrelin has been shown not to affect ethanol drinking (Lyons et al. 2008), although in this case the peptide may not have reached the brain to produce its central effects. However, when a ghrelin antagonist was administered peripherally, ethanol preference, intake, and locomotor activity induced by ethanol were all attenuated (Jerlhag et al. 2009; Kaur and Ryabinin 2010). Again, this...
effect may be related to a reduction in the rewarding effects of ethanol, inasmuch as the ghrelin antagonist has also been shown to reduce NAc DA levels and ethanol-conditioned place preference (Jerlhag et al. 2009).

The status of ghrelin has not been examined in alcohol-preferring rat or mouse models, although one study reported a reduction in alcohol-induced locomotor activity, NAc DA, and alcohol-conditioned place preference in mutant mice with a deletion of the gene that encodes ghrelin (Jerlhag et al. 2009).

The involvement of ghrelin in clinical alcoholism has also received support from the literature, suggesting that this peptide may play a more prominent role in abstinent than current alcoholic patients. For example, abstinent alcoholics have greater ghrelin levels than active drinkers (Kraus et al. 2005), and levels of this peptide have a positive association both with the time the patient has been abstinent (Kim et al. 2005) and with the level of craving for alcohol during withdrawal (Wurst et al. 2007). However, two separate clinical reports have demonstrated reduced ghrelin levels in current alcoholic patients (Addolorato et al. 2006) and a mutation in proghrelin in heavy alcohol users (Landgren et al. 2008).

These studies collectively demonstrate that ghrelin, which has a nonspecific role in the ingestion of several macronutrients, is involved in mediating the rewarding effects of ethanol intake.

Conclusions

Using a number of animal models of food and ethanol intake, researchers in the last few decades have discovered important factors involved in regulating the intake of these substances. Although the hypothalamus is classically associated with food intake due to its regulation of energy balance, more recent reports have suggested that nuclei in mesocorticolimbic regions also play a role due to their regulation of emotion and reinforcement.

A strikingly large number of neurochemical systems participate in driving food intake. In the hypothalamus, many of these systems have close associations with a specific macronutrient, whereas in mesocorticolimbic areas they appear to be more strongly related to general reward. Systems involved in fat intake appear to function in a positive feedback loop, further potentiating the consumption of this macronutrient, whereas systems involved in carbohydrate or protein intake are more often negatively regulated by ingestion of these nutrients.

Unlike food, alcohol is not necessary for survival, yet this calorie-containing drug of abuse functions through many of the same systems that evolved for food intake. In addition to increasing reward and reinforcement through the mesocorticolimbic regions, alcohol intake functions through neurochemicals in the hypothalamus and, remarkably, appears to have a special association with the same hypothalamic neuropeptides that control fat intake. This characteristic may in part explain how alcoholism can develop, acting through a system designed to maximize intake. Although it is doubtful that all drugs of abuse act through fat-related neurochemicals, it is likely that they act through many of the same systems that evolved to control food intake in general.

Based on the research described in this review, it may be possible to apply knowledge gained about the regulation of feeding behavior to the development of medications to treat alcohol use and drug addiction.

Acknowledgments

This research was supported by US Public Health Service grants AA12882 and DA21518.

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