REVIEW

The melanocortin pathway and control of appetite-progress and therapeutic implications

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Abstract

The initial discovery that *ob/ob* mice become obese because of a recessive mutation of the leptin gene has been crucial to discover the melanocortin pathway to control appetite. In the melanocortin pathway, the fed state is signaled by abundance of circulating hormones such as leptin and insulin, which bind to receptors expressed at the surface of pro-opiomelanocortin (POMC) neurons to promote processing of POMC to the mature hormone α-melanocyte-stimulating hormone (α-MSH). The α-MSH released by POMC neurons then signals to decrease energy intake by binding to melanocortin-4 receptor (MC4R) expressed by MC4R neurons to the paraventricular nucleus (PVN). Conversely, in the 'starved state' activity of agouti-related neuropeptide (AgRP) and of neuropeptide Y (NPY)-expressing neurons is increased by decreased levels of circulating leptin and insulin and by the orexigenic hormone ghrelin to promote food intake. This initial understanding of the melanocortin pathway has recently been implemented by the description of the complex neuronal circuit that controls the activity of POMC, AgRP/NPY and MC4R neurons and downstream signaling by these neurons. This review summarizes the progress done on the melanocortin pathway and describes how obesity alters this pathway to disrupt energy homeostasis. We also describe progress on how leptin and insulin receptors signal in POMC neurons, how MC4R signals and how altered expression and traffic of MC4R change the acute signaling and desensitization properties of the receptor. We also describe how the discovery of the melanocortin pathway has led to the use of melanocortin agonists to treat obesity derived from genetic disorders.

Key Words
▶ melanocortin
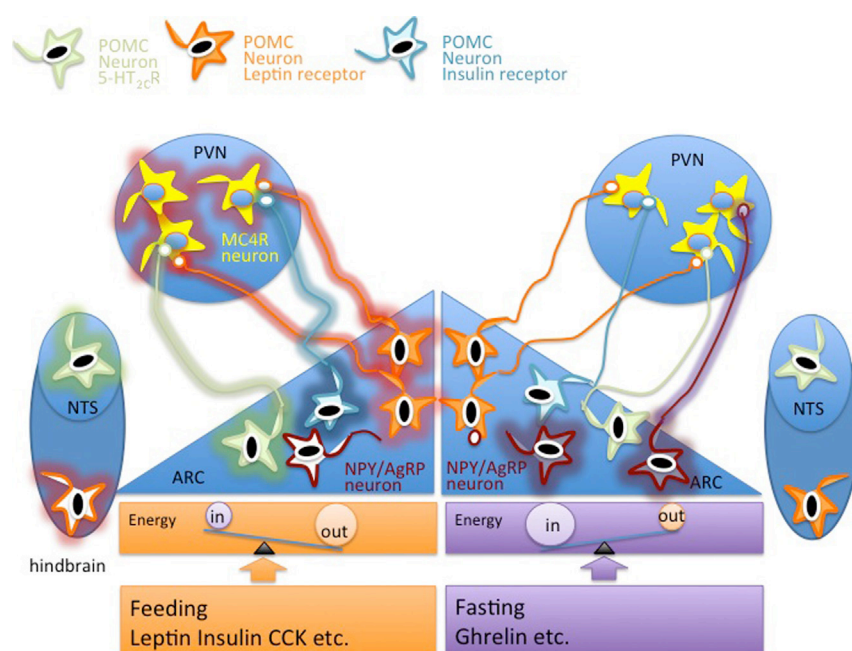▶ leptin
▶ hypothalamus
▶ MC4R
▶ AgRP
▶ appetite

Introduction

In the melanocortin system, hormones of the 'fed state' such as leptin and insulin, released in the bloodstream by adipocytes and by the β-cells of the pancreas, respectively, cross the blood-brain barrier to bind to leptin and insulin receptors on the surface of pro-opiomelanocortin (POMC) neurons to promote processing of POMC to the mature hormone α-melanocyte-stimulating hormone (α-MSH), which signals to decrease energy intake (Cone 2006, Ghamari-Langroudi *et al.* 2011, Morton *et al.* 2014, Gautron *et al.* 2015, Andermann & Lowell 2017).

In the fed state, leptin also binds to leptin receptors to inhibit secretion of AgRP and of neuropeptide Y (NPY) expressed by AgRP/NPY neurons. Conversely, in the 'starved state' AgRP/NPY neuron activity is increased by decreased circulation of leptin and insulin and by the orexigenic hormone ghrelin. Both POMC and AgRP/NPY neurons have their cell bodies in the arcuate nucleus of the hypothalamus and their axons project to the PVN. In the melanocortin pathway to regulate feeding, hypothalamic POMC neurons receive inhibitory signals

from cholinergic neurons localized to the dorsomedial hypothalamus (DMH) that project to the arcuate nucleus (Jeong *et al.* 2017), as well as from excitatory glutamatergic signals from steroidogenic factor (SF-1)-expressing neurons localized to the ventromedial hypothalamus (VMH) (Konner & Bruning 2012). To control feeding, AgRP/NPY neurons project in addition to the PVN to other brain regions such as the stria terminalis (BNST), the paraventricular nucleus of the thalamus (PVT) and the lateral hypothalamus (LH) (Betley *et al.* 2013). In the PVN, α-MSH released by POMC neurons interacts with melanocortin-4 receptor (MC4R) expressed by MC4R neurons to stabilize the receptor in an active conformation, with Gαq-mediated increase of intracellular calcium and decrease in food intake. AgRP antagonizes effects by α-MSH and also acts as inverse agonist to inhibit the constitutive activity of MC4R taking place in the absence of agonist. MC4R neurons localized to the brainstem and to the spinal cord signal by Gs-dependent increase of intracellular cAMP to increase energy expenditure (Li *et al.* 2016). MC4R neurons also signal by inducing, in a G-protein independent manner, closure and opening of the inwardly rectifying potassium channel, Kir7, in response to alpha-MSH and AgRP, respectively, thus modulating the firing activity of PVN neurons (Ghamari-Langroudi *et al.* 2015). In this review, we will describe the role of POMC, AgRP and MC4R/MC3R neurons in the melanocortin system to control appetite and weight as well therapeutic implications.

POMC neurons localized in the hypothalamus and hindbrain are essential for energy homeostasis

POMC neurons localized to the hypothalamus and to the hindbrain are essential for energy homeostasis (Mercer *et al.* 2013, Gautron *et al.* 2015, Dores *et al.* 2016, Toda *et al.* 2017, Caron *et al.* 2018) (Fig. 1). In POMC neurons, the POMC gene encodes a precursor polypeptide that undergoes cell-specific proteolytic cleavage to generate α-MSH (Toda *et al.* 2017). Mutations of POMC gene in both mice and humans lead to hyperphagia and obesity (Yaswen *et al.* 1999, Challis *et al.* 2004, Muller *et al.* 2016). Injury to POMC neurons such as that derived by ablation of the mitochondrial protein mitofusin 2 or that by increased secretion of tumor necrosis factor alpha (TNFα) from microglia in obesity also leads to disrupted energy balance with increased food intake (Schneeberger *et al.* 2013, Yi *et al.* 2017). Consistent with a role for POMC neurons in energy balance, postnatal ablation of POMC neurons in mice induces an obese phenotype. However, in mice with postnatal ablation of POMC neurons, obesity is unrelated to food intake and instead dependent on decreased energy expenditure (Greenman *et al.* 2013). POMC neuron activity is regulated by other neurons. In this respect, under fasting conditions, POMC neurons receive inhibitory inputs from AgRP/NPY neurons (Fig. 1) (Horvath *et al.* 1992, Cowley *et al.* 2001, Pinto *et al.* 2004, Cone 2006). Other inhibitory inputs originate from noradrenergic neurons localized to the locus

Figure 1

POMC neurons in the melanocortin system. In the fed state, the signal to stop eating and to increase energy expenditure is conveyed by leptin and insulin released in the bloodstream by adipocytes and by the β-cells of the pancreas, respectively. These hormones cross the blood-brain barrier to reach the arcuate nucleus (ARC) of the hypothalamus and promote firing (indicated by glow around the cell perimeter) of distinct populations of POMC neurons expressing the LepR and insulin receptor. Other populations of POMC neurons in the arcuate nucleus and in the nucleus of the solitary tract (NTS) express the serotonin receptor 5-HT$_{2c}$R. POMC neurons project to the paraventricular nucleus (PVN) to increase activity of MC4R neurons to decrease food intake and to increase energy expenditure. In the fasted state, POMC neurons in the arcuate nucleus are inhibited by decreased circulating leptin and insulin and by increased activation of AgRP/NPY neurons, which send inhibitory signals to reduce firing of POMC neurons and of MC4R neurons. References are in the main text.

coeruleus and the hindbrain that send projections to the hypothalamus (Bouret & Richmond 2015, Varazzani *et al.* 2015). Hypothalamic POMC neurons also receive inhibitory signals from cholinergic neurons localized to the dorsomedial hypothalamus (DMH) that project to the arcuate nucleus (Jeong *et al.* 2017). The activity of hypothalamic POMC neurons is also modulated by sex hormones. In this respect, POMC neurons in the arcuate nucleus are responsive to estradiol administration to reduce food intake and body weight (Steyn *et al.* 2018).

POMC neurons and leptin signaling

POMC neurons are activated by leptin, a peptide hormone secreted by adipocytes, and more efficiently so by subcutaneous fat rather than by the omental fat and in a manner proportional to adipocyte size (Masuzaki *et al.* 1995, Van Harmelen *et al.* 1998). A pioneering discovery in the control of energy balance was the finding that mutations in the *ob/ob* mice and *db/db* mice induce obesity (Coleman 1973, 1978). It was later found that the *ob/ob* mice become obese because of a recessive mutation of the leptin gene (Zhang *et al.* 1994). Also, patients with leptin deficiency due to mutations of the leptin gene are obese. Although such leptin deficiency is rare in the population, it provides evidence that the hormone is essential for energy homeostasis in humans (Montague *et al.* 1997, Clement *et al.* 1998). Additional evidence that leptin is essential for energy homeostasis is the finding that delivery of leptin to mice and to humans with leptin deficiency corrects obesity (Halaas *et al.* 1995, Pelleymounter *et al.* 1995, Farooqi *et al.* 1999). The identification of the leptin receptor (LepR) stemmed from the observation that iodinated leptin binds to the brain choroid plexus (Devos *et al.* 1996). The LepR was cloned from a cDNA expression library derived from the murine choroid plexus and found to belong to the IL-6 receptor family (Tartaglia *et al.* 1995). However, both obese *db/db* mice and fatty Zucker rats, which have elevated circulating leptin, have normal binding of leptin in the choroid plexus, comparable to that of lean rodents (Halaas *et al.* 1995, Devos *et al.* 1996). It was later discovered that the LepR has multiple splice forms and that the *db/db* mice and fatty Zucker rats become obese because of mutations which affects the intracellular domain of the long form of receptor, LepR, which is expressed in the hypothalamus including the POMC neurons (Chen *et al.* 1996, Chua *et al.* 1996, Iida *et al.* 1996, Lee *et al.* 1996, Phillips *et al.* 1996, Takaya *et al.* 1996, Cheung *et al.* 1997). Conversely,

the short form of LepR, expressed in the choroid plexus, can bind to leptin, but cannot signal (Bjorbaek *et al.* 1997, Ghilardi & Skoda 1997).

Administration of leptin to brain slices induces depolarization of POMC neurons and reduces inhibition of POMC neurons by the AgRP/NPY neurons (Cowley *et al.* 2001). Deletion of LepR specifically in POMC neurons by using the Cre/Lox system finds that expression of LepR in these neurons is essential to body weight homeostasis (Balthasar *et al.* 2004). Interestingly, it has been recently found that POMC neurons expressing LepR are required for the fasting-induced fall in leptin levels (Caron *et al.* 2018). LepR signals through multiple pathways (Fig. 2A and B) (Flak & Myers 2016, Toda *et al.* 2017, Wauman *et al.* 2017). During leptin signaling, LepR, expressed at the plasma membrane as a dimer, activates receptor-associated Janus kinase 2 (JAK2) to phosphorylate LepR at Tyr_{1138}, which then binds to signal transducer and activator of transcription 3 (Stat3). Stat3 is then phosphorylated by JAK2 to function as transcription factor (Bjorbaek *et al.* 1997, Ghilardi & Skoda 1997, Li & Friedman 1999, Banks *et al.* 2000, Bahrenberg *et al.* 2002). Activation of Stat3 by LepR is essential to control food intake (Bates *et al.* 2003, Cui *et al.* 2004, Gao *et al.* 2004, Buettner *et al.* 2006, Zhang & Scarpace 2009). Binding of leptin to LepR also leads to downstream activation of Rho-kinase 1 (ROCK1), which phosphorylates and activates JAK2 in a pathway that is essential for leptin signaling to maintain energy homeostasis (Huang *et al.* 2012). Binding of leptin to LepR also leads to JAK2 interaction with SH2-Bβ, which in turn promotes insulin receptor substrate 1 (IRS1)- and IRS2-mediated activation of the phosphatidylinositol 3-kinase (PI3K) pathway (Kellerer *et al.* 1997, Kim *et al.* 2000, Anderwald *et al.* 2002, Carvalheira *et al.* 2003, Duan *et al.* 2004, Li *et al.* 2007, Wauman *et al.* 2017). The PI3K signaling pathway is essential for leptin-induced depolarization and firing of POMC neurons (Hill *et al.* 2008, Kwon *et al.* 2016). PI3K pathway also promotes phosphorylation and translocation of forkhead box protein O1 (FOXO1) from the nucleus to the cytosol, an effect that promotes transcription of POMC and increased expression of carboxypeptidase E (CPE) with increased processing of POMC to α-MSH, and suppression of food intake (Kim *et al.* 2006, Plum *et al.* 2009, Kwon *et al.* 2016). The PI3K signaling pathway to control food intake and weight gain includes the atypical protein kinase C λ (aPKC λ) (Dorfman *et al.* 2017*b*). An effect by leptin is to induce POMC neuron depolarization through a cation channel (Cowley *et al.* 2001). In this respect, leptin-dependent signaling through the PI3K pathway activates
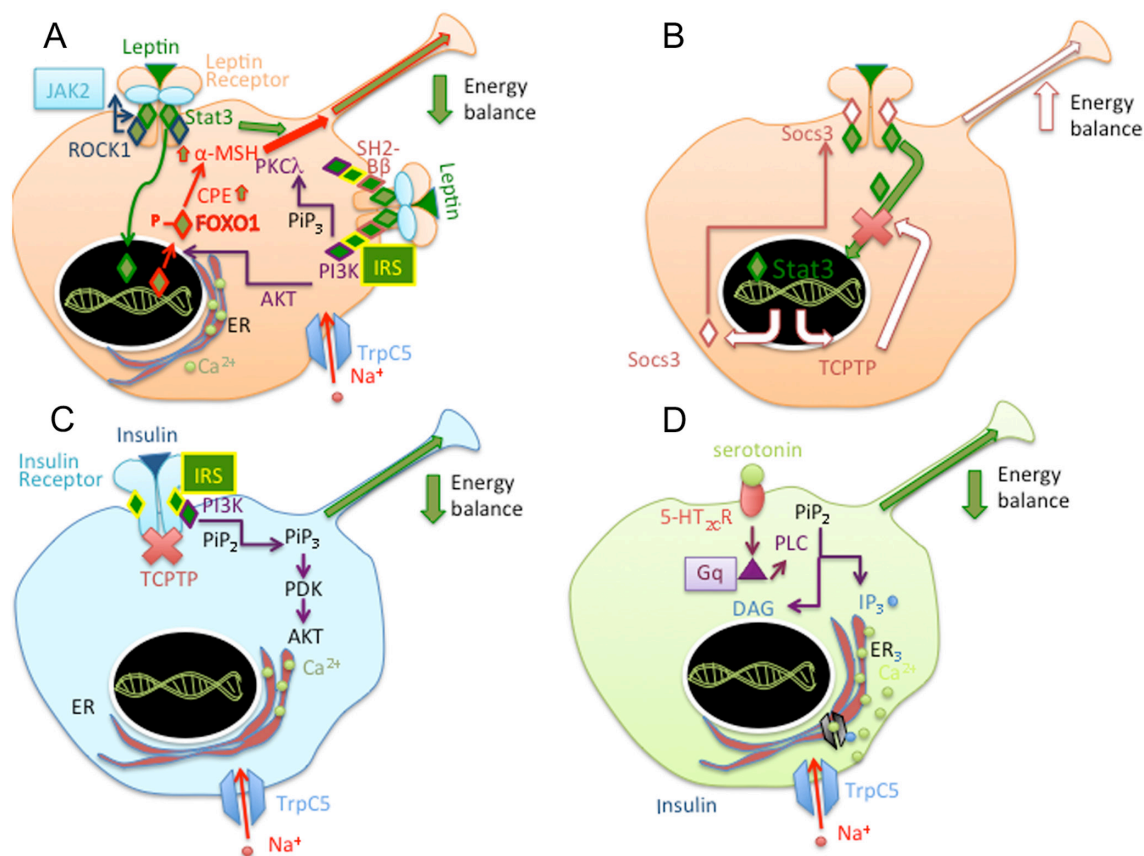
Figure 2

POMC neurons express leptin, insulin and serotonin receptors. (A) In the fed state, leptin bound to LepR expressed by POMC neurons in the arcuate nucleus and release of α-MSH hormone by multiple pathways initiated by activation of JAK2, a process that involves LepR-dependent activation of ROCK1. In one pathway initiated by JAK2, the kinase phosphorylates STAT3 to function as transcription factor. STAT3 promotes expression of the polypeptide POMC and processing of the pro-hormone to α-MSH. Binding of leptin to LepR induces another JAK2-dependent pathway where SH2-Bβ and IRS1 are recruited to activate the PI3K pathway. PI3K generates PIP_3 from PIP_2 at the plasma membrane. PIP_3 recruits and activates of PKC including the atypical PKCλ. PI3K signaling leads to opening of TrpC5 to allow inward flux of Na^+ and neuronal firing. PI3K pathway also promotes phosphorylation and translocation of FOXO1 from the nucleus to the cytosol to promote transcription of POMC, increased processing of POMC to α-MSH and suppression of food intake. (B) Stat3 induces expression of factors involved in feedback inhibitory pathways, such as that of Socs3, which binds to LepR to inhibit receptor signaling. Stat 3 also induces expression of protein phosphatases such as TCPTP to terminate LepR to inhibit receptor signaling. (C) In other populations of POMC neurons, the insulin receptor signals through PI3 kinase pathway to induce flux of Na^+ into the cell through TrpC5 and neuronal firing. (D) A population of POMC neurons expresses the GPCR 5-HT$_{2C}$R. Binding of serotonin to HT$_{2C}$R induces Gq-dependent activation of PLC, generation of increased intracellular IP_3 and Ca^{2+}, and opening of TrpC5 to allow Na^+ into the cell and neuronal firing. Heterogeneity of POMC neurons expressing insulin, leptin and serotonin receptor is indicated by drawing cells expressing these receptors with different colors. References are in the main text.

the transient receptor potential cation 5 (TrpC5) and this effect is essential to decrease food intake and increase energy expenditure (Qiu *et al.* 2014, Gao *et al.* 2017).

The binding of leptin to LepR induces phosphorylation of the receptor also at Tyr$_{985}$, which controls phosphorylation of the protein tyrosine phosphatase SHP-2, downstream activation of extracellular-regulated kinases-1/2 (ERK1/2), and increased expression of cfos (Li & Friedman 1999, Banks *et al.* 2000, Bjorbak *et al.* 2000). An effect of STAT3 activation by the occupied LepR is to initiate the feedback inhibitory pathway to induce expression of suppressor of cytokine signaling-3 (Socs3)

mRNA (Fig. 2B). Socs3 inhibits LepR signaling by binding to LepR Tyr$_{985}$ (Bjorbak *et al.* 2000, Eyckerman *et al.* 2000). Socs3 expression may contribute to increased food intake during pregnancy (Zampieri *et al.* 2016). Other mechanisms to inhibit leptin signaling in feedback inhibitory pathway take place by dephosphorylation of LepR by the protein tyrosine phosphatases 1B (PTP1B) and the STAT-1 and STAT-3 phosphatase T-cell protein tyrosine phosphatase (TCPTP) (Zabolotny *et al.* 2002, White *et al.* 2009, Loh *et al.* 2011, Tsou *et al.* 2012). It has been recently proposed that leptin and insulin act on hypothalamic POMC neurons to increase energy expenditure by a pathway that involves

PTP1B and TCPTP, and leads to increased browning of white adipose tissue (Dodd *et al.* 2015, Zhang *et al.* 2015). While deletion of LepR in POMC neurons has mild effects on body weight (Balthasar *et al.* 2004), deletion of LepR in large populations of hypothalamic regions produces profound obesity and metabolic dysfunction (Rupp *et al.* 2018). It is likely that LepR expressed in other neuronal populations outside of the arcuate nucleus contribute to energy homeostasis. LepR is expressed by neurons localized to the hindbrain and to the brainstem (Barnes *et al.* 2010). LepR expressed in these brain regions contributes to energy homeostasis by controlling meal size (Kanoski *et al.* 2012) and mediates counter-regulatory responses to hypoglycemia during starvation (Flak *et al.* 2014, Flak & Myers 2016). POMC neurons in the LH, brainstem and hindbrain express glucose transporter type 2 (Glut2). In these POMC neurons outside of the arcuate nucleus, Glut2-dependent glucose sensing functions to control thermoregulation by increasing leptin sensitivity (Mounien *et al.* 2010). LepR expressed by non-neuronal cells such as the microglia is also implicated in control of energy homeostasis (Gao *et al.* 2018).

POMC neuron and signaling in response to feeding, insulin and serotonin

Insulin functions to regulate energy homeostasis in POMC neurons by signaling through receptors expressed in a different population of hypothalamic POMC neurons than that expressing LepR (Williams *et al.* 2010) (Fig. 2A and C). In the hypothalamus, insulin induces the association of PI3K with IRS-2, to promote Ser473 phosphorylation of Akt (Torsoni *et al.* 2003, Dodd & Tiganis 2017, Haeusler *et al.* 2018). Brain IRS-2 signaling functions in energy homeostasis (Taguchi *et al.* 2007) and IRS-2 signaling in POMC neurons controls blood pressure and heart rate (do Carmo *et al.* 2016). Insulin signal in POMC neurons suppresses appetite by a pathway that is similar to that of leptin because it also involves activation of PI3K (Xu *et al.* 2005b) (Hill *et al.* 2008) (Al-Qassab *et al.* 2009) and opening of TrpC5 with inward flow of Na$^+$ (Qiu *et al.* 2018b).

Feeding modulates termination of insulin signaling in POMC neurons. The protein phosphatase TCPTP dephosphorylates the insulin receptor and attenuates insulin signaling (Tiganis 2013). Feeding, by decreasing abundance of TCPTP, suppresses termination of insulin signal in POMC neurons. Conversely, fasting, by increasing TCPTP, promotes the termination of insulin signaling in POMC neurons (Dodd *et al.* 2018b). Thus, nutritional status modulates insulin responsiveness in POMC neurons. With this respect, hypothalamic POMC neurons are glucose sensitive and increase their firing rate in response to increased extracellular glucose concentration by a mechanism that involves increased ATP/ADP ratio, closure of K$^+$ ATP channels and cell depolarization (Ibrahim *et al.* 2003, Parton *et al.* 2007). Feeding is associated with firing of POMC neurons, an effect paralleled by increased generation of reactive oxygen species (ROS) in these neurons (Andrews *et al.* 2008, Horvath *et al.* 2009, Diano *et al.* 2011). Moreover, central delivery of ROS promotes firing by POMC neurons, and suppression of ROS formation in POMC neurons inhibits their activity (Diano *et al.* 2011). Therefore, nutrient sensing in POMC neurons appears to be mediated by the changes in ROS generation. Generation of ROS takes place prevalently in mitochondria, which undergo fission/fusion cycles in POMC neurons, depending on availability of nutrients (Dietrich *et al.* 2013, Schneeberger *et al.* 2013, Toda *et al.* 2016). Dynamin-related protein (Drp1) functions in mitochondrial fission (Nasrallah & Horvath 2014). In response to feeding, the activation of Drp1 is decreased, resulting in increased mitochondrial size, increased generation of ROS and neuronal activation (Santoro *et al.* 2017). These data indicate that changes in mitochondrial size in POMC neurons modulate neuronal activation by altering generation of ROS. Another population of hypothalamic POMC neurons that regulates both energy and glucose homeostasis has been found to express the serotonin (5-HT) receptor 2C receptor (5-HT2CR), which signals to induce activation of TrpC5 and of the mammalian target of rapamycin (mTOR) pathway (Fig. 2D) (Churruca *et al.* 2008, Lam *et al.* 2008, 2010, 2011, Sohn *et al.* 2011, Sohn & Williams 2012, Berglund *et al.* 2013, Gao *et al.* 2017, Barone *et al.* 2018). Consistent with the concept that different POMC neuron populations express LepR, insulin receptor and 5-HT2CR, single cell RNA sequencing analysis indicates that the population of POMC neurons residing in the arcuate nucleus is highly heterogeneous (Lam *et al.* 2017). Interestingly, estradiol increases the excitability of POMC neurons by increasing the efficacy by which insulin activates canonical TrpC5 channels (Qiu *et al.* 2018a). In the face of insulin and leptin affecting only a subpopulation of POMC neurons, noradrenaline instead decreases the activity of the POMC neurons through signaling by the α_{2A} adrenergic receptor in a large population of POMC neurons, perhaps to promote food intake in response to challenges that require energy (Paeger *et al.* 2017a). In addition to the POMC neurons

of the hypothalamus, other POMC neurons expressing 5-HT2CR may contribute to regulate appetite. In this respect, POMC neurons responsive to 5-HT2CR agonist localized to the nucleus of the solitary tract (NTS) control food intake (D'Agostino et al. 2018).

AgRP/NPY neurons promote feeding and function in adapted behavior under starvation

AgRP was originally identified as a peptide expressed by neurons in the mediobasal hypothalamus, which acts as antagonist of MC4R and of another member of the melanocortin receptor family expressed in brain, MC3R (Ellacott & Cone 2004). Ubiquitous expression of AgRP in transgenic mice induces obesity (Ollmann et al. 1997). In the hypothalamus, AgRP-expressing neurons co-express NPY and respond to orexigenic and anorexigenic signals from the periphery to regulate feeding (Broberger et al. 1998, Hahn et al. 1998, Cowley et al. 1999, van den Top et al. 2004). Central delivery of AgRP induces increased feeding (Joppa et al. 2007). Food deprivation induces increased expression of NPY and AgRP mRNA in the AgRP/NPY neurons, while refeeding restores levels of these peptides (Swart et al. 2002). In humans, abundance of AgRP and NPY correlates with body mass index (Alkemade et al. 2012). Food deprivation increases

activity of AgRP/NPY neurons (Takahashi & Cone 2005). Nutrients are necessary and sufficient for the reduction of AgRP/NPY neuron activity and this effect is proportional to the amount of calories being obtained (Betley et al. 2013, Mandelblat-Cerf et al. 2015, Chen et al. 2015b, Su et al. 2017). AgRP/NPY neurons can also control feeding under conditions of appetite suppression (Padilla et al. 2016). The activity of AgRP/NPY neurons is modulated within the brain by excitatory neurons that originate from the PVN and express thyrotropin-releasing hormone (TRH) (Krashes et al. 2014), by excitatory glutamatergic inputs (Liu et al. 2012), and by postsynaptic AMPK-dependent synaptogenesis and spinogenesis (Kong et al. 2016).

Projections from the AgRP/NPY neurons converge with those from POMC neurons to MC4R neurons in the PVN to integrate control food intake and energy expenditure (Fig. 3) (Cowley et al. 1999, 2001, Aponte et al. 2011, Atasoy et al. 2012, 2014). AgRP/NPY neurons project also to POMC neurons of the arcuate nucleus and to neurons localized to the dorsomedial nucleus of the hypothalamus and to the rostral telencephalon and to the pons to control feeding (Broberger et al. 1998, Bagnol et al. 1999, Haskell-Luevano et al. 1999, Legradi & Lechan 1999, Singru et al. 2007). AgRP/NPY neurons also project to BNST and to the lateral hypothalamic area to control, in addition to feeding, also insulin sensitivity in brown adipose tissue (Steculorum et al. 2016) (Fig. 3). Under starvation, projections of AgRP/NPY neuron to the BNST,
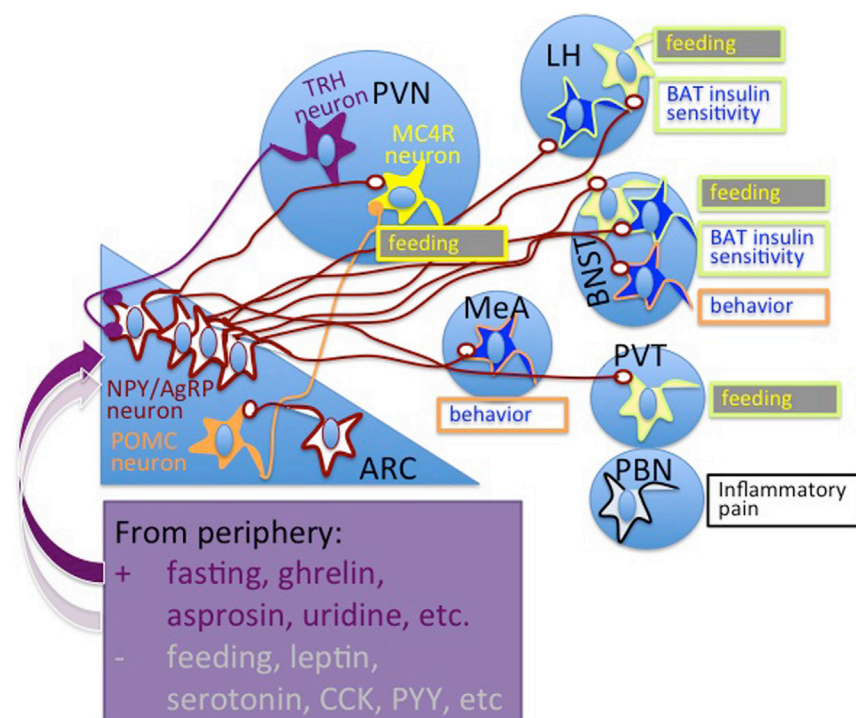
Figure 3

AgRP/NPY neurons drive food intake. Fasting and circulating hormones released by the stomach induce activity of AgRP/NPY neurons localized to the arcuate nucleus (ARC) of the hypothalamus. To promote feeding, subpopulations of AgRP/NPY neurons send projections to: the paraventricular nucleus of hypothalamus (PVN), to synapse with MC4R neurons; and to neurons in the lateral hypothalamus (LH), bed nucleus of the stria terminalis (BNST) and the paraventricular nucleus of the thalamus (PVT). Other projections to neurons in LH, medial amygdala (MeA), LH and parabrachial nuclei control insulin sensitivity in brown adipose tissue (BAT) and suppress inflammatory pain in hunger condition. 'Behavior' refers to the behavior induced by the nutritional status of the organism such as modulation of aggression, fear and exploration to find food. References are in the main text.

to the medial nucleus of the amygdala (MeA), control adapted behavior such as modulation of aggression, fear and exploration to find food by releasing NPY (Betley *et al.* 2013, Dietrich *et al.* 2015, Burnett *et al.* 2016, Padilla *et al.* 2016). During hunger, AgRP neuron projections to the PBN inhibit inflammatory pain by signaling through NPY (Alhadeff *et al.* 2018). During satiety, activation of AgRP neurons mimics hunger by a pathway to the insular cortex via the paraventricular thalamus (PVT) and basolateral amygdala (Livneh *et al.* 2017).

Fast and slow signals to induce feeding originate from subpopulations of arcuate nucleus AgRP/NPY neurons and by GABAergic neurons of the LH

Specific activation of AgRP/NPY neurons by photostim-ulation of channelrhodopsin-2 evokes a voracious feeding response within minutes (Aponte *et al.* 2011). Subpopulations of AgRP/NPY neurons appear to be sufficient to promote food intake and do so by redundant pathways (Betley *et al.* 2013). In addition to AgRP and NPY, AgRP/NPY neurons release GABA, which is essential to energy homeostasis (Tong *et al.* 2008, Wu & Palmiter 2011, Krashes *et al.* 2013). Feeding responses evoked by designer receptors exclusively activated by designer drugs (DREADD) technology activation indicate that NPY and GABA released by AgRP/NPY neurons, convey fast signals to induce feeding, while AgRP instead induces feeding with effects that are delayed and more prolonged as compared to those by NPY and GABA (Krashes *et al.* 2011, 2013, 2016). Ablation of AgRP neurons results in starvation and activation of neurons in brain regions innervated by AgRP neurons (Wu *et al.* 2008). Conversely, experiments where the AgRP/NPY neurons were ablated by making them selectively sensitive to diphtheria toxin or by expression of a neurotoxic form of ataxin-3, indicate that AgRP/NPY neurons are essential to control feeding in the adult mice, but not in neonatal mice, and thus suggesting compensatory pathways to promote food intake (Bewick *et al.* 2005, Gropp *et al.* 2005, Luquet *et al.* 2005, Tan *et al.* 2014). Similarly, mice where a Cre-lox strategy was used to induce progressive degeneration of hypothalamic neurons that express AgRP are normal, again suggesting existence of compensatory pathways (Xu *et al.* 2005*a*). AgRP-null mice instead have normal food intake, body weight and energy expenditure with reduced body weight after 6 months of age (Wortley *et al.* 2005). Also transgenic mice with AgRP and NPY double-knockout have

normal body weight and feeding response to starvation (Qian *et al.* 2002), suggesting the existence of compensatory mechanisms to regulate energy homeostasis. In the hypothalamus other GABAergic neurons, besides the AgRP/NPY neurons, contribute to food intake. In this respect, feeding can also be induced by the GABAergic neurons localized to the LH and projecting to the PVN (Mangieri *et al.* 2018).

Different roles of AgRP/NPY neurons in homeostatic and hedonic feeding

In the brain, multiple pathways function to control food intake, of which one is homeostatic feeding and the other is non-homeostatic, hedonic feeding (Pandit *et al.* 2013, Sternson 2016). The brain reward circuit includes many brain areas, such as the ventral tegmental area (VTA), the nucleus accumbens, the LH, the amygdala, the striatum and the prefrontal cortex (Pandit *et al.* 2011, 2013). In mice, AgRP/NPY neurons transmit negative valence signals (Betley *et al.* 2015). When mice are fed normal chow, food caloric intake is the main parameter to decrease the activity of AgRP/NPY neuron activity and limit food intake (Su *et al.* 2017). Conversely, when mice are instead fed a highly palatable diet, AgRP/NPY neurons become dispensable for feeding and other neural circuits sensitive to emotion and stress take place to control food intake (Denis *et al.* 2015).

Signaling in AgRP/NPY neurons

It is well established that AgRP/NPY neurons are activated by fasting and by ghrelin and are inhibited by leptin and by feeding (Pinto *et al.* 2004, van den Top *et al.* 2004, Yang *et al.* 2011, Hashiguchi *et al.* 2017, Mani *et al.* 2018). However, the mechanism by which fasting promotes AgRP/NPY activation is yet to be completely understood. Ghrelin is a peptide hormone secreted by the stomach under conditions of food deprivation that stimulates food intake and adiposity (Tschop *et al.* 2000, Wren *et al.* 2000, Nakazato *et al.* 2001, Toshinai *et al.* 2001, Wren *et al.* 2001, Kim *et al.* 2003, Shaw *et al.* 2005, Yang *et al.* 2011). AgRP neurons express the ghrelin receptor, also named growth hormone secretagogue receptor (GHsr), to mediate orexigenic as well as glucoregulatory actions of ghrelin (Zigman *et al.* 2006, Wang *et al.* 2014, Mani *et al.* 2017). Experiments using brain slices indicate that GHsr is essential for ghrelin response to increase firing

activity of AgRP/NPY neurons (Chen *et al.* 2017*b*). Ghrelin induces activation of AgRP neurons by a mechanism that is dependent on mitochondrial uncoupling protein 2 (UCP2) and involves proliferation of mitochondria and increased mitochondrial respiration (Andrews *et al.* 2008, Horvath *et al.* 2009, Diano 2013). Such ghrelin-dependent activation of AgRP/NPY neurons is driven by increased β-oxidation of fatty acids, which takes place by a mechanism that involves activation of 5' AMP-activated protein kinase (AMPK). Active AMPK inhibits of acetyl-CoA carboxylase (ACC) activity resulting in decreased production of malonyl-CoA and increased fatty acid transport into the mitochondrial matrix by brain-specific carnitine palmitoyltransferase-1c (CPT-1c) (Price *et al.* 2002, Obici *et al.* 2003, Obici & Rossetti 2003, Horvath *et al.* 2009). Increased fatty acid oxidation is paralleled by increased mitochondrial respiration, with increased generation of ROS and ROS quenching by UCP2 (Andrews *et al.* 2008). These data indicate that mitochondrial function is essential for ghrelin signaling to reduce appetite. However, the function mitochondrial CPT-1c appears more complex than to promote food intake by ghrelin signaling in the AgRP/NPY neurons. This is because CPT-1c KO mice, while leaner than control mice, gain more weight upon exposure to high-fat diet than control mice, without increasing their food intake (Wolfgang *et al.* 2006, Wolfgang *et al.* 2008). The role of ghrelin to control energy homeostasis is not yet completely understood because mice with loss of function mutations of the ghrelin system do not have altered body weight but, instead, altered glucose metabolism and insulin sensitivity (Sun *et al.* 2003, 2008, De Smet *et al.* 2006, McFarlane *et al.* 2014, Mani & Zigman 2017). Other hormones, such as leptin and insulin, may control activity of AgRP/NPY neurons in feeding and metabolism. In this respect, leptin signals to reduce food intake by inducing exclusion of Foxo1 from the nucleus of AgRP/NPY neurons and downstream expression of a purinergic G-protein-coupled receptors (GPCR), Gpr17 (Kitamura *et al.* 2006, Ren *et al.* 2012, Ren *et al.* 2015). Insulin signaling also takes place in AgRP/NPY neurons to decrease their activity by inducing neuronal hyperpolarization. These effects control hepatic glucose production (Konner *et al.* 2007). Insulin signaling in AgRP/NPY neurons is suppressed by TCPTP. Fasting induces TCPTP in AgRP/NPY neurons (Dodd *et al.* 2018*a*). Feeding instead promotes the degradation of TCPTP. Reduced TCPTP enhances AgRP/NPY neuron insulin sensitivity and promotes downstream effects such as repression of hepatic gluconeogenesis (Dodd *et al.* 2018*a*). In addition to ghrelin, leptin and insulin, other factors

such as serotonin, cholecystokinin (CCK) and peptide YY (PYY), released by the gastrointestinal tract in the general circulation, mediate effects of feeding to inhibit AgRP/NPY neurons (Beutler *et al.* 2017). It has been found that asprosin, a hormone released by adipocytes under fasting conditions, in addition to stimulating glucose output from the liver (Romere *et al.* 2016), also crosses the blood-brain-barrier to activate the AgRP/NPY neurons via a cAMP-dependent pathway and to inhibit POMC neuron activity by a pathway that involves the release of GABA by the AgRP/NPY neurons (Duerrschmid *et al.* 2017). It has been recently discovered that, during fasting, plasma levels of the pyrimidine nucleoside uridine is increased, and that such elevated plasma uridine is required for the changes in thermoregulation and glucose metabolism that occur during fasting (Deng *et al.* 2017). In the brain, extracellular uridine is converted to UTP (Ipata *et al.* 2010). Interestingly, UDP activates AgRP/NPY neurons by a pathway that is initiated by purinergic receptor 6 (P2Y6) signaling (Steculorum *et al.* 2015). Thus the uridine and uridine nucleotides may be novel regulators of body metabolism and function of AgRP/NPY neurons. In addition to AgRP/NpY neurons, ghrelin promotes activation of somatostatin neurons in the hypothalamic tuberal nucleus to promote feeding by inhibiting downstream neurons in the PVN and to the BNST (Luo *et al.* 2018).

MC4R neurons

MC4R is a member of a family of melanocortin GPCRs cloned in 1992 that also include MC1R, MC2R, MC3R and MC5R (Chhajlani & Wikberg 1992, Mountjoy *et al.* 1992, Gantz *et al.* 1993*a*,*b*, Cone 2005). MC4R is predominantly expressed in the brain, where it localizes to many areas (Gantz *et al.* 1993*b*, Mountjoy *et al.* 1994, Williams *et al.* 2000, Liu *et al.* 2003, Gautron *et al.* 2010, Rossi *et al.* 2011, Cui *et al.* 2012, Sohn *et al.* 2013, Shah *et al.* 2014) (Fig. 4) and in neuroendocrine cells of the intestine (Panaro *et al.* 2014). MC4R is essential for appetite control as knockout mice lacking MC4R have hyperphagia and obesity (Fan *et al.* 1997, Huszar *et al.* 1997). MC4R mutations are associated with human obesity (Vaisse *et al.* 1998, Yeo *et al.* 1998, Farooqi *et al.* 2000, Vaisse *et al.* 2000, Lubrano-Berthelier *et al.* 2006, Farooqi & O'Rahilly 2008, Stutzmann *et al.* 2008) this association is controversial in the case of MC3R mutations (Tao 2010*b*). The MC4R expressed in single-minded 1 (Sim1) and in MC4R glutamatergic neurons of the PVN of hypothalamus and
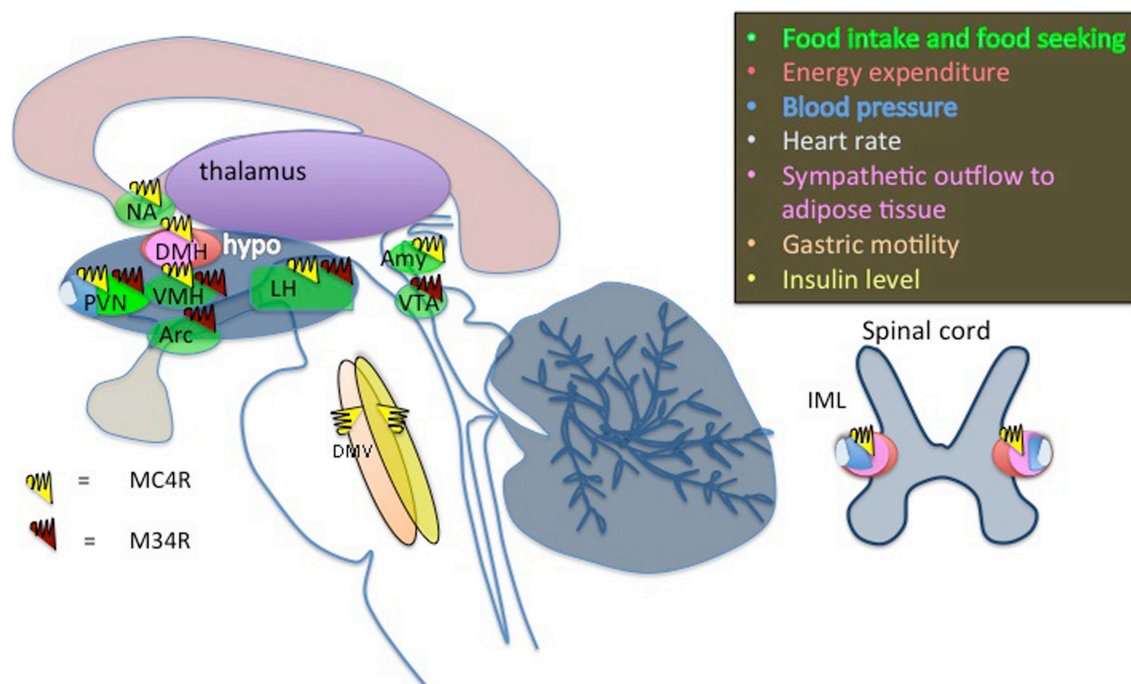
Figure 4
Localization and function of MC4R and MC3R in the central nervous system. Amy, amygdala; DMH, dorsomedial nucleus of the hypothalamus; DMV, dorsal motor nucleus of the vagus; IML, intermediolateral nucleus of the spinal cord; LH, lateral hypothalamus; NA, nucleus accumbens; PVN, paraventricular nucleus of hypothalamus; VMH, ventromedial nucleus of the hypothalamus; VTA, ventral tegmental area. References are in the main text.

the amygdala functions to control food intake (Balthasar *et al.* 2005, Shah *et al.* 2014, Garfield *et al.* 2015) (Fig. 4). In the pathway to control appetite, populations of POMC neurons residing in the arcuate nucleus activate MC4R neurons in the PVN (Mercer *et al.* 2013, Gautron *et al.* 2015, Dores *et al.* 2016, Toda *et al.* 2017, Caron *et al.* 2018). Also activation of glutamate-releasing neurons that reside in the arcuate nucleus and co-express oxytocin receptor rapidly cause satiety (Fenselau *et al.* 2017). These oxytocin receptor-expressing neurons engage MC4R neurons in the PVN through a fast glutamatergic transmission that is potentiated by α-MSH released from the POMC neurons. Other neurons, localized to the NTS and expressing CCK directly stimulate the activity of MC4R neurons in the PVN to signal (D'Agostino *et al.* 2016). It has been recently proposed that the bone specific hormone lipocalin-2 (LCN2) suppresses appetite by crossing the blood-brain barrier and binding to MC4R in the PVN and VMH (Mosialou *et al.* 2017, Mera *et al.* 2018). MC4R neurons are inhibited by the AgRP/NPY neurons residing in the arcuate nucleus (Bagnol *et al.* 1999, Cowley *et al.* 1999, Legradi & Lechan 1999, Cowley *et al.* 2001, Aponte *et al.* 2011, Atasoy *et al.* 2012, 2014). In genetic rodent models of obesity with elevated leptin levels, abundance of melanocortin receptors is reduced in the NA and in VTA,

which are areas of the brain involved in the reward circuit (Lindblom *et al.* 2000) (Fig. 4). In the VTA, dopaminergic neurons that regulate palatable feeding are responsive to α-MSH and project to neurons of the NA (Lindblom *et al.* 2000, Szczypka *et al.* 2000, Lindblom *et al.* 2002, Roseberry 2013, Panaro *et al.* 2014, Pandit *et al.* 2015, Roseberry *et al.* 2015, Yen & Roseberry 2015). MC4R regulates other functions in addition to appetite. Administration of the MC4R and MC3R agonist melanotan II (MTII) to the CNS of rats activates the hypothalamic melanocortin system and increases sympathoexcitation in the kidney and brown adipose tissue (Haynes *et al.* 1999). Specifically, MC4R expressed in neurons of the dorsomedial nucleus of the hypothalamus (DMH) and MC4R expressed in preganglionic cholinergic sympathetic neurons of the CNS localized in the intermediolateral nucleus (IML) of the spinal cord control both sympathetic outflow to adipose tissue and energy expenditure (Haynes *et al.* 1999, Chen *et al.* 2004, Shrestha *et al.* 2010, Enriori *et al.* 2011, Rossi *et al.* 2011, Berglund *et al.* 2014, Rezai-Zadeh *et al.* 2014, Andermann & Lowell 2017) (Fig. 4). MC4R neurons are also implicated in glucose homeostasis. In this respect, preganglionic cholinergic sympathetic neurons of the intermediolateral nucleus of the spinal cord (IML) that express MC4R control glucose output from

liver (Rossi *et al.* 2011, Berglund *et al.* 2014). Lorcaserin, a 5-HT2CR agonist used as anti-obesity drug, decreases glycemia by acting on MC4R cholinergic neurons (Burke *et al.* 2017). Interestingly, an association has been found between MC4R genotype and postpartum glycemic changes in humans, thus highlighting the relevance of MC4R in glucose metabolism in humans (de Carvalho *et al.* 2017). Cholinergic parasympathetic MC4R neurons, such as those localized in the dorsal motor nucleus of the vagus (DMV) control insulin levels and gastric motility (Rossi *et al.* 2011, Richardson *et al.* 2013, Sohn *et al.* 2013). In the PVN of the hypothalamus, in addition to food intake, melanocortin receptors modulate sympathetic outflow, blood pressure and heart rate (Kuo *et al.* 2003, Tallam *et al.* 2005, Li *et al.* 2006, 2013, Skibicka & Grill 2009). In the PVN, MC4R also appears essential for hypertension in the offspring of obese rats (Samuelsson *et al.* 2016). MC4R agonists increase blood pressure by targeting cholinergic neurons, including the sympathetic preganglionic neurons of the IML (Sohn *et al.* 2013). In the IML, MC4R neurons also control heart rate (Iwasa *et al.* 2013) (Fig. 4). In the hindbrain, MC4R neurons control heart rate, but not blood pressure (do Carmo *et al.* 2015) (2017).

MC3R neurons

In the brain, MC3R is abundantly expressed in the arcuate nucleus, VMH, central linear nucleus of raphe and dopaminergic neurons of the VTA; MC3R is instead moderately expressed in the lateral hypothalamic area and in the PVN (Roselli-Rehfuss *et al.* 1993, Gantz *et al.* 1993*b*, Xia & Wikberg 1997, Jegou *et al.* 2000, Lippert *et al.* 2014). In the arcuate nucleus, MC3R is expressed in subpopulations of both POMC and AgRP/NPY neurons (Bagnol *et al.* 1999, Jegou *et al.* 2000). The obesity induced by MC3R knockout in mice is less profound than that by knockout of MC4R and mice lacking both MC3R and MC4R are heavier than mice lacking only MC4R, suggesting distinct roles to control food intake (Butler *et al.* 2000, Chen *et al.* 2000, Butler & Cone 2003, Butler *et al.* 2017). Moreover, MC3R knockout mice do not develop fatty liver disease or severe insulin resistance like the MC4R mice (Ellacott *et al.* 2007, You *et al.* 2016). MC3R regulates normal fasting response (Marks *et al.* 2003, Renquist *et al.* 2012), adaptation to restricted feeding (Sutton *et al.* 2010, Girardet *et al.* 2017), food anticipatory activity (Girardet *et al.* 2014, Vaanholt *et al.* 2015) and enhanced motivation to acquire food during nutrient scarcity (Mavrikaki *et al.* 2016).

MC3R also functions to regulate nutrient partitioning in fat and liver tissues under conditions of fasting (Renquist *et al.* 2012). MC3R expressed in AgRP/NPY neurons functions to regulate inhibitory GABA release onto MC4R neurons in a pathway to tune energy balance from one set point to another (Bagnol *et al.* 1999, Cowley *et al.* 2001, Ghamari-Langroudi *et al.* 2018).

MC4R and MC3R signaling

MC4R and MC3R are GPCR that, in the presence of the natural agonist α-MSH, couple to Gs and AgRP antagonizes this effect (Tao 2010*a*). In cells expressing exogenous MC4R, exposure to the natural agonist, α-MSH, induces activation of adenylate cyclase and increased production of cAMP (Gantz *et al.* 1993*a*) (Fig. 5). The increased concentration of cAMP induced by MC4R is thought to activate exchange protein directly activated by cAMP (EPAC), leading to ERK1/2-dependent phosphorylation of the transcription factor cAMP response element (CRE) binding protein (CREB), increased transcription of cFos and reduced phosphorylation and activity of AMPK (Glas *et al.* 2016, Yang & Tao 2016). In this respect, intracerebroventricular delivery of the MC3R/MC4R synthetic agonists induces anorexia as well as activation of transcription by CREB and expression of cFos in the PVN (Thiele *et al.* 1998, Benoit *et al.* 2000, Harris *et al.* 2001, Lee *et al.* 2001, Sarkar *et al.* 2002, Lu *et al.* 2003, Rowland *et al.* 2010). On the other hand, other factors in addition to MC4R may modulate CREB-dependent control of energy homeostasis in the PVN. In this respect, it has been reported that, while lack of CREB in the Sim1 neurons of the PVN causes murine obesity, such effect can also take place in the absence of MC4R signaling (Chiappini *et al.* 2011).

In addition to agonist-dependent coupling to Gs and generation of cAMP, MC4R can couple constitutively to both Gs and Gi. Constitutive coupling of MC4R to Gs in cells can be detected by exposure to the natural antagonist AgRP, thus indicating that the hormone is also an inverse agonist (Nijenhuis *et al.* 2001, Oosterom *et al.* 2001). Importantly, mutations that modulate the constitutive activity of MC4R to increase Gs signaling are linked with obesity in humans, thus suggesting that MC4R constitutive signaling is physiologically relevant (Vaisse *et al.* 2000, Srinivasan *et al.* 2004, Proneth *et al.* 2006). Interestingly, for some MC4R mutants with altered constitutive activity, AgRP can act as a biased agonist to promote ERK1/2 activation (Wang & Tao 2011, Mo &
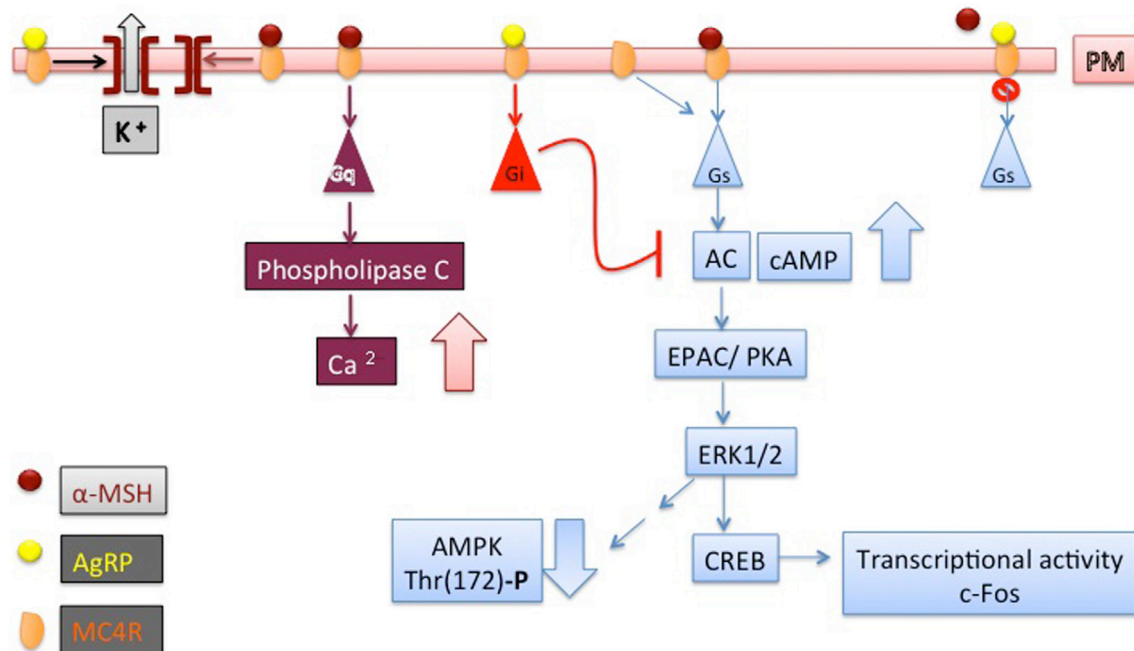
Figure 5

MC4R signaling. Binding of α-MSH to MC4R promotes receptor signal through Gs with activation of adenylate cyclase (AC) and increased generation of intracellular cAMP, followed by activation of PKA, EPAC, ERK1/2, CREB and increased transcription of c-Fos as well as decreased AMPK activity. AgRP antagonizes these effects. The Gs signal induced by MC4R likely takes place in the dorsomedial hypothalamus (DMH) to control energy expenditure. AgRP can also act a biased agonist to promote MC4R signal by Gi. MC4R can couple constitutively to both Gs and Gi, and AgRP blocks such signal, acting as an inverse agonist. MC4R in a complex with α-MSH also couples to Gq and induces activation of phospholipase C and increased intracellular cytosolic calcium. The Gq signal likely takes place in the paraventricular nucleus (PVN) of the hypothalamus to control food intake. MC4R in a complex with α-MSH closes the Kir7.1 channel to induce depolarization of MC4R neurons in a G-protein independent manner. AgRP acts as a biased agonist by opening the Kir7.1 channel to induce hyperpolarization of MC4R neurons. References are in the main text.

Tao 2013). It has been found that, in hypothalamic cells, MC4R can also couple to Gi and that MC4R constitutive activity through Gs and Gi/o can inhibit L-type voltage-gated calcium channels in neurons (Buch *et al.* 2009, Agosti *et al.* 2017). Moreover, MC4R activation by the synthetic agonist MTII inhibits presynaptic N-type calcium channels in amygdaloid complex neurons (Agosti *et al.* 2014). Thus, signaling of MC4R by Gs and Gi controls calcium channel activity in neurons. In addition to Gs and Gi, MC4R couples to Gq (Peters & Scott 2009) and induces increased intracellular cytosolic calcium (Nickolls *et al.* 2005, Newman *et al.* 2006, Li & Lytton 2014). In this respect, it appears that peptide MC4R agonists induce both cAMP accumulation and calcium mobilization, while non-peptide agonists have blunted ability to induce calcium mobilization, thus indicating biased agonism (Nickolls *et al.* 2005). The ability of MC4R to signal through a specific pathway likely depends on the cell type where the receptor is being expressed. In this respect, it appears that Gsα signaling in the dorsomedial nucleus of the hypothalamus (DMH) and, to a lesser degree, in the PVN is important for regulation of energy expenditure

(Chen *et al.* 2009, 2012, 2017a). It also appears that, in mice, Gs expressed in MC4R cells regulates in addition to energy expenditure, also food intake, insulin sensitivity and cold-induced thermogenesis. Such effect may take place by mechanisms that include release of PYY from enteroendocrine cells of the intestine expressing MC4R (Panaro *et al.* 2014, Podyma *et al.* 2018). Mice with PVN-specific loss of Gqα and G11α have hyperphagia and obesity and are relatively insensitive to delivery of MC4R agonist in the PVN, which would normally reduce food intake (Li *et al.* 2016). These findings indicate that signaling by MC4R to regulate appetite in the PVN is dependent upon Gqα and G11α. Mutations of adult type 3 adenylyl cyclase (Adcy3), a member of the adenylyl cyclase family that mediates Gs signaling, leads to obesity in mice (Wang *et al.* 2009). Humans with variants of Adcy3 are also obese (Stergiakouli *et al.* 2014, Wu *et al.* 2016). Interestingly, tagged MC4R-GFP co-localizes with Adcy3 at the primary cilia of PVN neurons, while obesity-associated MC4R variants impair localization of the receptor to cilia (Siljee *et al.* 2018, Tian *et al.* 2018). These observations suggest that MC4R signaling though

Adcy3 at cilia of hypothalamic neurons is essential for energy homeostasis (Varela & Horvath 2018). It also appears that α-MSH increases firing in MC4R neurons of the PVN in a G-protein independent manner by inducing depolarization through closure of the inwardly rectifying potassium channel, Kir7.1. Moreover, AgRP can act as a biased agonist by opening the Kir7.1 channel to induce hyperpolarization of MC4R neurons (Ghamari-Langroudi et al. 2015, Litt et al. 2018). These data indicate that different MC4R signals may control food intake and energy expenditure at specific brain locations and that MC4R-dependent control of ion channel activity may contribute to receptor signal. MC4R activity can also be modulated by accessory factors. MRAP2 is a MC4R-interacting factor that is co-expressed with the receptor in the hypothalamus, localizes to the plasma membrane and endoplasmic reticulum (ER) and potentiates MC4R function. Deficiency of MRAP2 leads to obesity (Chan et al. 2009, Agulleiro et al. 2013, Asai et al. 2013, Sebag et al. 2013). Experiments where MRAP2 was overexpressed postnatally in MC4R neurons also indicate that MRAP2 functions to potentiate MC4R neurons (Bruschetta et al. 2018). It has recently been found that MRAP2 affects other hypothalamic functions in addition to MC4R signaling, thus implicating multiple pathways to obesity by MRAP2 deficiency (Chaly et al. 2016, Novoselova et al. 2016). Nevertheless, the data offer evidence that interactions of MC4R with accessory factors along the secretory pathway modulate receptor function.

Intracellular traffic of MC4R and response to melanocortin receptor agonists

Studies based on undifferentiated cells, neuronal cells and immortalized hypothalamic cells indicate that desensitization of MC4R takes place by a process where, upon prolonged agonist exposure, the receptor routes to the lysosomes, instead of being made available at the cell membrane (Gao et al. 2003, Shinyama et al. 2003, Mohammad et al. 2007, Granell et al. 2013). MC4R is internalized at the same rate in the presence or absence of α-MSH agonist (Mohammad et al. 2007). This constitutive endocytosis of MC4R is fast, by taking place with a $t_{1/2}$ of approximately 3 min, is dependent on clathrin and membrane cholesterol and is necessary to maintain receptor function (McDaniel et al. 2012, Molden et al. 2015). The rapid constitutive internalization and cycling back to the cell surface of MC4R is a specific feature of

MC4R, as most other GPCRs, including the archetypal β2-adrenergic receptor, are endocytosed more efficiently in the presence of agonist (McDaniel et al. 2012). Agonist exposure induces binding of β-arrestin to the β2-adrenergic receptor, receptor internalization and desensitization (Drake et al. 2006). In the case of MC4R, desensitization instead takes place because, in the presence of agonist, a population of constitutively recycling receptor is retained in the intracellular localization and routes to lysosomes (McDaniel et al. 2012, Granell et al. 2013). A factor implicated in MC4R intracellular traffic to lysosomes is Mahogunin Ring Finger-1, a RING domain-containing ubiquitin ligase, which also competes with Gsα to bind to MC4R (Perez-Oliva et al. 2009, Overton & Leibel 2011). Desensitization of MC4R may also take place *in vivo* and manifest itself as 'tachyphylaxis' to chronic, continuous MC4R treatment with cessation of weight loss (Pierroz et al. 2002, Bluher et al. 2004). Such loss of receptor function upon chronic exposure to MC4R agonists could limit their effects to reduce food intake and body weight. The unselective MC4R agonist peptide MTII, the selective MC4R agonist peptides BIM-22511 and LY2112688, as well as the selective MC4R agonist THIQ, a small molecule, regulate food intake, energy expenditure, weight loss, sexual function and cardiovascular function (Adan et al. 1999, Kuo et al. 2002, Martin et al. 2002, Van der Ploeg et al. 2002, Greenfield et al. 2009, Kumar et al. 2009, Kievit et al. 2013). In response to exposure to all of these agonists, MC4R desensitizes to the same extent as in response to the natural agonist, α-MSH (Molden et al. 2015). Interestingly, by using intermittent, rather than constant, delivery of MTII in rodents, it is possible to prolong the effects of MC4R agonists to reduce food intake, even if this treatment does not prevent tachyphylaxis (Zhang et al. 2010, Cote et al. 2017). It is also becoming evident that, in the face of common desensitization properties by different melanocortin receptor agonists, temporal effects induced by such agonists vary. With respect to MC1R, it has been found that, unlike α-MSH, MTII and another non-selective agonist active toward MC1R, 4-norleucine, 7-D-phenylalanine-α-MSH, have prolonged biological activity to darken frog skin (Sawyer et al. 1980). With respect to MC4R, chronic treatment of obese primates with the selective MC4R agonist setmelanotide (also called RM-493), but not with another selective agonist LY2112688, results in persistent weight loss in nonhuman primates (Kievit et al. 2013). In neuronal cells and in immortalized hypothalamic neurons where fluctuation of intracellular cAMP are measured by a temporally resolved Forster resonance energy transfer assay MTII, BIM-22511

and THIQ can induce prolonged MC4R cAMP signaling after agonist withdrawal, while other MC4R agonists, such as α-MSH and LY2112688, do not have such property (Molden *et al.* 2015). It is possible that more persistent effects induced by some MC4R agonists to modulate energy homeostasis *in vivo* are linked to their biological property to induce prolonged receptor signal, rather than differences in the extent by which MC4R would desensitize.

Pharmacological chaperones and folding of MC4R

Recently, it has been discovered that it is possible to change the conformation of GPCRs by intracellular delivery of agonists and antagonists (Conn & Ulloa-Aguirre 2011). Many MC4R variants linked to obesity in humans have defective function because they are retained intracellularly (Ho & MacKenzie 1999, Lubrano-Berthelier *et al.* 2003, Nijenhuis *et al.* 2003, Tao & Segaloff 2003, Ju *et al.* 2018). Intracellular retention of obesity-linked MC4R variants is dependent on their localization to the ER as misfolded, ubiquitinated proteins (Granell *et al.* 2010, Rene *et al.* 2010). Such misfolded MC4R mutants can be rescued by (a) pharmacological chaperones, namely lipophilic compounds that can enter cells and serve as a molecular scaffold to assist proper folding of misfolded proteins; (b) by chemical chaperones, which likely modulate the folding capacity of the ER; (c) by inhibitors of ubiquitination, which inhibit protein degradation by the proteasome (Rene *et al.* 2010, Meimaridou *et al.* 2011, Granell *et al.* 2012, Huang & Tao 2014, Tao & Conn 2014, 2018, Tao & Huang 2014). Interestingly, setmelanotide promotes weight loss in obese individuals expressing MC4R variants (Collet *et al.* 2017) (Table 1). Setmelanotide, when tested in cells, also acts as a pharmacological chaperone to promote receptor expression and function at the cell surface (Collet *et al.* 2017) (Table 1). Changing conformation of newly synthesized MC4R along the secretory pathway may also affect the receptor properties not only to signal, but also to desensitize. In this respect, co-expressing α-MSH together with MC4R in the ER can rescue an obesity-linked variant retained in the ER and stabilize the wild-type receptor in an active conformation that does not route to lysosomes nor desensitizes (Granell *et al.* 2013). These observations indicate that MC4R conformation and ability to signal can be modulated by interactions with agonist in the ER.

The influence of diet-induced obesity on the melanocortin signaling

Obesity by high-fat feeding induces damage to several regions of the hypothalamus implicated in energy homeostasis

In male mice, exposure to high-fat diet induces hypothalamic injury with inflammation, gliosis and neuronal loss in the arcuate nucleus, medial eminence and LH (De Souza *et al.* 2005, Moraes *et al.* 2009, Velloso *et al.* 2009, Thaler *et al.* 2012, Dorfman & Thaler 2015, Dorfman *et al.* 2017*a*, Yi *et al.* 2017). Female rodents exposed to HF diet, while having less severe hypothalamic injury and adverse metabolic consequences than male mice, nevertheless develop obesity (Hong *et al.* 2009, Atamni *et al.* 2016, Dorfman *et al.* 2017*a*, Chowen *et al.* 2018, Qiu *et al.* 2018*a*). In the PVN of the hypothalamus, Sim1 neurons are essential to control energy homeostasis and include the population of MC4R neurons that regulate appetite (Balthasar *et al.* 2005). In mice exposed to HF diet, while injury to POMC neurons and microgliosis in arcuate nucleus is specific to male mice, injury to Sim1 neurons of the PVN is a shared feature, taking place both in male and female mice (Nyamugenda *et al.* 2019). Differently than in other regions of the hypothalamus, damage to Sim1 neurons by exposure to high-fat diet is not paralleled by microgliosis (Nyamugenda *et al.* 2019). These contributions indicate, within the hypothalamus, region-specific mechanisms of neuronal damage by exposure to high-fat diet.

Obesity by high-fat feeding induces local activation and expansion of resident glia in the mediobasal hypothalamus, as well as recruitment of bone-marrow-derived myeloid cells

Obesity induces activation of the innate immune system and inflammation, which impacts many organs including adipose tissue, pancreas, liver, skeletal muscle and the brain (Saltiel & Olefsky 2017). Feeding rodents a high-fat diet induces, in hypothalamus, increased activation of c-Jun N-terminal kinase (JNK) and of nuclear factor-kappaB (NFκB) and expression of proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) (De Souza *et al.* 2005). Already within the first week of high-fat diet exposure, reactive gliosis and neuronal injury is detectable in the arcuate nucleus of the hypothalamus (Thaler *et al.* 2012, Morari *et al.* 2014). Obesity induced by exposure to high-fat diet is associated with increased entry into the central nervous

Table 1 Natural and synthetic agonists of MC4R.

	hMC4R EC50 *in vitro* (nM)	MC4R desensitizes (MC4R †) MC4R does not desensitize (MC4R ♣) After agonist withdrawal: MC4R able to signal (MC4R ★) MC4R unable to signal (MC4R X) Increased expression of MC4R at cell surface (MC4R ◉)	Decreased body weight (↓BW) Decreased food intake (↓FI) Increased blood pressure (↑BP) Increased blood glucose (↑BG) Improved glucose metabolism (GM★) Increased heart rate (↑HR)	Increased energy expenditure (↑EE) Increased insulin sensitivity (↑IS) Increased lipolysis in white adipose tissue (↑WATL) Decreased fat (↓Fat)	Increased neurogenesis (↑Neuro) Anti-inflammatory effects (↓Inf)
α-MSH	4.69 (Kievit *et al*. 2013)	MC4R †; MC4R X (*in vitro*) (Molden *et al*. 2015)	↓BW (h, only normal weight humans) intranasal delivery (Hallschmid *et al*. 2008) ↓BW (r) ICV delivery (McMinn *et al*. 2000)	↑WATL (h) intranasal delivery (Wellhoner *et al*. 2012)	
NDP-α-MSH (MTI)	0.0752 (Kievit *et al*. 2013)	NT	↓FI ICV delivery (Koegler *et al*. 2001)	↑IS (r) in hypothalamus ICV delivery (Chai *et al*. 2009)	↓Inf (*in vitro*) (Carniglia *et al*. 2016) ↑Neuro (r) (Giuliani *et al*. 2015)
MTII	0.0542 (Kievit *et al*. 2013)	MC4R †; MC4R ★ (*in vitro*) (Molden *et al*. 2015)	↓BW; ↓FI (r) ICV and IP delivery (Fan *et al*. 1997) (Bluher *et al*. 2004; Cote *et al*. 2018)	↑EE (r) IP delivery (Podyma *et al*. 2018)	
LY2112688	0.0857 (Kievit *et al*. 2013)	MC4R †; MC4R X (*in vitro*) (Molden *et al*. 2015)	↑BP; ↑HR (h) (Greenfield *et al*. 2009) ↑BP ; ↑HR (nhp) (Kievit *et al*. 2013)	↑WATL *In vitro* human explants (Moller *et al*. 2015)	
Setmelanotide (BIM-22493)	0.27 (Kievit *et al*. 2013) (Kumar *et al*. 2009)	MC4R ◉ (*in vitro*) (Collet *et al*. 2017)	↓BW ; no ↑BP (h) s.c. delivery, obese humans with LepR deficiency (Clement *et al*. 2018) s.c. delivery obese humans with MC4R deficiency (Collet *et al*. 2017) ↓BW ; ↓FI ; no ↑BP ; GM★ (nhp) (Kievit *et al*. 2013)	↑EE (h) s.c. delivery, obese humans (Chen *et al*. 2015*a*,*b*) ↑EE ↓Fat (nhp) (Kievit *et al*. 2013)	
Setmelanotide and glibencamide			↓BW ; ↓FI; GM★ (r) (Clemmensen *et al*. 2015)	↑EE ; ↓Fat (r) (Clemmensen *et al*. 2015)	
α-MSH-ER (intracellular α-MSH)	NT	MC4R ♣; MC4R ★ (*in vitro*) (Granell *et al*. 2013)			
MC4-NN2-0453 (peptide 19)	88 (Conde-Frieboes *et al*. 2012)	NT	No ↓BW ; no ↑BP (h) s.c. delivery, obese humans (Royalty *et al*. 2014)	↑IS (r) (Fosgerau *et al*. 2014)	
MC4-NN1-0182 (peptide 11)	62 (Conde-Frieboes *et al*. 2012)	NT	↓BW; GM★(r) (Fosgerau *et al*. 2014)		
RO27-3225	NT		↓FI (r) ICV and IP delivery (Benoit *et al*. 2000)		↓Inf (r) IP delivery (Chen *et al*. 2018)

ER, endoplasmic reticulum; h, humans; ICV, intracerebroventricular; IP, intraperitoneal; LepR, leptin receptor; nhp, nonhuman primates; NT, not tested, r, rodents; s.c., subcutaneous; WAT, white adipose tissue.

system of monocytes with the phenotype of activated microglia/macrophage (Buckman *et al.* 2014). The mediobasal hypothalamus includes the arcuate nucleus, the medial eminence and the anterior part of the PVN (Timper & Bruning 2017). In diet-induced obesity (DIO), the recruitment of bone-marrow-derived myeloid cells in the mediobasal hypothalamus follows the earlier stage of hypothalamic inflammation, when hypothalamus-resident microglia are activated (Valdearcos *et al.* 2017). In mice exposed to high-fat diet, depletion of resident microglia from the mediobasal hypothalamus abolishes inflammation, reduces neuronal stress, suppresses food intake, and decreases weight gain (Valdearcos *et al.* 2014). Moreover, inhibition of microglia expansion in mice exposed to high-fat diet by central delivery of the antimitotic drug arabinofuranosyl cytidine inhibits hypothalamic and systemic inflammation and decreases weight gain (Andre *et al.* 2017). Inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB)-dependent signaling in microglia reduces both local microgliosis and recruitment of bone-marrow-derived myeloid cells in the mediobasal hypothalamus (Valdearcos *et al.* 2017). Moreover, even in mice that are not exposed to high-fat diet, deletion of a negative regulator of NFκB, A20, induces hypothalamic microgliosis and increases food intake and weight gain (Valdearcos *et al.* 2017). Thus, in the mediobasal hypothalamus of mice treated with high-fat diet, expansion and activation of resident microglia by the NFκB pathway promotes recruitment of myeloid cells from the circulation and neuronal injury. In DIO, upregulation of astrocytic inhibitor of nuclear factor kappa-B kinase β (IKKβ, also called IKK2)/NFκB pathway in astrocytes impairs astrocytic plasticity and promotes systemic adverse effects such as glucose intolerance, increased blood pressure, increased body weight and adiposity (Zhang *et al.* 2017). Conversely, knockout of IKKβ in astrocytes protects mice on a high-fat diet from further weight gain by decreasing food intake and increasing energy expenditure (Douglass *et al.* 2017). It appears that male and female astrocytes respond differently to exposure to high-fat diets (Chowen *et al.* 2018). In addition to microglia and astrocytes, the hypothalamus contains, especially the in median eminence, other resident glial cells such as monocyte-derived macrophages, which undergo expansion in mice exposed to high-fat diet (Gao *et al.* 2014, Kalin *et al.* 2015, Lee *et al.* 2018). Thus, under chronic exposure to high-fat diet, activation of astrocytes, microglia, and macrophages residing in the hypothalamus and recruitment of activated bone-marrow-derived myeloid cells from the

periphery disrupts hypothalamic control of energy homeostasis. Importantly, increased gliosis appears also in the hypothalamus of obese humans (Thaler *et al.* 2012, Dorfman & Thaler 2015).

Role of increased saturated fatty acids and lipoproteins to induce microglia and astrocyte activation

In the arcuate nucleus, toll-like receptor-4 (TLR4) is predominantly expressed in microglia. In DIO, increased levels of circulating saturated fatty acids, such as palmitic acid, activate TLR4 and modulate activity of neurons in the arcuate nucleus and feeding behavior (Milanski *et al.* 2009, Reis *et al.* 2015). Palmitic acid-induced activation of TLR4 in microglia has been found to promote phosphorylation and nuclear translocation NF-κB, increased secretion of proinflammatory cytokines, generation of nitric oxide (NO) and increased formation of ROS (Wang *et al.* 2012). Conversely, knockdown of TLR4 in the arcuate nucleus of obese rats fed high-fat diet, improves glucose homeostasis, attenuates insulin resistance, and reduces hepatic steatosis and adipocyte hypertrophy (Zhao *et al.* 2017). Moreover, TAK-242, a TLR4 signaling inhibitor, decreases microglial activation and improves neurogenesis in mice fed fat diet (Moser *et al.* 2018). These data indicate that, in the hypothalamus of obese mice, activation of TLR4 in microglia by excess circulating saturated fatty acids plays a major role to regulate hypothalamic inflammation by initiating the NF-κB pathway to alter glucose metabolism, to induce insulin resistance and to promote feeding. In obese mice, activation of GFAP-expressing glia promotes firing from orexigenic AgRP/NPY, but not from anorexigenic POMC neurons, and evokes feeding (Chen *et al.* 2016). Thus activation of astrocytes mediated by IKKβ/NF-κB signaling has effects to alter activity of arcuate nucleus neurons involved in appetite control. Changes in lipoprotein abundance in DIO may also promote astrocytosis and disrupt glucose metabolism and energy homeostasis. With this respect, decreased circulating high-density lipoprotein (HDL), a risk factor for cardiovascular disease, correlates with obesity (Woudberg *et al.* 2016). When loss of HDL is simulated in mice by knockout of Apolipoprotein A1 (apoA-I), there is hypothalamic astrogliosis paralleled by disrupted hypothalamic mitochondrial function as well as by increased hepatic triglyceride content and glucose output (Gotz *et al.* 2018). These data indicate that, in obesity, dyslipidemia with increased circulating saturated fatty acids and loss of HDL promotes hypothalamic

inflammation and altered mitochondrial function in the hypothalamus.

Glial activation in DIO changes the architecture of the hypothalamus

Glia is involved in the regulation of neuronal differentiation, proliferation and synaptogenesis during development (Nedergaard *et al.* 2003, Clarke & Barres 2013, Argente-Arizon *et al.* 2015) (2017). In adult rodents exposed to high-fat diet, reactive gliosis modifies the architecture of the hypothalamus in the vicinity of POMC and AgRP/NPY neurons. In rats that are obese by being exposed to high-fat diet, but not in rats that are diet-resistant, reactive gliosis in the arcuate nucleus reduces the accessibility of POMC and AgRP/NPY neurons to blood vessels and decreases synapses to the POMC neurons (Horvath *et al.* 2010). High-fat feeding induces activation of hypothalamic macrophages, which promotes nitric oxide synthase (iNOS)-dependent changes in blood-brain barrier permeability, as well as altered glucose metabolism (Lee *et al.* 2018). Therefore in obesity, inflammation-induced changes in the properties of blood-brain-barrier may contribute to neuronal injury.

In DIO, changes in the cyto-architecture of the hypothalamus appear to include reduced adult neurogenesis. With this respect, it has been reported that murine adult astrocytes instruct stem cells to adopt a neuronal fate (Song *et al.* 2002) and astrocytes themselves are, at least in some regions of the brain such as the ventricular-subventricular zone, precursors to neural stem cells (Doetsch *et al.* 1999, Paul *et al.* 2017). Neurogenesis has been found to take place in multiple brain regions, including the adult hypothalamus and hippocampus (Lindqvist *et al.* 2006, Migaud *et al.* 2010). In the hypothalamus, tancytes residing in the median eminence function to generate neurons (Lee *et al.* 2012). In obesity, impaired neurogenesis affects multiple neuronal populations including POMC and AgRP neurons (Park *et al.* 2010, Lee *et al.* 2012, Li *et al.* 2012, McNay *et al.* 2012). Thus, in DIO, hypothalamic inflammation and neuronal injury is paralleled by decreased renewal of neurons relevant to energy homeostasis (Argente-Arizon *et al.* 2015).

Disrupted mitochondrial function in DIO impairs activation of POMC neurons in response to feeding

Feeding controls mitochondrial function in hypothalamic neurons (Jin & Diano 2018). Increased glucose, by promoting increased ATP production in POMC neurons, enhances neuronal activation. Conversely, the activity

of mitochondrial uncoupling protein 2 (UCP2) impairs glucose-stimulated ATP production in POMC neurons by promoting proton leak (Parton *et al.* 2007, Ma *et al.* 2012). In POMC neurons, glucose-dependent generation of ROS is essential for neuronal firing (Andrews *et al.* 2008, Horvath *et al.* 2009, Diano *et al.* 2011). However, in DIO, glucose sensing by POMC neurons is impaired (Parton *et al.* 2007). With this respect, in hypothalamic POMC neurons of mice exposed to high-fat diet, expression of peroxisome proliferator-activated receptor γ (PPAR-γ) is increased and cell peroxisome population is also increased, leading to decreased levels of ROS and suppressed neuronal firing (Diano *et al.* 2011). Thus, it appears that, in DIO, a mechanism of impaired glucose-dependent activation of POMC neurons is reduced levels of ROS. This is different than in AgRP/NPY neurons where suppression of ROS instead promotes the activity of AgRP/NPY neurons and feeding (Diano *et al.* 2011). Recently, it has been found that, when mitochondrial respiration is mildly impaired in POMC neurons of obese mice exposed to high-fat diet, mitochondrial fatty acid utilization is improved, ROS generation is maintained and neuronal activation continues to take place (Timper *et al.* 2018).

Mitochondrial architecture regulates mitochondrial function in POMC neurons. With this respect, lack of mitofusin-1 (MFN1) in POMC neurons impairs mitochondrial elongation induced by feeding and alters mitochondrial respiration with excessive generation of ROS (Ramirez *et al.* 2017). Exposure to high-fat diet disrupts mitochondrial network with changes in mitochondrial morphology and decreases contact sites between mitochondria and ER in POMC neurons (Diano 2011, Schneeberger *et al.* 2013). In mice exposed to high-fat diet, hypersecretion of tumor necrosis factor (TNF)-α by activated glia changes mitochondrial morphology and induces mitochondrial stress in POMC neurons (Yi *et al.* 2017). Moreover, exposure to high-fat diet impairs, in POMC neurons, calcium uptake in mitochondria, resulting in an increased level of cytosolic calcium and decreased excitability of these neurons (Paeger *et al.* 2017*b*). These data converge to indicate that, in DIO, disrupted mitochondrial function with altered generation of ROS and increased cytosolic calcium suppresses nutrient-dependent excitability of POMC neurons.

Disrupted ER function in DIO impairs processing of POMC to α-MSH

In the arcuate nucleus of the hypothalamus of mice exposed to high-fat diet secretion of α-MSH from POMC

neurons is impaired (Enriori *et al.* 2007). Moreover, in POMC neurons of mice exposed to high-fat diet, there is ER stress with defective processing of POMC precursor protein to generate α-MSH (Cakir *et al.* 2013) as well as decreased mitochondria-ER contact sites (Schneeberger *et al.* 2013). Loss of α-MSH in POMC neurons of mice exposed to high-fat diet impairs whole body glucose homeostasis by inducing, even before the onset of weight gain, increased hepatic gluconeogenesis (Schneeberger *et al.* 2015). When ER stress takes place, induction of the unfolded protein response with activation of the transcription factor spliced XBP1S) branch of the unfolded protein response (UPR) promotes restoration of ER homeostasis (Fu *et al.* 2012). In mice that are obese by high-fat diet feeding, induction of the UPR by overexpression of transcription factor spliced X-box binding protein 1 (Xbp1s) in POMC neurons protects against weight gain and suppresses hyperinsulinemia, hyperleptinemia and glucose production (Williams *et al.* 2014). These studies indicate that, in DIO, resolution of ER stress in POMC neurons is a target to reverse obesity and altered metabolism.

The onset of selective neuronal resistance to insulin and leptin in obesity

In DIO, systemic inflammation with increased levels of the inflammatory cytokines TNF-α and IL-6 parallels the onset of insulin and leptin resistance (Fu *et al.* 2012). With this respect, signaling by insulin and leptin is inhibited by components of inflammatory pathways such as IKKβ and c-JNK (Haeusler *et al.* 2018). Expression of constitutively active IKKβ in hypothalamic neurons induces forced activation of the IKKβ/NFκB pathway, increased expression of SOCS3, and, thereby, resistance to insulin and leptin signaling (Zhang *et al.* 2008). Conversely, inactivation of SOCS3 in cells expressing the LepR restores leptin and insulin sensitivity in mice exposed to high-fat diet (Pedroso *et al.* 2014). Therefore, in DIO, systemic and local inflammation induces increased expression of SOCS3 in POMC neurons, resulting in leptin and insulin resistance. Leptin signaling by STAT3 is required for leptin regulation of energy balance (Bates *et al.* 2003). When mice are fed long-term a high-fat diet, there is region-specific onset of leptin resistance in the arcuate nucleus with defective STAT3 signaling and increased expression of SOCS3, which functions in physiological termination of the leptin signal (Munzberg *et al.* 2004) (Fig. 2). Interestingly, also chronic activation of STAT3 in murine POMC neurons promotes,

even when mice are fed chow diet, inhibition of both leptin and insulin signaling, upregulation of SOCS3 expression and increased weight gain (Ernst *et al.* 2009). Together, these findings indicate that, in obesity, disrupted insulin signaling by inflammation disrupts the STAT3/SOCS3 signaling in POMC neurons, thereby resulting in insulin and leptin resistance. In AgRP neurons, the activation of the IKKβ pathway increases action potential firing, without causing leptin resistance or obesity. Differently, inflammatory cytokines also activate another pathway, initiated by JNK1. Activation of the JNK1 pathway results in increased firing by AgRP neurons and results in leptin and insulin resistance in these neurons as well as in peripheral tissues (Tsaousidou *et al.* 2014). Thus, leptin resistance in AgRP neurons by activation of JNK1 may contribute to the onset of systemic insulin resistance in obesity.

In DIO, changes in other factors than increased SOCS3 levels may contribute to attenuation of the leptin signal and, thereby, leptin resistance. With this respect, the protein phosphatase PTP1B dephosphorylates JAK2 to terminate leptin signaling in POMC neurons, thereby regulating energy homeostasis (Bence *et al.* 2006, Banno *et al.* 2010). Also TCPTP attenuates leptin signaling by dephosphorylating STAT3 (Loh *et al.* 2011) (Fig. 2B). In DIO, levels of both PTP1B and TCPTP are elevated (Dodd *et al.* 2015). Conversely, combined deficiency of PTP1B and TCPTP in POMC neurons enhances insulin and leptin signaling and prevents weight gain in mice fed high-fat diet. Under these conditions, prevention of weight gain takes place because browning of white fat is increased and energy expenditure is enhanced (Dodd *et al.* 2015). Thus, in DIO, alteration of mechanisms to terminate leptin signaling results in hypothalamic resistance to anorexigenic hormones. Atypical PKCλ functions in the PI3K signaling pathway induced by leptin (Fig. 2A). Male mice exposed to high-fat diet have increased propensity to develop obesity, glucose intolerance and insulin resistance when lacking PKCλ in POMC neurons (Dorfman *et al.* 2017*b*). Together these data indicate that alterations of leptin and insulin signaling at multiple steps including both factors implicated in the onset of the signal and signal termination may contribute to hypothalamic resistance to these hormones in obesity.

Neurons in the VMH project excitatory synaptic inputs to POMC neurons in the arcuate nucleus (Sternson *et al.* 2005). SF-1-expressing neurons in the VMH regulate feeding and anxiety (Klockener *et al.* 2011, Viskaitis *et al.* 2017). In the SF-1 neurons, insulin activates the PI3K signaling pathway with downstream activation of

K⁺ATP channel, resulting in neuronal hyperpolarization and neuronal silencing (Klockener *et al.* 2011). In mice fed high-fat diet, but not in mice fed control low fat diet, lack of insulin receptor signaling in SF-1 neurons protects against weight gain and impaired glucose homeostasis, and increases activation of POMC neurons. These data indicate that, in DIO, increased insulin sensitivity of SF-1 neurons may contribute to loss of excitatory glutamatergic inputs to POMC neuron with loss of POMC neuron activity (Klockener *et al.* 2011, Timper & Bruning 2017). These studies indicate that onset of insulin resistance in populations of hypothalamic neurons outside of the arcuate nucleus contribute to energy homeostasis and glucose metabolism.

Mice with DIO are resistant to the anorectic actions of leptin in POMC and AgRP neurons. Leptin increases sympathetic nerve activity resulting in increased thermogenesis taking place in interscapular brown adipose tissue (Enriori *et al.* 2011). This leptin-dependent increase of sympathetic outflow to the brown adipose tissue originates from neurons in dorsomedial hypothalamus and is increased in DIO (Enriori *et al.* 2011). Thus, in obesity, selective leptin sensitivity, taking place in neurons of the dorsomedial hypothalamus may underlie other systemic features associated with excessive weight gain, such as increased sympathetic outflow, blood pressure and heart rate.

Lipid stress and MC4R desensitization

MC4R constitutive traffic makes the receptor particularly sensitive to changes in cell environment. For example, depletion of membrane cholesterol, a condition that takes place in the diabetic hypothalamus (Suzuki *et al.* 2010), appears to inhibit constitutive traffic of MC4R when modeled in cultured neuronal cells and immortalized hypothalamic neurons. Under such conditions, MC4R rapidly loses its ability to signal (McDaniel *et al.* 2012). Moreover, lipid stress by obesity and high-fat feeding induce injury to hypothalamic neurons and inflammation (De Souza *et al.* 2005, Moraes *et al.* 2009, Thaler *et al.* 2012). Lipid stress modeled in cultured neuronal cells and immortalized hypothalamic neurons by increasing the concentration of extracellular palmitate induces loss of MC4R protein abundance and function (Cragle & Baldini 2014). However, in diet-induced obese mice, the melanocortin system is instead over-responsive to MC4R agonists (Bluher *et al.* 2004, Enriori *et al.* 2007). An unexpected consequence of lipid stress is the disruption

of endocytosis following MC4R localization to clathrin-coated sites and inhibition of receptor desensitization. Such effects may underlie increased effectiveness of MC4R agonists in obesity (Cooney *et al.* 2017).

Melanocortin agonists and *in vivo* studies

MC4R is considered as a target for anti-obesity therapy. However the receptor controls multiple pathways that include, in addition to energy homeostasis, also glucose metabolism, blood pressure and heart rate. In this respect, the first round of peptides being synthesized were 4-norleucine, 7-ᴅ-phenylalanine-alpha-melanocyte-stimulating hormone (NDP-α-MSH, also called melanotan I) and MTII (Hadley & Dorr 2006). NDP-α-MSH and MTII have superpotent activity as compared to that of α-MSH, are resistant to proteases and have prolonged activity (Sawyer *et al.* 1980, Al-Obeidi *et al.* 1989*a*,*b*, Hadley & Dorr 2006) (Table 1). However, α-MSH and the relatively non-selective synthetic agonists NDP-α-MSH and MTII as well as the more selective agonist LY2112688, have side effects of increasing blood pressure and heart rate in rodents, nonhuman primates and humans (Dunbar & Lu 2000, Hill & Dunbar 2002, Ni *et al.* 2006, Greenfield *et al.* 2009, Greenfield 2011, Rinne *et al.* 2012, Kievit *et al.* 2013). These effects are likely to be the result of activation of the sympathetic nervous system by the melanocortin receptor agonists (Fig. 4 and related text) and therefore preclude their use to treat obesity (Greenfield *et al.* 2009). These compounds have been patented and clinical trials have been carried out to study their effects to promote skin tanning and to improve sexual function in male and females (Hadley & Dorr 2006). However, potential risks to develop melanoma related to the use of unlicensed MTII have been reported (Hjuler & Lorentzen 2014). Nevertheless, the first round of melanocortin receptor agonists have been the starting point to synthesize many other agonists and antagonists with elevated selectivity toward MC4R and MC3R (Hruby 2016, Ericson *et al.* 2017). In this respect, chronic treatment of obese nonhuman primates with the selective MC4R agonist setmelanotide, but not with LY2112688, results in persistent weight loss without concomitant effects to increase blood pressure and heart rate and, instead, improvement of cardiovascular function (Kievit *et al.* 2013) (Table 1). Importantly, setmelanotide, delivered to severely obese individuals with leptin deficiency, induces durable decrease in hyperphagia and body weight loss (Clement *et al.* 2018). Moreover, obese patients with POMC deficiency have also

durable decrease of hyperphagia and substantial weight loss upon treatment with setmelanotide (Kuhnen *et al.* 2016, Muller *et al.* 2016, Collet *et al.* 2017). In this respect, a phase 2 clinical trial is undergoing to study the use of setmelanotide to treat patients with rare genetic disorders of obesity (ClinicalTrials.gov Identifier: NCT03013543). A phase 3 clinical trial is undergoing to study the use of setmelanotide to treat patients with LepR deficiency (ClinicalTrials.gov Identifier: NCT03287960). In obese individuals, short-term administration of setmelanotide increases resting energy expenditure and promotes fat oxidation (Chen *et al.* 2015*a*). In mice with diet-induced obesity, simultaneous agonism at the GLP-1 receptor and MC4R induces body weight loss and improves glucose tolerance and cholesterol metabolism (Clemmensen *et al.* 2015). Interestingly, some melanocortin agonists such as NDP-α-MSH and RO27-3225 have effects to reduce inflammation and promote neurogenesis, which may prove relevant to counteract injury to the hypothalamus in obesity (Giuliani *et al.* 2015, Carniglia *et al.* 2016, Chen *et al.* 2018). These studies suggest that, in addition to obesity due to POMC or leptin deficiency, setmelanotide, in combination with GLP-1 agonists, may also be beneficial for treatment of obesity and diabetes. In conclusion, discoveries on the melanocortin system in the last two decades have revealed an unexpected complexity by which appetite control takes place and indicate possible therapeutic targets to reduce obesity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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