

The Role of the Melanocortin System in Metabolic Disease: New Developments and Advances

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Abstract

Obesity is increasing in prevalence across all sectors of society, and with it a constellation of associated ailments including hypertension, type 2 diabetes, and eating disorders. The melanocortin system is a critical neural system underlying the control of body weight and other functions. Deficits in the melanocortin system may promote or exacerbate the comorbidities of obesity. This system has therefore generated great interest as a potential target for treatment of obesity. However, drugs targeting melanocortin receptors are plagued by problematic side effects, including undesirable increases in sympathetic nervous system activity, heart rate, and blood pressure. Circumnavigating this roadblock will require a clearer picture of the precise neural circuits that mediate the functions of melanocortins. Recent, novel experimental approaches have significantly advanced our understanding of these pathways. We here review the latest advances in our understanding of the role of melanocortins in food intake, reward pathways, blood pressure, glucose control, and energy expenditure. The evidence suggests that downstream melanocortin-responsive circuits respon-

sible for different physiological actions do diverge. Ultimately, a more complete understanding of melanocortin pathways and their myriad roles should allow treatments tailored to the mix of metabolic disorders in the individual patient.

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Introduction

Obesity is increasing in prevalence across all sectors of society, and is now considered to be a global epidemic. Nearly 35% of adults in the USA are obese [1], with 6.3% of the population being severely obese [2]. Obesity is associated with a constellation of serious disorders, including hypertension [3], type 2 diabetes [3], and depression [4]. Given these links, it is not surprising that obesity is also associated with higher all-cause mortality [5]; estimates for the annual number of excess deaths attributable to obesity in the USA vary from 111,909 to 365,000 [6, 7]. These facts have sparked intensive research into the causes and consequences of obesity.

The modern era of obesity research is only 20 years old, dating from the discovery of the adipokine leptin and leptin-responsive neurons in the brain [8, 9]. Arguably, the most critical neural system identified to date underly-

ing the control of body weight is the melanocortin system. In fact, the melanocortin system may contribute to the development or severity of many health problems associated with obesity, including hypertension, type 2 diabetes, and eating disorders. Gaining a complete understanding of the function of the melanocortin system is therefore critical.

In the past decade, conditional knockout strategies have been used to delete melanocortin-related genes in targeted neuronal populations. However, observational studies and simple gene knockout experiments have drawbacks. For instance, altering gene expression may not affect neuronal activity. In addition, functional redundancies and rewiring of neurocircuitry due to genetic manipulation may present a misleading picture of the importance of the targeted gene.

Recent, novel experimental approaches are changing our picture of the melanocortin system along with our understanding of brain function generally. Technologies developed within the past 5 years, such as optogenetics and the chemogenetic “designer receptors exclusively activated by designer drugs” (DREADD) system, are helping to establish a causal relationship between cellular activity in the brain and physiological responses [10–12]. These techniques allow an investigator to turn on or off specific cell populations acutely using genetic manipulation and either photostimulation (optogenetics) or pharmacotherapy (DREADD). In addition, new techniques to visualize and record neuronal activity in awake, behaving animals are revealing a previously unappreciated complexity in the function of neurons *in vivo*. It is now possible to do extracellular recording from multiple neurons simultaneously using an optic fiber-coupled multielectrode probe (or “optetrode”) introduced into a targeted nucleus of the brain [13]. This technology is particularly powerful in conjunction with the optogenetic identification of neuron types. Alternatively, neuronal activity can be monitored following the introduction of a calcium-sensitive, fluorescent reporter. A fiber-optic probe may then be used to record changes in the fluorescence of a neuronal population, or the fluorescence of individual neurons can be recorded with a lens attached to a miniature microscope mounted on the head of the animal (e.g., a mouse).

In some cases, findings from studies using these modern techniques contrast with the effect of gene ablation, providing new information on how the melanocortin system functions. These approaches have also been complemented by more sophisticated genetic approaches that allow gene deletion or reactivation at the time and location of an investigator’s choosing. Using this new in-

formation, we are beginning to develop a larger, more integrated view of the function of the melanocortin system.

The Melanocortin System

Melanocortins, of which α -MSH is a classic example, are products of the proopiomelanocortin (POMC) gene. The brain’s melanocortin system consists of the neurons that express POMC, the central nervous system (CNS) circuits that sense melanocortins, and the neurons that produce agouti-related peptide (AgRP), an endogenous antagonist of melanocortins [14]. Two recognized neuronal populations expressing POMC exist in the brain, although low levels of POMC mRNA have been reported in other CNS regions [15–17]. A small population resides in the nucleus of the solitary tract (NTS) in the brainstem [18]. The second, largest population resides in the hypothalamus, specifically in the arcuate nucleus (ARC), and coexpresses cocaine- and amphetamine-regulated transcript [18]. Note that this population is not homogeneous. Subpopulations of these neurons express GABA, glutamate, and various receptors; these subgroups may project to different areas and serve different functions [19–22]. In addition, POMC neurons may also exhibit age-dependent changes in amino acid phenotype [23].

The 5 melanocortin receptors (MC1R to MC5R) were named in the order of their cloning. MC3R and MC4R are considered the neural melanocortin receptors (MCRs), due to their high expression in the brain [24–28]. MC4Rs show a broader expression pattern than MC3Rs, being found in around 100 brain nuclei.

The highest expression of MC4Rs is seen in the hypothalamus and the brainstem. Second-order neurons in the hypothalamus expressing MC4Rs that have been identified to date include oxytocin, thyrotropin-releasing hormone (TRH), and corticotrophin-releasing hormone synthesizing neurons in the paraventricular nucleus of the hypothalamus (PVH) [28, 29]. Several brainstem regions that receive direct projections from POMC neurons of the ARC also express MC4Rs. These include sympathetic preganglionic neurons in the intermediolateral cell column (IML) [18, 30], the dorsal motor nucleus of the vagus (DMV), which contains parasympathetic preganglionic cells, and the NTS, which controls output from DMV cells [31]. MC4R agonists directly inhibit the activity of parasympathetic preganglionic neurons in the DMV and excite the activity of sympathetic preganglionic neurons in the IML [32].

MC3Rs are restricted to the hypothalamus and limbic structures. The highest expression levels occur in the ARC, ventromedial hypothalamus, ventral tegmental area (VTA), and medial habenula [24]. Interestingly, the MC3R is the only MCR expressed by POMC and AgRP/neuropeptide Y (NPY) neurons in the ARC [33, 34]. These MC3Rs allow melanocortins to act pre- and post-synaptically to reduce POMC neuronal activity, forming an “ultrashort” autoinhibitory feedback loop [34–36].

MCRs are G protein-coupled receptors with a high level of constitutive activity [37]. Receptor activation can ultimately increase neuronal excitability, facilitate neurotransmitter release, regulate how neurons integrate synaptic input, and alter synaptic strength and connectivity [38, 39]. For instance, MC4Rs can increase the amount of GABA released by neurons projecting to the PVH. The MC4R is located on the terminals of axons in the PVH [40]. α -MSH was found to potentiate GABA-mediated responses in PVH neurons by acting presynaptically; in addition, the action of α -MSH was blocked by AgRP [40]. The ability to alter synaptic strength may play a role in many functions of melanocortins, including their influence on body weight and reward pathways [41]. However, when stimulated continuously, the MC4R undergoes desensitization and internalization [42].

The neuronal population expressing AgRP is restricted to the medial ARC and coexpresses NPY [43, 44]. AgRP and POMC neurons project to similar areas of the forebrain, but AgRP neurons do not send projections to the brainstem [45]. AgRP neurons also provide direct inhibitory input to POMC neurons via NPY receptors [36]. AgRP inhibits the basal activity of MC4Rs [46] and acts as a competitive antagonist that prevents binding of melanocortins. AgRP also regulates the type of G protein that is associated with the MC4R. While α -MSH stimulates increased coupling of the receptor with $G_{s\alpha}$, AgRP stimulates coupling of the receptor to the $G_{i/o\alpha}$ subunit, which inhibits adenylate cyclase and reduces cellular cAMP levels [47].

The pathways described above permit melanocortins and their endogenous antagonist, AgRP, to influence multiple physiological processes, including control of blood pressure, serum glucose levels, energy expenditure, and food intake.

Role in Food Intake

It has been known for 30 years that melanocortins have a potent and long-lasting inhibitory effect on feeding [48]. However, optogenetics has revealed differences in

how hypothalamic and hindbrain POMC populations suppress food intake. Acute activation of NTS POMC neurons produces an immediate inhibition of feeding behavior [49]. The NTS projects to the parabrachial nucleus (PBN) (Fig. 1a) [50, 51], which in turn projects to other areas that influence energy balance, including the lateral hypothalamic area (LHA), amygdala, VTA, and nucleus accumbens (NA) [52–54]. In contrast, arcuate POMC activation has delayed effects on feeding that require MC4Rs [11, 49, 55]. An important caveat is that optogenetic stimulation may not recapitulate more subtle, short-term actions of ARC POMC neurons on feeding initiation. Mandelblat-Cerf et al. [56] measured the electrophysiological activity of ARC POMC neurons in behaving animals; as described below, their results suggest that at least some POMC neurons may act on faster timescales to reduce the drive to seek food.

Interestingly, the drive to eat is generally stronger than systems promoting satiety. For instance, blocking MC4Rs has a more profound effect than stimulating them; the former causes a weight loss of 10–15% before ceasing to be effective, while the latter causes a weight gain of 25% in a similar amount of time and continues to cause hyperphagia as long as the antagonists are infused [57–60]. Thus, the primary purpose of the melanocortin system may be to prevent starvation rather than obesity.

Fasting excites AgRP neurons [61, 62], and both NPY and AgRP stimulate appetite and food-seeking behavior [63, 64]. In recent years, novel techniques to manipulate and visualize AgRP neuronal activity have helped to advance our understanding of these neurons substantially. Photostimulation [11] or DREADD activation [10] of AgRP neurons, which are GABAergic, results in unopposed and immediate onset of hyperphagia. However, optrode recording found that while the onset of feeding in fasted mice reduced firing rates of AgRP neurons, it did not abolish their activity; the firing rates remained elevated relative to those in mice without caloric deficiency following ad libitum night feeding [56]. This residual, persistent spiking may reflect a sustained drive to consume food, while additional circuits downstream of AgRP neurons prevent continued feeding.

AgRP gene deletion subtly reduces long-term weight gain, primarily by promoting thyroid hormone production [65]. Likewise, AgRP inhibits paraventricular TRH neurons via MC4Rs [66–68]. The mild hyperthyroidism in AgRP knockout mice, however, was not accompanied by altered spontaneous food intake [65, 69]. Furthermore, activating AgRP neurons can increase food intake independently of MCRs, suggesting that AgRP release is

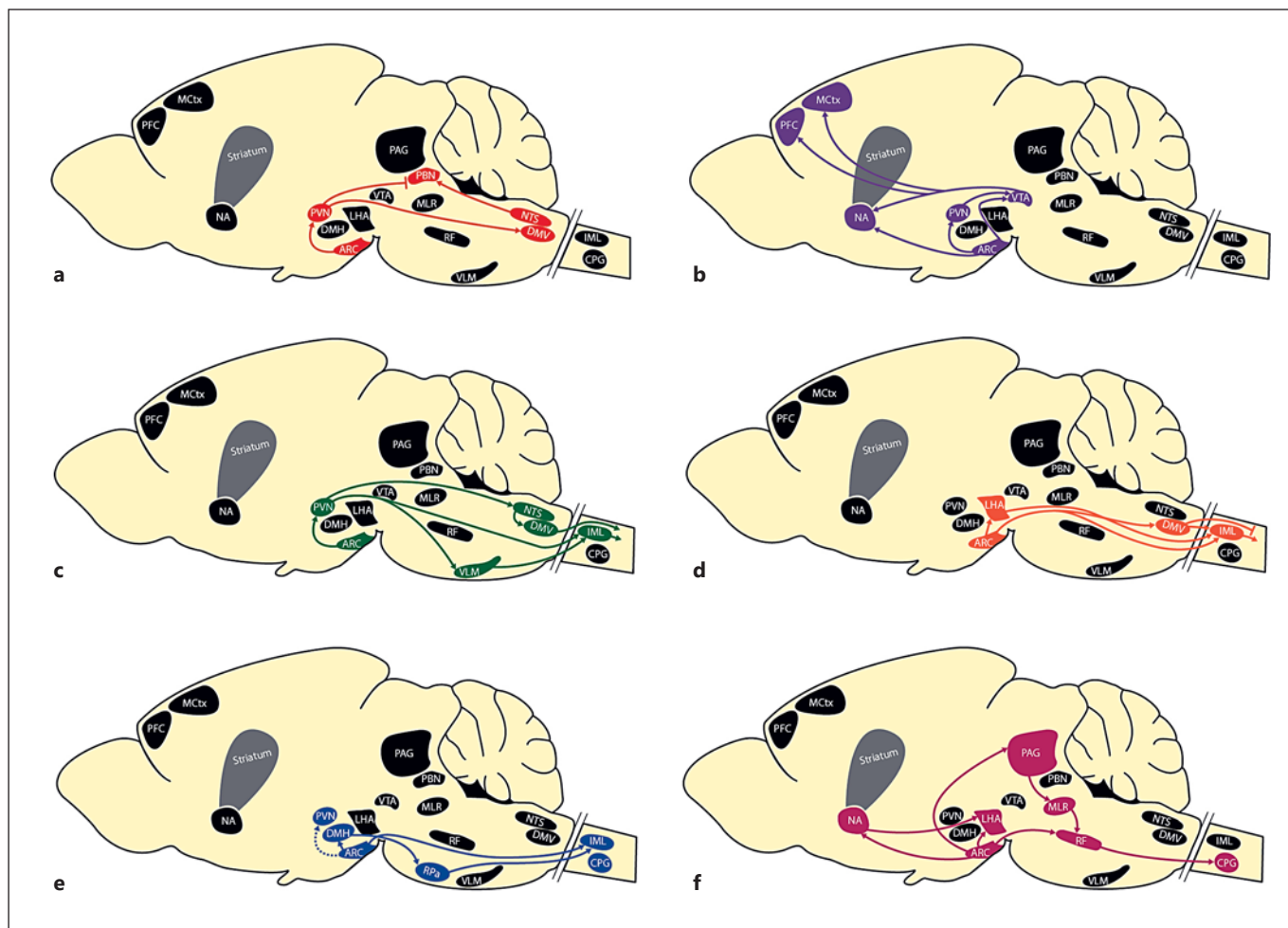


Fig. 1. Neuronal pathways whereby melanocortins alter energy balance. Melanocortin projections affect food intake (a), reward pathways (b), blood pressure (c), glucose homeostasis (d), basal energy expenditure (e), and locomotor activity (f). Primarily first- and second-order connections from proopiomelanocortin populations in the ARC and NTS are shown (see text for details). ARC, arcuate nucleus of the hypothalamus; CPG, central pattern generators; DMH, dorsomedial nucleus of the hypothalamus; DMV,

dorsal motor nucleus of the vagus; IML, intermediolateral nucleus; LHA, lateral hypothalamic area; MCtx, motor cortex; MLR, mesencephalic locomotor region; NA, nucleus accumbens; NTS, nucleus of the solitary tract; PAG, periaqueductal gray; PBN, parabrachial nucleus; PFC, prefrontal cortex; PVN, paraventricular nucleus of the hypothalamus; RF, reticular formation; VLM, ventrolateral medulla; VTA, ventral tegmental area.

not necessary for these effects [11]. These results imply that another product of AgRP neurons is important for appetite stimulation. Indeed, blocking PVH GABA or NPY receptors inhibits food intake following AgRP neuron activation [55]. Thus, it appears that AgRP neurons suppress the effects of POMC neurons through 3 mechanisms: they employ direct local GABAergic inhibition of POMC neurons and their PVH targets, AgRP-mediated antagonism at MCRs, and NPY receptor-mediated signaling to functionally counteract MCR signaling. The timescale of these mechanisms differs, however; GABA

and NPY can each rapidly stimulate feeding, while a few hours are required for AgRP to stimulate feeding by blocking MC4Rs [70].

The ability of POMC and AgRP neurons to sense and respond to circulating markers of energy balance such as insulin, leptin, glucose, and ghrelin led to the hypothesis that the main role of these neurons is to match feeding to caloric needs [71]. Recent studies have confirmed that AgRP and POMC neuron activity in part reflects slow changes in energy balance that occur over the course of the day. Mandelblat-Cerf et al. [56] used optetrode elec-

trophysiological recording in awake, behaving mice to measure absolute activity levels of identified AgRP neurons. Slow, opposite changes in AgRP and putative POMC neuron spiking were seen across hours, as the energy balance gradually changed.

An unexpected result from recent research on AgRP neuron activity is that not only do these neurons respond to internal cues signaling energy status, but they also respond within seconds to food consumption, the sight of food, or even to cues that predict food [72–74]. These timescales are inconsistent with a purely homeostatic role in feeding. Rather, these changes must reflect input from neurons that process information about the immediate availability and attractiveness of food in the environment. These fast changes may decrease the drive to continue the search for food, or they may predict meal consumption. Motivation and prediction of rewards are subserved in the brain by the mesocorticolimbic pathway; these results will therefore be discussed further in the next section.

Mc4r mutations cause the most common form of monogenic obesity in humans [75]. Mc3r mutations that reduce sensitivity to melanocortins appear to have been selected for in human populations facing frequent food scarcity [76]. Mc3r or Mc4r deficiency also results in hyperphagia and obesity in mice [77, 78]. Identifying the key MCR-expressing neurons involved in appetite regulation has been a focus of intensive research recently. MC4R-expressing neurons in the PVH are critical for regulating food intake; re-expression of MC4Rs on PVH SIM1-positive neurons in mice otherwise lacking MC4Rs abolishes hyperphagia and reduces their obesity [79–81]. *Sim1* is expressed in most neurons of the PVH, some of which produce the neuroendocrine peptides oxytocin, arginine vasopressin, corticotropin-releasing hormone, TRH, and somatostatin. Studies using anatomical, pharmacogenetic, and optogenetic approaches suggest that PVH oxytocin neurons regulate acute feeding [55, 82, 83]. However, the importance of melanocortin-sensing oxytocin pathways to satiety is not yet settled [81, 84].

Until recently, it was believed that MC4R-expressing PVH neurons promote satiety by projecting to and activating neurons in the NTS [85, 86], increasing their sensitivity to gut-derived, afferent vagal satiety signals [87, 88]. However, channelrhodopsin-assisted circuit mapping has found that melanocortin-responsive PVH neurons target the preganglionic vagal motor neurons in the DMV and not the NTS (Fig. 1a) [81]. MC4Rs in the NTS may therefore respond to melanocortins produced locally to reduce food intake [89]. Instead, the PVH-DMV

projection may modulate gut reflexes and gastric processes [90].

Shah et al. [81] found that the *Sim1* neurons responsible for suppressing food intake synapse onto neurons expressing calcitonin gene-related protein (CGRP) in the PBN, a key region for transmitting taste information to the cortex and forebrain regions such as the central nucleus of the amygdala that modulate perception and behavior [91, 92]. The lateral PBN has been implicated in taste [93, 94], learned taste aversion [95], and appetite suppression in response to illness or nausea [96–99]. Inhibiting CGRP neurons reduces nausea and increases food intake during illness but does not increase feeding under standard conditions [55, 100]. The PBN satiety center is normally suppressed by GABAergic input from AgRP neurons (Fig. 1a) [99], permitting feeding when appropriate. However, when AgRP neurons are killed in adult mice, CGRP neurons become hyperactive and the mice cease to eat, resulting in starvation [99, 100]. Inhibiting CGRP neurons in the PBN prevents this starvation [99, 100]. Shah et al. [81] found that more than half of the PBN neurons receive input from glutamatergic PVH *Sim1* neurons. Furthermore, the majority of the PBN-projecting PVH neurons have MC4Rs.

These results were recently supported using optogenetic techniques. Using mice expressing Cre recombinase in MC4R neurons, Garfield et al. [101] found that optical activation and inhibition of PVH MC4R neurons exerted a bidirectional control of feeding via projections to the lateral PBN. These cells were found to mediate AgRP neuron-driven hunger. It is possible that this pathway allows melanocortins to induce appetite suppression by amplifying nausea or the discomfort of extreme fullness. However, mice showed a preference for activation of this circuit if they were calorically depleted. Given that fed mice showed neither an aversion to nor a preference for activation of this pathway [101], it is likely that the PVH MC4R to lateral PBN connection affects satiety in a manner unrelated to sensations of pleasure or pain.

Actions in Reward Pathways

The VTA contains the dopaminergic cell bodies of the mesocorticolimbic dopamine system, the reward circuitry of the brain. This limited number of dopamine neurons project widely, permitting coordinated responses to new rewarding stimuli (Fig. 1b). Dopamine influences the value placed on goals by acting in the orbital prefrontal cortex [102]. It also modulates memory consolidation

in the amygdala and hippocampus [103, 104]. In addition, dopamine encodes new motor programs that will facilitate obtaining a reward via the core region of the NA and dorsal striatum [105]. The dorsal striatum also plays a critical role in the anticipation and processing of aversive stimuli [106] and in altering future behavior following negative reinforcement [107], although these functions may not be primarily dopamine dependent [108, 109]. Finally, dopamine confers motivational salience or “wanting” to a reward (as opposed to the hedonic aspect or “liking” of a reward) by acting in the NA shell region [110, 111].

Melanocortins have the ability to alter dopaminergic reward pathways. MC4R signaling within dopamine-responsive neurons in the NA and dorsal striatum leads to morphological changes in dendritic spines that contribute to addictive behavior [15, 112]. In addition, both POMC and AgRP neurons of the ARC project to the VTA [113, 114] and NA [45] (Fig. 1b). Indeed, α -MSH stimulates dopamine release in the NA when microinjected into the VTA [114, 115]. High levels of MC3R are found in the NA and VTA [24]. In addition, the MC3R – but not the MC4R – was recently reported to be expressed in up to a third of dopaminergic neurons of the VTA [116]. Recently, arcuate POMC neurons projecting to VTA dopaminergic neurons have been shown to modulate motivation for palatable food via MC3Rs [117]. Others have reported the MC4R is also present within the VTA [26, 118]. Within the VTA, AgRP neurons synapse on dopamine neurons [114]. Reduced activity of AgRP neurons facilitates VTA dopamine neuron activation of the NA [114]. Notably, the regulation of the VTA by AgRP neurons appears to be mediated by GABA rather than MCRs [114]. In addition, the PVH projects strongly to the VTA [119], potentially allowing second-order influences of melanocortins on that system. Thus, these effects in the VTA allow the melanocortin system to influence the overall level of dopamine released.

Glutamatergic projections from neurons in the prefrontal cortex and dopaminergic projections from the VTA enter the NA to synapse on medium spiny neurons (MSNs). These neurons express either the dopamine D₁ or the dopamine D₂ receptor, which are G_s- and G_i-coupled receptors, respectively. Dopamine released by stimuli diffuses into the synaptic cleft, where it excites D₁-expressing MSNs of the direct striatonigral pathway and inhibits D₂-expressing MSNs of the indirect striatopallidal pathway, leading to activation of targeted motor cortical areas (Fig. 1b) [120, 121]. The emergence of genetic technologies to target discrete populations of neurons

coupled with optogenetic and pharmacogenetic tools to manipulate their activity has provided a means for dissecting the contributions of these neuronal populations to various aspects of motivated behavior.

Dopamine neurons in the VTA exhibit 2 different patterns of dopamine release: (1) basal levels of dopamine release due to tonic firing, which activates high-affinity D₂ receptors, and (2) bursts of elevated dopamine release due to phasic firing, which activates low-affinity D₁ receptors [122–124]. These release patterns reflect 2 different functions of dopamine release. When animals encounter unexpected rewards, dopamine neurons evoke a burst of phasic firings that activates the low-affinity D₁ receptor, which promotes new reward-directed learning [125, 126]. Recent optogenetic studies have shown that the reinforcing properties of dopamine, cocaine, amphetamine, and natural rewards result from dopamine excitation of D₁ MSNs [127–130]. Increased activity of D₁ receptor MSNs may be a critical component of the long-lasting neurological changes associated with drug sensitization and addiction [131].

The inhibition of the indirect pathway by the tonic firing of dopamine also increases motivation for reward and allows the activation of motor patterns necessary for its pursuit. Indeed, inhibition of D₂ MSNs using a chemogenetic approach enhances the motivation to obtain cocaine [132]. In this way, dopamine release promotes reinforcement both by activating D₁ MSNs and the direct pathway and by inhibiting D₂ MSNs and the indirect pathway [129]. However, the absence of an expected reward suppresses dopamine neurons and relieves the D₂ receptor-mediated inhibition of the indirect pathway. Optogenetic activation of D₂ MSNs, normally inhibited by dopamine, reduces self-administration of cocaine [127, 132, 133], decreases initiation of movement and increases freezing, and causes mice to react as though they have received a punishment [134].

Until recently, the main role of melanocortin modulation of the mesocorticolimbic pathways was thought to be promoting preference for palatable and calorie-dense food. Like D₁ receptor agonists, melanocortin agonists suppress food intake primarily through reduction in meal size, rather than meal frequency [135, 136]. Loss of MC4R signaling in mice produces hyperphagia, but it is associated with decreased effortful pursuit of palatable food [137–139]. Activation of MC4Rs on D₁ receptor MSNs decreases meal size, reinforces high-fat food preferences, and permits cocaine-induced anorexia [140, 141]. MC4R may also be expressed by D₂ receptor neurons in the NA [140], which drive the striatopallidal pathway. MC4R-de-

ficient mice decreased effortful responding to palatable food during restricted feeding, and this deficit was not rescued by re-expression of MC4R in D₁ receptor MSNs [140]. Thus, MC4R signaling may influence the function of both the direct and the indirect reward pathways. While global deletion of the MC3R decreases preference for sucrose in female mice, the mean meal size is similar to that in wild types even when the difficulty in obtaining food is increased [116]. Together, these findings suggest that the rewarding aspects of food consumption are blunted by loss of MC4R but not MC3R signaling.

New work, however, suggests that POMC and AgRP neurons may play a more fundamental role in motivation for obtaining food. A nonhomeostatic aspect of the function of AgRP neurons had previously been suggested by observations of a decrease in AgRP neuron *c-Fos* expression 2 h after consumption of a calorie-free meal [74]. Recently, Chen et al. [73] used *in vivo* fiber photometry to monitor bulk changes in population calcium activity, pooled from many ARC AgRP neurons in awake, behaving mice. Whereas in fasted mice AgRP neuronal activity was elevated, their AgRP neuronal activity decreased within mere seconds as soon as eating began. Conversely, POMC activity, predictably low in hungry mice, rose almost immediately as feeding started; this rise did not prevent the mice from continuing to eat eagerly. They also found the presentation of food is sufficient to rapidly reverse the activation state of these neurons induced by an energy deficit. This rapid regulation is modulated by food palatability and occurs before any food is consumed [73]. Finally, if food was removed partway through the meal, the suppressed AgRP neurons again increased their activity, and the POMC neurons reduced their activity [72, 73]. Similar results were found using endoscopy to measure calcium activity in individual AgRP neurons in awake mice [72]. In addition, *in vivo* electrophysiological recording demonstrated that roughly half of responsive ARC neurons were modulated within minutes following food cue presentation only; AgRP neurons show a cue-induced decrease, while others (presumably POMC neurons) show a cue-induced increase [56].

These findings are reminiscent of classic experiments showing that dopamine release is a predictive signal of reward [142]. Electrophysiological recording of neurons in the VTA of monkeys found a spike of activity in response to an unexpected reward with fruit juice. When trained to associate the juice reward with a conditioned stimulus such as a tone, the spike of activity shifted to immediately following the tone. However, when the conditioned stimulus was given without the expected reward,

the spike of activity was followed by a decrease in dopamine neuron firing below basal levels at the expected time of the reward. Thus, dopamine release encodes the expectation of a reward, and responds with a spike if the reward is better than expected and with a dip if it is worse [142]. Interestingly, dopamine is released by separate VTA neurons when unpleasant or aversive stimuli are encountered [143]. In humans, recent work suggests dopamine release reflects the confidence that a chosen behavior will lead to an expected outcome [144]. It therefore appears that dopamine release draws the individual's attention to surprising events, affecting the salience of objects and events. Potentially important stimuli seem more noticeable and more important, which assists decision-making. Note also that dopamine projections to the basal ganglia control motor patterns in response to these signals and projections to the cortex allow higher-order judgments about appropriate responses.

The parallels with the POMC and AgRP recording studies above are striking, including neuronal responsiveness to conditioned stimuli (the smell of food), modulation by the size of the reward (the palatability of food), and the generation of an error signal due to interruption of an anticipated reward (food removal). Further, there is evidence for a connection of AgRP and POMC neurons with motor pattern generators as well. The study by Mandelblat-Cerf et al. [56] demonstrated that in mice consuming liquid food, almost half of the ARC neurons were significantly modulated by individual licks or lick bouts. These changes in firing occurred approximately 1 s before or after lick/bout onset, suggesting that these changes could help drive the initiation of upcoming licks. If rapid POMC and AgRP effects are indeed dependent on dopamine release, one would expect POMC neurons to promote a dopamine release or action in response to positive stimuli, while AgRP neurons would oppose these effects. In support of this concept, Betley et al. [72] found that mice avoid situations leading to activation of AgRP neurons, suggesting that AgRP activity contributes to negative reinforcement of behaviors leading to aversive outcomes. Likewise, strong AgRP neuronal inhibition conditioned the preference for flavors and places.

It is clear that the older view that AgRP neuronal activity produces hunger that directly causes eating was overly simplistic. In a recent review, Seeley and Berridge [145] assert that POMC and AgRP neurons are likely to modulate and receive input from the brain reward circuitry that reacts to food cues and mediates motivation to eat. In other words, elevated AgRP (and low POMC) may “prime

the reactivity” of dopamine circuits to the sight, smell, and taste of food. In turn, the mesocorticolimbic circuitry must send feedback signals to the hypothalamus when food is encountered (or is unexpectedly absent), so that AgRP and POMC activity reflects the new incentive value of the food. Indeed, starvation signals generally increase mesocorticolimbic reactivity to food in both humans and rats [146–148]. Future experiments to test this and related models will require simultaneous manipulation of or recording from POMC/AgRP neurons and mesocorticolimbic pathways.

Role in Blood Pressure

The brainstem, especially the ventral medulla, has a key role in the maintenance of blood pressure. Sympathomedullary pathway activation is responsible for the stereotyped “fight-or-flight” response, which includes an increased heart rate and blood pressure, redirection of the blood flow away from digestive processes, and mobilization of stored energy. The NTS receives inhibitory baroreceptor afferents and serves a key role in stimulating inhibitory (blood pressure-lowering) pathways. Vasoconstriction and an increased heart rate and stroke volume are promoted by sympathetic output of the IML in the spinal cord. The limbic system, cerebral cortex, and hypothalamus directly or indirectly project to the IML to influence blood pressure (Fig. 1c). Activity of the IML is promoted by the rostral ventrolateral medulla, which in turn is tonically inhibited by GABAergic projections from the caudal ventrolateral medulla. NTS activation of the caudal ventrolateral medulla thus decreases blood pressure. A decreased heart rate/force is promoted by parasympathetic innervation from the DMV. The NTS promotes activity of the DMV, thus further decreasing blood pressure [149–151].

The melanocortin pathway has been implicated in obesity-related hypertension and increased sympathetic activity [58]. Unlike in high-fat diet-induced obese mice, blood pressure is not elevated in MC4R deficiency. Rather, rodents lacking MC4R often show reduced sympathetic nervous system (SNS) activity and blood pressure, even though they exhibit many other characteristics of the metabolic syndrome [59, 152, 153]. Compared with most obese patients, those deficient in MC4R show lower blood pressure and norepinephrine excretion even if they are very obese [154–156]. These observations have led to the hypothesis that obesity-related hypertension is MC4R dependent.

Preclinical and clinical studies have largely supported this idea. Chronic hypothalamic MCR activation can increase arterial pressure despite reduced food intake, whereas the inhibition of MCR significantly increases body weight without raising arterial pressure [57, 157]. Furthermore, acute central administration of α -MSH increases the mean arterial pressure and heart rate in wild-type animals but not in MC4R knockout mice [158, 159]. In normal animals, chronic MC4R antagonism slows the heart rate in a sustained manner and slightly reduces blood pressure despite hyperphagia and rapid weight gain [59, 160, 161]. MC4R antagonism can reduce blood pressure by 25–30 mm Hg in lean, spontaneously hypertensive rats, which are known to have increased SNS activity [59]. Correspondingly, an MC4R agonist increased systolic and diastolic blood pressure in obese volunteers [157]. These findings indicate that for excess weight gain to increase SNS activity and elevate blood pressure, MC4R activation is necessary.

Leptin links obesity, SNS overactivity, and high blood pressure. Elevating leptin levels of lean rodents to those seen in rodents with severe obesity causes chronic increases in blood pressure, secondary to adrenergic activity [162]. These chronic effects of hyperleptinemia on SNS activity and blood pressure regulation require an intact POMC neuron-MC4R axis; the ability of leptin to raise blood pressure requires both leptin receptors and MC4R in POMC neurons [152, 163, 164]. Recent work suggests that IRS2 signaling in POMC neurons is essential for the chronic actions of leptin to raise mean arterial pressure [165].

The PVH represents an important location for the convergence of neuronal inputs that control sympathetic outflow and cardiovascular activity through its projections to the rostral ventrolateral medulla and the intermediolateral column of the spinal cord (Fig. 1c) [166]. The PVH is also involved in prolonged excessive sympathetic activation; injection of an MCR agonist into the PVH of male rats increased renal sympathetic afferent activity and mean arterial pressure. These effects were attenuated but not blocked by AgRP or a selective MC4R antagonist, indicating that activation of both MC3Rs and MC4Rs in the PVH can increase sympathetic outflow and blood pressure [167]. Thus, MC3Rs in the PVH may exert a tonic excitatory effect on sympathetic activity.

Second-order melanocortin-responsive neurons are also located in the hindbrain. Some studies have found a reduced blood pressure and heart rate following microinjection of α -MSH or a synthetic agonist into the NTS of anesthetized, spontaneously hypertensive rats and nor-

motensive Sprague-Dawley rats [168, 169]. On the other hand, an α -MSH-expressing viral vector injected into the NTS increased sympathetic activity and the heart rate and reduced parasympathetic activity in mice [170]. Similarly, Iwasa et al. [171] observed an increased heart rate after α -MSH had been microinjected into the IML, the location of cholinergic sympathetic preganglionic neurons. Recent work shows that re-expressing MC4Rs specifically in cholinergic neurons renders MC4R null mice hypertensive without affecting their obesity [32]. These studies support the hypothesis that MC4Rs in sympathetic preganglionic neurons of the IML play an important role in obesity-associated hypertension.

Role in Glucose Control

Along with its role in the regulation of blood pressure, the melanocortin system influences glucose homeostasis and, in particular, insulin secretion, glucose utilization, and glucose production. Intracerebroventricular administration of an MCR or MC4R agonist reduces plasma insulin levels in leptin-deficient and lean mice and improves hepatic and skeletal insulin sensitivity [172–174]. Conversely, chronic intracerebroventricular administration of an MCR antagonist impairs hepatic and skeletal muscle insulin sensitivity regardless of the body weight [174]. Mutations in the human MC4R gene may lead to hyperinsulinemia when compared to obese control subjects [174], although the evidence is inconsistent [175]. Nevertheless, MC4Rs appear likely to influence the maintenance of normal glucose homeostasis. Additional studies are needed to examine the real-time effect on glucose homeostasis of activating or suppressing hypothalamic and hindbrain POMC neurons.

Recently there has been great interest in the downstream targets of POMC neurons that are responsible for the glucose-regulating actions of POMC neurons. Importantly, restoring MC4Rs in the PVH does not rescue from hyperglycemia or hyperinsulinemia; therefore, the PVH does not mediate actions of melanocortin on glucose homeostasis [79]. Rather, it appears that the activity of MC4Rs in brainstem autonomic regions prevents hyperglycemia and hyperinsulinemia. Recent studies evaluated 2 mouse models with MC4R expression restored specifically in just cholinergic DMV preganglionic parasympathetic neurons or in both cholinergic DMV preganglionic parasympathetic and IML sympathetic neurons. Reactivation of MC4R signaling in all cholinergic neurons ameliorated both hyperglycemia and hyperinsulinemia

[176]. By comparison, reactivation of MC4Rs in parasympathetic afferent neurons located in the nodose ganglia did not prevent hyperglycemia but did abolish hyperinsulinemia [176]. This finding suggests a role for the SNS in regulating insulin secretion; MC4Rs in the DMV may inhibit spontaneously active parasympathetic preganglionic neurons that stimulate insulin secretion (Fig. 1d) [176, 177]. Glucose intolerance was seen in both groups, suggesting insulin resistance [178]. Thus, these data suggest that MC4Rs expressed in preganglionic sympathetic neurons of the IML may mediate the ability of melanocortins to suppress blood glucose levels. It remains unknown whether NTS or ARC POMC neurons are the source of the endogenous ligand for the hindbrain MC4R. Interestingly, under hypoglycemic conditions, 100% of NTS neurons were found to depolarize in response to α -MSH, as opposed to 35% under normoglycemic conditions [179].

Leptin can have a profound effect on glucose homeostasis via the melanocortin system – for example, by increasing hepatic gluconeogenesis and by decreasing glycogenolysis [180]. Central blockade of MCRs in streptozotocin-induced diabetic rats blocked a leptin-mediated decrease in blood glucose [181]. These effects may be due to leptin's actions on POMC neurons, since mice lacking the leptin receptor (OBR) selectively in POMC neurons have strain-dependent glucose intolerance [182, 183]. Conversely, OBR reactivation in POMC neurons in OBR null mice normalized hepatic glucose production at a time point when their body weight was similar to that of whole-body *db* (leptin receptor null) mice [184, 185], indicating that POMC neuron OBRs can regulate glucose homeostasis independently of body weight. However, *db* mice produce very high levels of leptin [186, 187]; thus, activated POMC OBRs may reverse hyperglycemia only in response to abnormally strong signaling. Lower leptin levels could still inhibit the release of AgRP from AgRP neurons without greatly increasing the α -MSH release from POMC neurons. Indeed, a recent study provides strong evidence that AgRP neurons may have a dominant effect on glucose regulation by controlling glucagon production. In *ob* mice (which cannot produce leptin), deletion of leptin receptors from AgRP neurons, but not from POMC neurons, prevented the ability of low doses of leptin to correct hyperglycemia. Further, AgRP action on MCRs, but not NPY or GABA release by these neurons, mediated this effect [187]. Thus, although both AgRP and POMC neurons are involved in leptin's glucose-lowering actions in diabetic states, only AgRP neurons are required for these actions.

Melanocortin signaling in the LHA has recently been shown to regulate glucose tolerance and sympathetic nerve activity. Restoring expression of MC4Rs specifically in the LHA improves glucose intolerance in obese MC4R null mice without affecting body weight. This effect results from increased sympathetic nerve activity leading to increased uptake of Glut4 expression and glucose uptake by interscapular brown adipose tissue (BAT) (Fig. 1d) [188].

Role in Energy Expenditure

The melanocortin system also influences caloric expenditure. Several mechanisms involving heat generation, cellular metabolism, and locomotor activity have the potential to underlie this effect. The MC4Rs in the PVH do not mediate melanocortin's actions on energy expenditure [79]. Rather, these functions involve melanocortin-responsive autonomic neurons [189].

Melanocortins appear to induce a dual effect on body temperature; a well-studied, prolonged induction of hyperthermia follows a newly identified, rapid reduction in body temperature. The latter seems to be independent of MC4Rs and possibly dependent on a similar biphasic effect on heart rate [190, 191]. BAT plays a crucial role in adaptive thermogenesis in response to diet or environmental challenges. Chemogenetic stimulation of POMC neurons or their exposure to insulin and leptin promotes the conversion of white adipose tissue into BAT via sympathetic activation [192]. Melanocortins also modulate sympathetic outflow to affect BAT metabolism in order to promote energy expenditure [193]. Conversely, when administered to the dorsomedial nucleus of the hypothalamus, AgRP blunts the thermogenic effect of melanotan II [194]. ARC POMC neurons project directly to the IML, where they activate sympathetic preganglionic fiber cell bodies (Fig. 1e) [18, 32]. The IML in turn projects to postganglionic neurons innervating BAT [195–197]. Indeed, MC4Rs in sympathetic preganglionic neurons are required to regulate energy expenditure, including heat generation in response to excess caloric intake or cold exposure and conversion of white adipose tissue into thermogenic fat [178].

Another mechanism for controlling energy expenditure is modulating cellular energy use through the hypothalamic-pituitary-thyroid axis. Thyroid hormone signaling acts in concert with SNS stimulation to promote adaptive thermogenesis in BAT and to affect other tissues including white adipose tissue and skeletal muscle [198].

In general, hypothalamic melanocortins are believed to stimulate the thyroid axis, while AgRP inhibits it. As previously mentioned, AgRP null mice exhibit increased circulating thyroid hormone levels, leading to an increased metabolic rate and body temperature [65]. While α -MSH has the ability to increase thyrotropin production when infused intracerebroventricularly [199–201], MC4R mutations in humans have no impact on free thyroxin levels, although a tendency in some patients to paradoxically have higher thyrotropin levels has been observed [202].

MC4R is not required for the fasting-induced suppression of the central axis [203]. Interestingly, MC4R signaling regulates hepatic thyroid hormone metabolism during fasting – although whether this effect is direct or mediated by the CNS is not known [203]. Thus, melanocortin signaling indirectly lowers thyroid hormone levels and the metabolic rate during fasting by accelerating T_4 clearance.

The extent to which caloric expenditure through locomotor activity contributes to body weight in rodents is debated [204, 205]. In general, locomotion is initiated by the mesencephalic locomotor region (MLR) and its projections to the medial reticular formation (RF) in the lower brainstem (Fig. 1f). Signals relayed from the RF to the locomotor central pattern generator in the spinal cord trigger locomotor patterns. The MLR receives inputs from the thalamus and the basal ganglia. The latter select desired movements and influence voluntary exercise such as exploratory, spontaneous, conditioned, and drug-induced locomotor activity [206, 207]. The motor cortex in the forebrain can also drive locomotion via reciprocal connections with the thalamus and basal ganglia. It may also bypass these structures and communicate directly with the locomotor central pattern generator [208]. In addition, the ARC projects to the MLR via the periaqueductal gray and may also project directly to the RF and indirectly to the RF via the LHA or NA [141, 209].

Locomotor activity can involve pursuit of food when calories are needed or pursuit of nonnutritive activities during times of energy surplus. Decreased circulating levels of leptin, as would be seen during a famine, reduce general locomotor activity while increasing food-seeking/anticipatory behavior [210]. Indeed, leptin increases general locomotor activity, and this action requires arcuate neuronal targets [210, 211]. Evidence suggests an altered melanocortin release is involved in this effect. Deletion of leptin receptors on POMC neurons reduces locomotor activity [182]. In addition, restoring leptin receptors only in POMC neurons in *db* mice normalizes locomotor activity but not body weight [185]. Further-

more, locomotor activity of young, nonobese, but hyperleptinemic MC4R null males is significantly lower than that of wild-type males [212]. Therefore, melanocortin signaling via MC4Rs may underlie leptin's ability to promote general locomotor activity. In contrast, MC3R signaling may promote food anticipatory activity in a leptin-independent manner; MC3R null mice exhibit decreased food-anticipatory activity [213], but deleting MC3Rs in *ob* mice does not change their elevated food-seeking behavior.

Dietrich et al. [214] recently used a chemogenetic approach to demonstrate that activation of AgRP neurons in the absence of food triggers foraging and repetitive behaviors, which are reverted by food consumption. These stereotypic behaviors are coupled with an increased willingness to explore normally fear- or anxiety-provoking environments. These effects are most likely not due to altered melanocortin signaling, since NPY5 receptor signaling is necessary to mediate the repetitive behaviors after AgRP neuron activation [214]. As previously described, inhibiting AgRP activity provides a way to rapidly halt the energy-intensive activity of foraging when food is found [73].

Conclusions

The breadth of the physiological roles served by the melanocortin system is often overlooked. As we have described, this system controls many aspects of metabolism that can contribute to obesity and its comorbidities. Indeed, the metabolic syndrome may have a dysfunction of the melanocortin system at its core.

The ability of melanocortins to suppress feeding and increase energy expenditure has made MCRs the focus of the search for an antiobesity treatment [215]. In addition, the work described above showing that the melanocortin system influences circulating glucose levels has generated great interest in it as a potential target for treatment of obesity-related type 2 diabetes [216]. Not surprisingly, MCR-targeted drugs have been plagued by problematic side effects, including undesirable increases in SNS activity, heart rate, and blood pressure [158, 217]. Again, these outcomes are not off-target effects, but rather main physiological functions of melanocortins. These problems have thus far prevented the use of antiobesity drugs that target the activation of MC4R in the clinic [154, 218].

Recent work suggests one possible approach for circumnavigating this roadblock. MCRs are generally coupled to $G_s\alpha$ proteins that increase intracellular cAMP and

activate protein kinase A. $G_s\alpha$ does not mediate all of the actions of MC4R, however [219]. Recent studies also suggest that melanocortins act through $G_{q/11}\alpha$ pathways in the PVH to regulate food intake, linear growth, cholesterol metabolism, and *Crh* gene expression [220]. If, as suggested by these studies [220, 221], heart rate and blood pressure are mediated by $G_s\alpha$, a $G_{q/11}\alpha$ -biased MC4R agonists may be a potential treatment for obesity.

If this approach fails, a clearer picture of the precise neural circuits that mediate each of the actions of melanocortins may provide solutions. As we have seen, the evidence to date suggests that downstream melanocortin-responsive pathways do diverge and may be separable in animal models by using technologies to target specific second-order neuronal populations that express unique neuropeptides or other markers. Targeting pharmacological strategies to particular MCR-expressing circuits in humans, however, is much more challenging. Ultimately, the development of drugs free of unwanted side effects may require identifying other targets in second- or third-order neurons rather than MCRs themselves.

The ongoing effort to understand the function of these circuits forms part of the larger push within neuroscience to develop a more integrated view of circuit activity. Unraveling the complexity of neural communication in real time is a massive task. Technical advances in being able to manipulate neuronal activity in awake, behaving animals as well as new approaches to monitoring and interpreting neuronal activity as it occurs offer tremendous benefits. However, the need for neuroanatomy assisted by well-designed neural tracers, traditional electrophysiology, and purely genetic approaches will remain. Ultimately, a more complete understanding of melanocortin pathways and their myriad functions will allow treatments tailored to the mix of metabolic disorders in the individual patient.

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