Commentary

Targeting AgRP neurons to maintain energy balance: lessons from animal models

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Abbreviations:

ARC, Arcuate nucleus; POMC, Proopiomelanocortin; GABA, γ-Aminobutyric acid, LHA, lateral hypothalamic nucleus; VHA, ventromedial nuclei; PVH paraventricular**;** PBN, parabrachial nuclei; MSH, Melanocyte-stimulating hormone; CART, amphetamine-stimulating hormone transcript; ChR, channelrhodopsin; DREADDs, designer receptor exclusively activated by designer drugs, CNO, clozapine; PI3K, Phosphoinositide 3-kinase; PIP_3, phosphatidylinositol-3,4,5-triphosphate; FOXO1, forkhead box O1; BSX, Brain specific homeobox; BAT, brown adipose tissue; ICV, intracerebroventricular; FAs, Fatty acids; AMPK, AMP-activated protein kinase; ACC, acetyl-CoA carboxylase; CPT1, carnitine palmitoyltransferase 1; FAS, fatty acid synthase; MCD malonyl-CoA decarboxylase; SNS sympathetic nervous system; WAT, white adipose tissue; ATF4, activating transcription factor 4; UCP1, uncoupling protein 1.

Abstract

The current obesity epidemic is a major worldwide health and economic burden. In the modern environment, an increase in the intake of high-fat and high-sugar foods plays a crucial role in the development of obesity by disrupting the mechanisms governing food intake and energy balance. Food intake and whole-body energy balance are regulated by the central nervous system through a sophisticated neuronal network located mostly in the hypothalamus. In particular, the hypothalamic arcuate nucleus (ARC) is a fundamental center that senses hormonal and nutrient-related signals informing about the energy state of the organism. The ARC contains two small, defined populations of neurons with opposite functions: anorexigenic proopiomelanocortin (POMC)-expressing neurons and orexigenic Agouti-related protein (AgRP)-expressing neurons. AgRP neurons, which also co-produce neuropeptide Y (NPY) and γ-Aminobutyric acid (GABA), are involved in an increase in hunger and a decrease in energy expenditure. In this review, we summarize the key findings from the most common animal models targeting AgRP neurons and the tools used to discern the role of this specific neuronal population in the control of peripheral metabolism, appetite, feeding-related behavior, and other complex behaviors. We also discuss how knowledge gained from these studies has revealed new pathways and key proteins that could be potential therapeutic targets to reduce appetite and food addictions in obesity and other diseases.

Contents

1. Introduction

The hypothalamus is a region of the diencephalon located below the thalamus on each side of the third ventricle. It is composed of many small neuronal nuclei that integrate endocrine, nutritional and sensory signals, which culminate in the generation of precise neuroendocrine, behavioral, and autonomic responses aimed at controlling body homeostasis. Anatomically, the hypothalamus extends from the anterior commissure, lamina terminalis, and optic chiasm to the caudal limit of the mammillary bodies. Within these anatomical limits, hypothalamic nuclei are organized into the following zones and regions: preoptic area, composed of the medial and lateral preoptic nuclei; lateral zone, includes the lateral hypothalamic nucleus (LHA), and the tuberal nuclei; and the medial zone, subdivided into the anterior (or supraoptic) region, which contains supraoptic, paraventricular (PVH), suprachiasmatic, and anterior nuclei; the tuberal region, which contains ventromedial nuclei (VHM), the dorsomedial hypothalamic nucleus, and the arcuate nucleus (ARC); and finally the third region or mammillary part, which consists of the medial, intermediate, and lateral mammillary and posterior hypothalamic nuclei.

The first experiments connecting the hypothalamus with the regulation of energy balance and food intake were done by Hetherington and Ranson over 70 years ago. Their studies reported that lesions in the VHM nuclei of rats caused hyperphagia and obesity [1]. Later, Anand and Brobeck demonstrated in 1951 that bilateral destruction of the lateral portion of the LHA causes complete inhibition of food intake [2]. These results gave rise to the "dual-center hypothesis", which suggests that VHM and LHA are the hypothalamic centers that regulate satiety and appetite respectively [3]. Nonetheless, following studies have further established that ARC nuclei play a more crucial role in the regulation of food intake and energy balance.

92 The relevance of the ARC was identified with the discovery of the melanocortin system,
93 which plays a key role in a number of homeostatic processes [4,5], and reviewed elsewhere [6,7] The
94 melanocortin system consists of 1) melanocortin peptides (α-, β-, and γ-melanocyte-stimulating
95 hormone (MSH) and adrenocorticotropic hormone) derived from the precursor proopiomelanocortin
96 (POMC); 2) five melanocortin receptors (MCRs, MC1R-MC5R, MC3R and MC4R are particularly
97 involved in food intake energy balance regulation), which are widely expressed in the hypothalamus;
98 and 3) the endogenous melanocortin antagonist (AgRP). All these components are expressed mainly
99 in two populations of hypothalamic neurons: neurons that co-produce AgRP, NPY, and GABA
100 (AgRP/NPY neurons) [8,9] and neurons that co-produce POMC and cocaine and amphetamine-
101 stimulating hormone transcript (CART) (POMC/CART neurons) [10] Both are located at the base of
102 the hypothalamus in close proximity to fenestrated capillaries. POMC/CART neurons and
103 AgRP/NPY neurons exert opposite functions and are reciprocally regulated (Fig.1). While
104 POMC/CART neurons express anorexigenic peptides and have appetite-suppressing functions,
105 AgRP/NPY neurons express orexigenic peptides and have an appetite-stimulating role. NPY acts as
106 a neurotransmitter in the brain and is thought to have several functions besides food intake, including
107 regulation of fat storage [11]. The appetite-stimulating response to NPY is mediated by multiple NPY
108 receptor subtypes, which are all Gi protein-coupled receptors. There are six identified NPY receptors,
109 but the Y1 and Y5 isoforms are most strongly associated with the effect of NPY on feeding revised
110 in [12]. Furthermore, AgRP/NPY neurons also release AgRP neuropeptide, a melanocortin antagonist
111 that prevents the binding of α-MSH onto MC3R and MC4R, thus activating hunger [13]. AgRP/NPY
112 neurons also release GABA. GABA is an inhibitory neurotransmitter, and may exert its orexigenic
113 action through GABAergic-mediated inhibition of POMC/CART neurons [14].

In recent decades, many animal models have been used to establish the relevance of this system in food intake and energy balance regulation. In this review, we analyze the techniques and approaches used to generate these animal models and their contribution to dissecting the molecular mechanisms and specific roles of AgRP/NPY neurons (thereafter AgRP neurons) on the modulation of peripheral glucose and lipid metabolism, thermogenesis, food intake, feeding behaviors, and other complex behaviors beyond feeding. We believe that this comprehensive review will help researchers in the field in search of specific pharmacological strategies to fight against excessive eating, obesity, and derived diseases.

2. Animal models targeting the activity of AgRP neurons

One of the oldest genetic models that demonstrated the involvement of the agouti protein in energy balance regulation is the lethal yellow A^y mouse, which expresses the agouti protein in all tissues [15]. This mouse develops an obesogenic phenotype characterized by excessive food intake, hyperinsulinemia, and increased body weight. The study of A^y mice also showed that the agouti protein acts as an antagonist at MC1R and MC4R.. Subsequently, using knock-out techniques, the genetic deletion of MC4R (MC4R-KO) further contributed to the understanding of the melanocortin system and its role in energy homeostasis. MC4R-KO mice also display hyperphagia and obesity under free-feeding conditions [16,17]. Despite this, AgRP-deficient mice show normal food intake, body weight, and energy expenditure, and only a significant impairment in the regulation of body weight during aging [18]. Furthermore using the Cre-loxP system, a large number of genes have been deleted from AgRP neurons in an effort to dissect the molecular mechanisms involved in the main physiological roles of this set of neurons [14,19,20]. Taken together, these studies suggested that the

various components of the melanocortin system and specific AgRP genes might contribute differently to the regulation of food intake and energy balance, and thus motivated additional studies in the field.

In recent years, several biotechnological tools have been developed and applied to study the physiological role of AgRP neurons. The optogenetic approach combines genetic and optical methods to control and monitor the activity of single neurons in living tissues [21,22]. This groundbreaking technique is based on the expression of microbial opsins, such as channelrhodopsin (ChR) or halorhodopsin, which are light-gated proteins that directly regulate the flow of ions across the plasma membrane. Thus, in mice expressing opsins, light stimulation might inhibit or stimulate the activity of specific neuronal populations. The analogous pharmacosynthetic chemogenetic approach, known as the designer receptor exclusively activated by designer drugs (DREADDs), has also been used to control the activity of a single neuronal population in mice. The DREADDs system is based on the expression of a synthetic G-protein coupled receptor that responds to an exogenous inert ligand such as Clozapine-N-oxide (CNO, inert ligand). Indeed, DREADD technology has been widely used to control the activity of AgRP neurons by delivering CNO to the experimental animals [23,24].

While these techniques have exposed the physiological functions of AgRP neurons, the undeniable demonstration of their critical role in the control of energy balance came from the study of AgRP neuron-ablated mice. This model was engineered to express the diphtheria toxin receptor (DTR) specifically in AgRP neurons ($AgRP^{DTR}$), which caused the selective ablation of these neurons upon delivery of diphtheria toxin [25,26].

Altogether, the results obtained using the above approaches have identified AgRP neurons as a key hypothalamic neuronal population that not only modulates food intake and feeding-related behaviors, but also whole-body energy homeostasis, through the control of peripheral glucose and lipid metabolism and thermogenesis.

3. AgRP neurons regulate peripheral metabolism

3.1. AgRP neurons and glucose metabolism

Every organism needs to match energy intake and expenditure to be able to subsist. Therefore, from a homeostatic perspective, hunger must be understood as a response emitted from the central nervous system in response to whole-body energy balance. For example, Horowitz M and colleagues showed in 1998 that physiological changes in blood glucose levels affect appetite [27]. Indeed, several decades ago, it had already been demonstrated that specific hypothalamic neurons can sense blood glucose concentrations. Yet, the precise molecular mechanisms underlying this observation are still incomplete.

Activation of AgRP neurons promotes food seeking and intake. After a meal, blood glucose levels increase and, as a result, POMC neurons are activated. While the mechanisms underlying the excitatory actions of glucose on anorexigenic POMC neurons are clear, it is not completely understood yet if and how glucose could directly inhibit AgRP activity, as revised [28,29].

Neurons could sense glucose levels through the ATP-dependent potassium (K_{ATP}) channels, which control potassium flow to depolarize or hyperpolarize the neuron membrane depending on the levels of ATP generated from glucose catabolism [28,30] (Fig. 2). The extracellular concentration of glucose in the brain is approximately 2.5 mM during the fed state and 0.5-0.2 mM during the fasted or hypoglycemic condition. POMC neurons increase spike firing when the concentration of brain glucose changes from 0.1 mM to 2 mM [31], and that is dependent on K_{ATP} channel subunits [32]. Conversely, the expression of a mutated K_{ATP} channel subunit that was insensitive to the ATP in POMC neurons impaired the whole-body response to a systemic glucose load [30].

It is unclear whether K_{ATP} channels are also involved in the glucose-dependent modulation of AgRP neurons. Even though K_{ATP} channels are expressed in AgRP neurons, probably because some of these neurons share a common progenitor with POMC neurons, only a minority of AgRP neurons are glucose responsive [29,31,33,34]. Yet, AgRP neurons respond to insulin levels. Insulin hyperpolarizes AgRP neurons in wild-type mice but not in those lacking the insulin receptor in AgRP neurons specifically ($IR^{\Delta AgRP}$) [35]. Insulin inhibits AgRP neuronal activity by activating the phosphoinositide 3-kinase (PI3K) signaling pathway. The phosphatidylinositol-3,4,5-triphosphate (PIP_3) that is generated activates K_{ATP} channels, resulting in hyperpolarization and a decreased neuronal firing rate, with subsequent reduced release of AgRP [35]. PI3K activation ultimately leads to the exclusion of forkhead box O1 (FOXO1) transcription factor from the nucleus [36]. FOXO1 increases AgRP expression [37], thus insulin action in AgRP neurons ultimately decreases the expression of AgRP. This signaling pathway has also been described in POMC neurons, but in those neurons the exclusion of FOXO1 from the nucleus promotes POMC expression [38]. Taken together, these studies suggest that POMC neurons sense glucose levels, while AgRP neurons respond to changes in insulin concentration.

In addition, AgRP neurons influence peripheral glucose metabolism. For instance, the ablation of these neurons in adult $AgRP^{DTR}$ mice caused a reduction in blood glucose levels 48 hours after the administration of diphtheria toxin [39]. One of the molecular explanations for this phenotype is the decreased hepatic gluconeogenesis observed in this model [35,40]. Accordingly, the acute activation of AgRP neurons using either a chemogenetic or optogenetic approach caused an increase in blood glucose levels due to systemic insulin resistance and decreased glucose uptake in brown adipose tissue (BAT) [24]. At molecular level, several genes in AgRP neurons have been associated with this regulation of whole-body glucose homeostasis. For instance, the deletion of purinergic receptor 6

(P2Y6) specifically in AgRP neurons, improved systemic insulin sensitivity in obese mice [41] and similar results have been observed when the transcription factor activating transcription factor 4 (ATF4) was deleted specifically in AgRP neurons [42].

3.2. AgRP neurons and lipid metabolism

Fatty acids (FAs) are the primary fuel for most tissues under the fasting state. Therefore, FA levels are associated with the energy status of the organism. Importantly, FAs can cross the blood-brain barrier and be oxidized in the brain. These observations raise the question of whether FAs could be a signal to AgRP neurons. It has been shown that intracerebroventricular (icv) administration of long-chain FAs inhibits the expression of NPY and AgRP in the hypothalamus, and therefore food intake [43]. Very recently, it has been reported that GPR120 and GPR40, also known as Free fatty acid receptor 1, are expressed in the hypothalamus of mice. The majority of cells that express POMC and AgRP also express GPR40 [44]. This receptor binds free FAs, acting as a nutrient sensor [44,45]. Of note, the icv administration of TUG905, which is an agonist of GPR40, reduces body weight [44]. The fact that GPR40 is expressed in AgRP and POMC neurons suggests that these cells could sense the content of FAs in the hypothalamus to regulate food intake and peripheral energy metabolism accordingly.

Besides, most enzymes of the FA metabolic pathways are expressed in the hypothalamus, including AMP-activated protein kinase (AMPK) [46,47], acetyl-CoA carboxylase (ACC), carnitine palmitoyltransferase 1 (CPT1), fatty acid synthase (FAS), and malonyl-CoA decarboxylase (MCD) [48]. Of note, fasting stimulates hypothalamic AMPK and inhibits ACC and FAS activities, whereas re-feeding induces opposite changes [49,50]. Moreover, pharmacological and genetic manipulation of some of these genes/proteins has a profound impact on food intake and whole-body energy

227 homeostasis. For instance, icv administration of the FAS inhibitor cerulenin reduces feeding and
228 causes weight loss due to the reduction of NPY and AgRP expression in the hypothalamus [51]. The
229 anorectic effect of this drug requires the accumulation of malonyl-CoA in the hypothalamus, which
230 in turn inhibits CPT1A, a key regulatory enzyme of the FA oxidation pathway. In agreement with
231 these observations, the long-term increase of FA oxidation in the ventromedial hypothalamus, through
232 the expression of a mutated and permanently active form of CPT1A, caused hyperphagia and
233 increased body weight in rats [52]. Future studies are needed to elucidate whether enzymes from the
234 FA metabolism pathway in specific ARC neuronal populations, *i.e.* AgRP or POMC neurons, play a
235 role in the regulation of food intake and peripheral energy metabolism.
236 Additionally, the hypothalamus regulates peripheral lipid metabolism and adiposity. For
237 instance, disruption of melanocortin signaling in the brain promotes lipid uptake, triglyceride
238 synthesis, and fat accumulation in white adipose tissue (WAT) [53]. Conversely, stimulation of MCRs
239 reduces adiposity [54]. Within the hypothalamus, the activity of AgRP neurons impacts peripheral
240 lipid metabolism. In fact, the activation of AgRP neurons promotes lipogenesis via the sympathetic
241 nervous system (SNS) [55]. Additionally, the deletion of carnitine acyltransferase specifically in
242 AgRP neurons increased FA utilization and attenuated the switch to glucose utilization after re-
243 feeding [56]. Moreover, the deletion of ATF4 specifically in AgRP neurons increases lipolysis in
244 epididymal WAT, thus promoting fat loss [42]. Taken together, these results indicate that targeting
245 the activity of AgRP neurons might be a good strategy to reduce adiposity in certain pathological
246 conditions characterized by excessive fat accumulation, such as obesity.
247
248 *3.3. The role of AgRP neurons in thermogenesis*

One of the first experiments that linked food intake with thermogenesis was completed by Rothwell A and Stock M in 1997. The study demonstrated that food consumption increases the activity of BAT through the SNS, which could be an important compensatory mechanism in case of energy surplus [57]. In addition, caloric restriction and feeding-fasting cycles also regulate body temperature and thermogenesis in BAT [58]. While it is known that sympathetic activity in BAT and WAT controls heat production and energy homeostasis [59], the same neurons that regulate energy intake may also control feeding-induced thermogenesis.

Adipose tissue can be classified as WAT, which stores energy in the form of triglycerides, and BAT, which is highly oxidative and contains abundant mitochondria that oxidize FAs to generate heat via the BAT specific uncoupling protein 1 (UCP1). In response to specific stimuli, certain WAT depots can undergo a process known as browning where the tissue takes on characteristics of BAT, including the expression of UCP1. The icv infusion of AgRP gradually suppressed sympathetic tone in BAT thus decreasing thermogenesis [60]. Using the optogenetic approach, acute activation of AgRP neurons strongly inhibited the expression of thermogenic genes, including *Ucp1*, in retroperitoneal WAT, and to a lesser extent in inguinal WAT, which indicates that AgRP neurons suppress the browning of WAT [61]. While the inhibition of AgRP neuronal activity by selectively deleting the O-GlcNAc transferase caused WAT browning in those mutant mice [61]

The hormone ghrelin, which is released from the empty stomach, acts on AgRP neurons through its receptor (GHS-R) to activate food consumption. Deletion of GHS-R specifically in AgRP neurons (*AgRP-Cre*;*Ghsr*$^{f/f}$ mice) caused increased energy expenditure in mice fed a HFD, and enhanced thermogenic activation in both BAT and subcutaneous WAT [62]. Overall, the role of AgRP neurons in thermogenesis could be an important target to increase energy expenditure and thus fight against obesity.

4. The role of AgRP neurons in food intake

There are numerous neurons distributed in the brain that influence appetite [63], yet AgRP neurons within the hypothalamus are probably the neuronal population that has the strongest and most closely documented association with feeding behavior [64,65]. The first evidence supporting the role of AgRP neurons in food intake control came from experiments showing that AgRP and NPY injection in the brain increase food intake. It was also drawn from the observation the AgRP and NPY release is higher in brain sections of food-deprived mice [66,67].

These early results have been confirmed recently by using the innovative cell type-specific approaches mentioned in Section 2 of this review. For example, optogenetic and pharmacogenetic activation of AgRP neurons result in a robust increase in food intake, even in fed mice [68,69]. Moreover, ablation of AgRP neurons in adult, but not neonatal, mice results in an acute reduction of feeding and starvation [25,26,70]**.** The differences observed between neonatal and adult mice suggest that network-based compensatory mechanisms can occur in neonates but not in the adult stage [71]. Surprisingly, deletion of the entire *Agrp* coding sequence using BAC-based targeting vectors did not generate differences in food intake or body weight in adult mice before 6 months of age [38]. Likewise, deletion of *Npy* had little effect on body weight control [72,73]. These observations suggest that GABA, also produced by AgRP neurons, might be a critical neurotransmitter regulating food intake and energy balance. Actually, disruption of GABA signaling by selective inactivation of the vesicular GABA transporter gene (*Vgat*) in AgRP neurons caused a lean phenotype and protected mice from diet-induced obesity [14]. Accordingly, chronic and subcutaneous delivery of bretazenil (a $GABA_A$ receptor partial agonist) prevented starvation in AgRP neuron-ablated mice [74].

GABA is an inhibitory neurotransmitter and thus GABAergic input from AgRP neurons inhibits the activity of several neuronal populations within the brain, including POMC neurons from the ARC. This crosstalk between orexigenic and anorexigenic neurons in the ARC is essential for the precise regulation of food intake [65]. In addition, GABAergic projections from AgRP neurons also suppress the activity of neurons in the PVH [75]. Because activation of PVH neurons decreases feeding [76], it has been suggested that AgRP neurons increase food intake in part through the GABAergic inhibition of PVH neurons. Another important GABAergic target of AgRP neurons is the parabrachial nuclei (PBN). **However, in this nucleus the effect of GABA promotes feeding, since administration of bretazenil (a GABA(A) receptor partial agonist) into the PBN prevents starvation in AgRP neuron-ablated mice [74]**.

Independently of the neurotransmitter released, there are many areas in the brain under the control of AgRP neurons that regulate feeding behavior. Elegant optogenetic experiments by Brüning and colleagues confirmed previous studies showing that AgRP neurons control neurons in the PVH to influence feeding. Likewise, they showed that LHA and the anterior bed nucleus of the stria terminalis (aBNST) dorsomedial nucleus were activated by AgRP projections to promote feeding, while the dorsal vagal complex, dorsal raphe nucleus and aBNST ventral lateral nucleus were not [24]. Overall, this study suggested that AgRP neurons orchestrate the activation and inactivation of various brain areas to promote food seeking and intake.

Now the question is: what is the mechanism by which AgRP neurons are activated upon starvation? A large amount of evidence indicates that ghrelin action on these neurons leads to their activation. Ghrelin is a 28-amino acid peptide produced in the stomach. Ghrelin blood levels increase preprandially and decrease postprandially. Ghrelin increases food intake by activating its receptor GHS-R in AgRP neurons [77,78] (Fig .3). Of note, ghrelin administration increases AgRP firing in

brain slices from control mice but not from mice lacking *Ghsr* specifically in AgRP neurons ($Ghsr_{AgRP}$-/-) [79]. Ghrelin increases the mRNA levels of NPY and AgRP in AgRP neurons, yet the molecular mechanism remains partially unclear [80]. It has been suggested that the AMPK-CPT1A-UCP2 axis is a relevant pathway in the ARC, which is involved in ghrelin's control of food intake [81]. AMPK is a downstream target of GHS-R, and upon its activation it inhibits ACC, thus decreasing the intracellular levels of malonyl-CoA. Malonyl-CoA is the physiological inhibitor of CPT1A, which is the rate-limiting step in the mitochondrial FA oxidation pathway. Increased FA oxidation generates reactive oxygen species (ROS) and subsequent uncoupling protein-2 (UCP2) activity. Using a mouse model lacking *Ucp2* in all cells, it had been suggested that UCP2 mediates ghrelin's actions on AgRP neurons by lowering free radicals [82]. However, cell-type specific studies are needed to clarify the role of UCP2 in AgRP neurons in regulating food intake.

Another proposed candidate to mediate ghrelin's orexigenic action in the ARC is the NAD+-dependent class III deacetylases SIRT1. *Sirt1* is expressed in the hypothalamus, its function is redox dependent, and it is induced by negative energy balance. In fact, central administration of ghrelin increases SIRT1 activity in the hypothalamus and blockade of SIRT1 abolished the orexigenic effect of this hormone [83]. Another study showed that the Sirtuin 1/p53 pathway is essential for the orexigenic action of ghrelin [84]. Of note, cell-type specific ablation of Sirt1 ($Sirt1_{AgRP}$-/- mice) in AgRP neurons determined that this neuronal population mediates the action of SIRT1 on energy balance: $Sirt1_{AgRP}$-/- mice show decreased electric responses of AgRP neurons to ghrelin, decreased food intake, decreased lean mass, fat mass, and body weight [83].

Finally, ghrelin stimulates AgRP and NPY neuropeptide expression through the activation of transcriptional factors, such as Brain specific homeobox (BSX) protein. BSX needs to interact with another two transcription factors to activate *Agrp* and *Npy* mRNA expression: FOXO1 for the *Agrp*

gene and the phosphorylated cAMP response-element-binding protein (pCREB) for the *Npy* gene [85]. Yet, the activation of BSX downstream of the AMPK-ACC-CPT1A-UCP2 axis remains unclear. Nonetheless, it has been demonstrated that FOXO formed a complex with SIRT1 in response to oxidative stress [86] and enhances FOXO transcriptional activity [87], but so far there is no evidence that all these events occur specifically in neuronal cells.

5. AgRP neurons regulate complex behaviors beyond feeding

As reviewed in the previous sections, hypothalamic neurons that co-produce NPY, AgRP, and GABA are fundamental in the regulation of food intake and peripheral metabolism. In addition, the study of animal models targeting the activity of AgRP neurons has revealed that these cell populations also coordinate other complex behaviors beyond feeding. Mice with compromised AgRP neuronal excitability (*Sirt1*$_{AgRP}$-/- mice) exhibit greater locomotor activity and exploratory behavior than control littermates during an open field test (OFT) [88]. The same is true in AgRP-neuron-ablated mice. Conversely, brain infusion of AgRP in rats reduces locomotor activity [89]. The OFT is an approach/avoidance assay based on the fact that a novel environment (an open field box) evokes both anxiety and exploration in the experimental animal [90]. Therefore, the increased exploratory behavior observed in *Sirt1*$_{AgRP}$-/- and AgRP-neuron-ablated mice suggests that activity of AgRP neurons regulates anxiety-like behaviors in rodents. In addition, *Sirt1*$_{AgRP}$-/- mice and AgRP-neuron-ablated mice show an increased response and preference for cocaine, suggesting that AgRP neurons also affect reward circuits [88]. Exploring this possibility revealed that AgRP fibers innervate the ventral tegmental area (VTA) in the midbrain, where dopaminergic neurons responsible for reward seeking are located [88]. Intriguingly, the activation of AgRP neurons also decreases anxiety during an OFT and an elevated plus-maze (EPM) test [91]. During an EPM test, anxiety-like behaviors

363 inversely correlate with the time that animals spend in the open arms of an EPM apparatus comprised
364 of two open and two enclosed arms elevated from the floor [92]. The fact that the activation and
365 ablation of AgRP neurons causes an apparently similar decrease in anxiety-like behaviors requires
366 further investigation. However, it also highlights the role of hypothalamic AgRP neurons in regulating
367 complex behavior. Additionally, activation of hypothalamic AgRP neurons in the absence of food
368 stimuli triggers foraging and other stereotypic behaviors [91]. Of note, some aspects of these
369 stereotypic behaviors are not seen in food-deprived animals, which suggests that subpopulations of
370 AgRP neurons have different functions. Altogether, these observations revealed a previously
371 unsuspected role for hypothalamic AgRP neurons in controlling complex behaviors beyond feeding
372 in mice.
373
374 **6. Pharmacological modulation of AgRP neurons**
375 New insights into the neuronal circuit to control feeding behaviors and energy homeostasis are
376 essential to devise specific pharmacological strategies that could be useful to treat obesity and its
377 complications.
378 Many receptors expressed in AgRP neurons have been identified to understand which
379 molecule could be useful for pharmacological manipulation (Table 1). A recent finding revealed that
380 norepinephrine (NE) activates AgRP neurons due to α1A-Ars, which suggests an orexigenic influence
381 of NE in ARC nuclei [93]. Furthermore, Uridine-diphosphate (UD) and its receptor P2Y6 have
382 recently been identified as regulators of AgRP neurons that promote feeding through the activation
383 of these neurons [41]. Interestingly, AgRP neurons express angiotensin AT_{1A} receptor and the lack of
384 this receptor exhibits attenuated BAT sympathetic nerve activity in response to leptin, which suggests
385 that some AT_{1A} agonists and antagonists could be useful to modulate energy expenditure [94]. Many

386 of these receptors belong to the GPCR family receptors with distinct coupling G protein associated.
387 It seems that Gs protein has a strong association with food intake because the activation of Gs-coupled
388 receptor expressed by AgRP neurons leads to a robust increase in food intake [95]**.** Alternatively, key
389 regulatory factors of AgRP activation such as Sirt1 could be interesting targets to control food intake.
390 It has been demonstrated that the Sirt1 inhibitor (EX-527) injected peripherally or icv decreased food
391 intake during the dark cycle and ghrelin-induced food intake [83]. Altogether, this indicates that there
392 is a need for further studies to discover new therapeutic targets and their efficient drugs.

393 **However, the aforementioned receptors and ligands belong to a ubiquitous system and**
394 **therefore new approaches are necessary to target exclusively specific neuronal populations,**
395 **such as AgRP. One possibility is the use of chemogenetic tools. Usually, these technologies**
396 **involve genetically targeting the sensitivity to an otherwise inert small ligand. For example, the**
397 **group of Sternson has developed chemogenetic technologies (such as the pharmacologically**
398 **selective actuator modules/pharmacologically selective effector molecule - PSAM/PSEM) that**
399 **could be applied in conjunction with gene therapies in humans to target specific cell populations.**
400 **Noteworthy, PSAM/PSEM has been already used in mice to silence specifically AgRP neurons,**
401 **which resulted in a suppression of food intake and learning enhancement [96]. In addition,**
402 **chemogenetic approaches have been used in mice to induce dimerization of proteins,**
403 **destabilization of domains or inhibition of engineered kinases in a cell-specific manner [97].**

404 **Alternatively, enormous research efforts have been focused on brain drug delivery to**
405 **develop new therapies for brain disease. Recent studies have analysed new tools to enhance the**
406 **delivery of drugs across the blood brain barrier and drug uptake by neurons. Some of these**
407 **tools include nanocarriers that transport bioactive substances to a target site [98].**
408 **Unfortunately, currently none of these strategies has accomplished a cell-specific drug**

delivery. Nonetheless, these technologies provide an exciting prospect for the future development of human therapies.

7. Conclusions and future directions

The mouse genetic revolution has shown repeatedly that hypothalamic neurons are crucial in the regulation of appetite and whole-body energy balance. In particular, data from all these animal studies have highlighted AgRP and POMC neurons as key components of the neuronal circuits that control food intake and peripheral energy metabolism. In this review, we have focused deliberately on AgRP neurons because of the vast number of animal models targeting this specific neuronal population. In summary, all these *in vivo* studies have established that AgRP neurons integrate interoceptive, nutritional, endocrine, and sensory information to coordinate goal-directed behavior, such as food seeking and intake. Thus, activation of AgRP neurons stimulates feeding as well as a rapid decrease in energy expenditure. At the molecular level, several genes and proteins have been identified as potential players controlling AgRP neuronal function. These include receptors for hormones, such as insulin and ghrelin, enzymes of the FA metabolism pathway, SIRT1, and transcription factors such as ATF4. Of note, many of these molecules might be potential targets in the treatment of eating disorders, *i.e.* anorexia nervosa or compulsive eating, and metabolic diseases such as obesity.

Moving forward, it remains crucial to question whether differences in AgRP neuronal function exist between individuals vulnerable or invulnerable to developing disorders characterized by dysregulated energy balance. Moreover, future studies are needed to determine whether specific subpopulations of AgRP neurons are responsible for the regulation of food intake and other aspects of energy homeostasis. Lastly, considering the heterogeneity of the hypothalamus and the opposite functions of different hypothalamic neuronal populations, we believe that future efforts must be focused on

developing cell-type specific drug delivery methods, in order to design efficient and safe treatments for eating disorders, obesity, and related pathologies.

Disclosure statement

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References

[1] A.W. Hetherington, S.W. Ranson, The relation of various hypothalamic lesions to adiposity in the rat, J. Comp. Neurol. 76 (1942) 475–499. doi:10.1002/cne.900760308.

[2] B.K. Anand, J.R. Brobeck, Hypothalamic control of food intake in rats and cats., Yale J. Biol. Med. 24 (1951) 123–40.

[3] J.K. Elmquist, C.F. Elias, C.B. Saper, From lesions to leptin: hypothalamic control of food intake and body weight., Neuron. 22 (1999) 221–32. doi:S0896-6273(00)81084-3 [pii].

[4] P.S. Singru, E. Sánchez, C. Fekete, R.M. Lechan, Importance of melanocortin signaling in refeeding-induced neuronal activation and satiety, Endocrinology. 148 (2007) 638–646. doi:10.1210/en.2006-1233.

[5] A.A. Van Der Klaauw, J.M. Keogh, E. Henning, C. Stephenson, S. Kelway, V.M. Trowse, N. Subramanian, S. O'Rahilly, P.C. Fletcher, I.S. Farooqi, Divergent effects of central melanocortin signalling on fat and sucrose preference in humans, Nat. Commun. 7 (2016) 1–5. doi:10.1038/ncomms13055.

[6] K.G. Mountjoy, Pro-Opiomelanocortin (POMC) Neurones, POMC-Derived Peptides, Melanocortin Receptors and Obesity: How Understanding of this System has Changed Over the Last Decade, J. Neuroendocrinol. 27 (2015) 406–418. doi:10.1111/jne.12285.

[7] E.J.P. Anderson, I. Cakir, S.J. Carrington, R.D. Cone, M. Ghamari-Langroudi, T. Gillyard, L.E. Gimenez, M.J. Litt, Regulation of feeding and energy homeostasis by α-MSH, J. Mol. Endocrinol. 56 (2016) T157–T174. doi:10.1530/JME-16-0014.

[8] G.S. Kelly J, GABA and hypothalamic feeding systems. II. A comparison of GABA, glycine and actylcholine agonists and their antagonists.Pharmacol Biochem Behav, Pharmacol

473 Biochem Behav. 11 (1979) 647–52.
474 [9] S.P.K. John T. Clark, Pushpa S. Kalra, William R. Crowley, Neuropeptide Y and human
475 pancreatic polypeptide stimulate feeding behavior in rats, Endocrinology. 115 (1984) 427–
476 429. doi:org/10.1210/endo-115-1-427.
477 [10] J. Douglass, a a McKinzie, P. Couceyro, PCR differential display identifies a rat brain
478 mRNA that is transcriptionally regulated by cocaine and amphetamine., J. Neurosci. 15
479 (1995) 2471–2481.
480 [11] K. Yang, H. Guan, E. Arany, D.J. Hill, X. Cao, Neuropeptide Y is produced in visceral
481 adipose tissue and promotes proliferation of adipocyte precursor cells via the Y1 receptor,
482 FASEB J. 22 (2008) 2452–2464. doi:10.1096/fj.07-100735.
483 [12] C.W. Shaun P. Brothers, Therapeutic potential of neuropeptide Y (NPY) receptor ligands,
484 EMBO Mol. Med. 2 (2010) 429–439. doi:10.1002/emmm.201000100.
485 [13] Y. Chen, Z.A. Knight, Making sense of the sensory regulation of hunger neurons, BioEssays.
486 38 (2016) 316–324. doi:10.1002/bies.201500167.
487 [14] Q. Tong, C.-P. Ye, J.E. Jones, J.K. Elmquist, B.B. Lowell, Synaptic release of GABA by
488 AgRP neurons is required for normal regulation of energy balance, Nat. Neurosci. 11 (2008)
489 998. doi:org/10.1038/nn.2167.
490 [15] Margaret M. Dickies, A new viable yellow mutation in the house mouse, J. Hered. 53 (1962)
491 84–86. doi:org/10.1093/oxfordjournals.jhered.a107129.
492 [16] D.C. Albarado, J. McClaine, J.M. Stephens, R.L. Mynatt, J. Ye, A.W. Bannon, W.G.
493 Richards, A.A. Butler, Impaired coordination of nutrient intake and substrate oxidation in
494 melanocortin-4 receptor knockout mice, Endocrinology. 145 (2004) 243–252.
495 doi:10.1210/en.2003-0452.

[17] D. Huszar, C.A. Lynch, V. Fairchild-Huntress, J.H. Dunmore, Q. Fang, L.R. Berkemeier, W. Gu, R.A. Kesterson, B.A. Boston, R.D. Cone, F.J. Smith, L.A. Campfield, P. Burn, F. Lee, Targeted Disruption of the Melanocortin-4 Receptor Results in Obesity in Mice, Cell. 88 (1997) 131–141. doi:10.1016/S0092-8674(00)81865-6.

[18] K.E. Wortley, K.D. Anderson, J. Yasenchak, A. Murphy, D. Valenzuela, S. Diano, G.D. Yancopoulos, S.J. Wiegand, M.W. Sleeman, Agouti-related protein-deficient mice display an age-related lean phenotype, Cell Metab. 2 (2005) 421–427. doi:10.1016/j.cmet.2005.11.004.

[19] Y. He, G. Shu, Y. Yang, P. Xu, Y. Xia, C. Wang, K. Saito, A. Hinton, X. Yan, C. Liu, Q. Wu, Q. Tong, Y. Xu, A Small Potassium Current in AgRP/NPY Neurons Regulates Feeding Behavior and Energy Metabolism, Cell Rep. 17 (2016) 1807–1818. doi:10.1016/j.celrep.2016.10.044.

[20] A. Üner, G.H.M. Gonçalves, W. Li, M. Porceban, N. Caron, M. Schönke, E. Delpire, K. Sakimura, C. Bjørbæk, The role of GluN2A and GluN2B NMDA receptor subunits in AgRP and POMC neurons on body weight and glucose homeostasis, Mol. Metab. 4 (2015) 678–691. doi:10.1016/j.molmet.2015.06.010.

[21] L. Fenno, O. Yizhar, K. Deisseroth, The Development and Application of Optogenetics, Annu. Rev. Neurosci. 34 (2011) 389–412. doi:10.1146/annurev-neuro-061010-113817.

[22] K. Deisseroth, Optogenetics: 10 years of microbial opsins in neuroscience, Nat. Neurosci. 18 (2015) 1213. http://dx.doi.org/10.1038/nn.4091.

[23] M.J. Krashes, S. Koda, C. Ye, S.C. Rogan, A.C. Adams, D.S. Cusher, E. Maratos-Flier, B.L. Roth, B.B. Lowell, Rapid, reversible activation of AgRP neurons drdives feeding behavior in mice, J. Clin. Invest. 121 (2011) 1424–28. doi:10.1172/JCI46229DS1.

[24] S.M. Steculorum, J. Ruud, I. Karakasilioti, H. Backes, L.E. Ruud, K. Timper, M.E. Hess, E.

519 Tsaousidou, J. Mauer, M.C. Vogt, L. Paeger, S. Bremser, A.C. Klein, D.A. Morgan, P.
520 Frommolt, P.T. Brinkkötter, P. Hammerschmidt, T. Benzing, K. Rahmouni, F.T. Wunderlich,
521 P. Kloppenburg, J.C. Brüning, HHS Public Access, 165 (2016) 125–138.
522 doi:10.1016/j.cell.2016.02.044.AgRP.
523 [25] Q. Wu, M.P. Howell, M.A. Cowley, R.D. Palmiter, Starvation after AgRP neuron ablation is
524 independent of melanocortin signaling, Proc. Natl. Acad. Sci. 105 (2008) 2687–2692.
525 doi:10.1073/pnas.0712062105.
526 [26] M. Saito, T. Iwawaki, C. Taya, H. Yonekawa, M. Noda, Y. Inui, E. Mekada, Y. Kimata, A.
527 Tsuru, K. Kohno, Diphtheria toxin receptor–mediated conditional and targeted cell ablation
528 in transgenic mice, Nat. Biotechnol. 19 (2001) 746. doi:org/10.1038/90795.
529 [27] A.J. Physiol, R. Integr, C. Physiol, I.M. Chapman, E.A. Goble, G.A. Wittert, J.E. Morley, K.
530 Beckoff, C.G. Macintosh, J.M. Wishart, A. Howard, M. Horowitz, K.L. Jones, G. Liver,
531 I.A.N.M. Chapman, M. Ian, G.A. Wit-, Effect of intravenous glucose and euglycemic insulin
532 infusions on short-term appetite and food intake appetite in the elderly, (1998) 596–603.
533 [28] D. Burdakov, S.M. Luckman, A. Verkhratsky, Glucose-sensing neurons of the hypothalamus,
534 Philos. Trans. R. Soc. B Biol. Sci. 360 (2005) 2227–2235. doi:10.1098/rstb.2005.1763.
535 [29] B.F. Belgardt, T. Okamura, J.C. Brüning, Hormone and glucose signalling in POMC and
536 AgRP neurons, J. Physiol. 587 (2009) 5305–5314. doi:10.1113/jphysiol.2009.179192.
537 [30] L.E. Parton, C.P. Ye, R. Coppari, P.J. Enriori, B. Choi, C.-Y. Zhang, C. Xu, C.R. Vianna, N.
538 Balthasar, C.E. Lee, J.K. Elmquist, M.A. Cowley, B.B. Lowell, Glucose sensing by POMC
539 neurons regulates glucose homeostasis and is impaired in obesity, Nature. 449 (2007) 228.
540 doi:org/10.1038/nature06098.
541 [31] M. Claret, M.A. Smith, R.L. Batterham, C. Selman, A.I. Choudhury, L.G.D. Fryer, M.

542 Clements, H. Al-qassab, H. Heffron, A.W. Xu, J.R. Speakman, G.S. Barsh, B. Viollet, S.
543 Vaulont, M.L.J. Ashford, D. Carling, D.J. Withers, Jci0731516, 117 (2007).
544 doi:10.1172/JCI31516.suming.
545 [32] N. Ibrahim, M.A. Bosch, J.L. Smart, J. Qiu, M. Rubinstein, O.K. Rønnekleiv, M.J. Low, M.J.
546 Kelly, Hypothalamic proopiomelanocortin neurons are glucose responsive and express
547 KATPchannels, Endocrinology. 144 (2003) 1331–1340. doi:10.1210/en.2002-221033.
548 [33] S.L. Padilla, J.S. Carmody, L.M. Zeltser, Pomc-expressing progenitors give rise to
549 antagonistic neuronal populations in hypothalamic feeding circuits, Nat. Med. 16 (2010) 403.
550 http://dx.doi.org/10.1038/nm.2126.
551 [34] E. Sanz, A. Quintana, J.D. Deem, R.A. Steiner, R.D. Palmiter, G.S. McKnight, Fertility-
552 Regulating Kiss1 Neurons Arise from Hypothalamic Pomc-
553 Expressing Progenitors, J. Neurosci. 35 (2015) 5549 LP-5556.
554 http://www.jneurosci.org/content/35/14/5549.abstract.
555 [35] A.C. Könner, R. Janoschek, L. Plum, S.D. Jordan, E. Rother, X. Ma, C. Xu, P. Enriori, B.
556 Hampel, G.S. Barsh, C.R. Kahn, M.A. Cowley, F.M. Ashcroft, J.C. Brüning, Insulin Action
557 in AgRP-Expressing Neurons Is Required for Suppression of Hepatic Glucose Production,
558 Cell Metab. 5 (2007) 438–449. doi:10.1016/j.cmet.2007.05.004.
559 [36] T. Kitamura, Y. Feng, Y. Ido Kitamura, S.C. Chua Jr, A.W. Xu, G.S. Barsh, L. Rossetti, D.
560 Accili, Forkhead protein FoxO1 mediates Agrp-dependent effects of leptin on food intake,
561 Nat. Med. 12 (2006) 534. doi:org/10.1038/nm1392.
562 [37] L. Varela, M.J. Vázquez, F. Cordido, R. Nogueiras, A. Vidal-Puig, C. Diéguez, M. López,
563 Ghrelin and lipid metabolism: Key partners in energy balance, J. Mol. Endocrinol. 46 (2011)
564 43–63. doi:10.1677/JME-10-0068.

565 [38] W. Ma, G. Fuentes, X. Shi, C. Verma, G.K. Radda, W. Han, FoxO1 negatively regulates
566 leptin-induced POMC transcription through its direct interaction with STAT3, Biochem. J.
567 466 (2015) 291 LP-298. doi:10.1042/BJ20141109.
568 [39] E. Gropp, M. Shanabrough, E. Borok, A.W. Xu, R. Janoschek, T. Buch, L. Plum, N.
569 Balthasar, B. Hampel, A. Waisman, G.S. Barsh, T.L. Horvath, J.C. Brüning, Agouti-related
570 peptide-expressing neurons are mandatory for feeding, Nat. Neurosci. 8 (2005) 1289–1291.
571 doi:10.1038/nn1548.
572 [40] H. Inoue, W. Ogawa, A. Asakawa, Y. Okamoto, A. Nishizawa, M. Matsumoto, K.
573 Teshigawara, Y. Matsuki, E. Watanabe, R. Hiramatsu, K. Notohara, K. Katayose, H.
574 Okamura, C.R. Kahn, T. Noda, K. Takeda, S. Akira, A. Inui, M. Kasuga, Role of hepatic
575 STAT3 in brain-insulin action on hepatic glucose production, Cell Metab. 3 (2006) 267–275.
576 doi:10.1016/j.cmet.2006.02.009.
577 [41] S.M. Steculorum, K. Timper, L. Engström Ruud, N. Evers, L. Paeger, S. Bremser, P.
578 Kloppenburg, J.C. Brüning, Inhibition of P2Y6 Signaling in AgRP Neurons Reduces Food
579 Intake and Improves Systemic Insulin Sensitivity in Obesity, Cell Rep. 18 (2017) 1587–1597.
580 doi:10.1016/j.celrep.2017.01.047.
581 [42] J. Deng, F. Yuan, Y. Guo, Y. Xiao, Y. Niu, Y. Deng, X. Han, Y. Guan, S. Chen, F. Guo,
582 Deletion of ATF4 in AgRP neurons promotes fat loss mainly via increasing energy
583 expenditure, Diabetes. 66 (2017) 640–650. doi:10.2337/db16-0954.
584 [43] S. Obici, Z. Feng, K. Morgan, D. Stein, G. Karkanias, L. Rossetti, Central Administration of
585 Oleic Acid Inhibits Glucose Production and Food Intake, Diabetes. 51 (2002) 271 LP-275.
586 doi:org/10.2337/diabetes.51.2.2.271.
587 [44] N.R.V. Dragano, C. Solon, A.F. Ramalho, R.F. de Moura, D.S. Razolli, E. Christiansen, C.

588 Azevedo, T. Ulven, L.A. Velloso, Polyunsaturated fatty acid receptors, GPR40 and GPR120,
589 are expressed in the hypothalamus and control energy homeostasis and inflammation, J.
590 Neuroinflammation. 14 (2017) 1–16. doi:10.1186/s12974-017-0869-7.
591 [45] C.F. Burant, Activation of GPR40 as a therapeutic target for the treatment of type 2 diabetes,
592 Diabetes Care. 36 (2013) 175–179. doi:10.2337/dcS13-2037.
593 [46] M. López, R. Nogueiras, M. Tena-Sempere, C. Diéguez, Hypothalamic AMPK: a canonical
594 regulator of whole-body energy balance, Nat. Rev. Endocrinol. 12 (2016) 421.
595 doi:org/10.1038/nrendo.2016.67.
596 [47] M.K.Q. Huynh, A.W. Kinyua, D.J. Yang, K.W. Kim, Hypothalamic AMPK as a Regulator of
597 Energy Homeostasis, Neural Plast. 2016 (2016). doi:10.1155/2016/2754078.
598 [48] Z.J. JulianSwierczynski, Elzbieta Goykea, Justyna Korczynska, Acetyl-CoA carboxylase and
599 fatty acid synthase activities in human hypothalamus, Neurosci. Lett. 444 (2008) 209–211.
600 doi:10.1016/jneulet.2008.08.046.
601 [49] M. López, R. Lage, A.K. Saha, D. Pérez-Tilve, M.J. Vázquez, L. Varela, S. Sangiao-
602 Alvarellos, S. Tovar, K. Raghay, S. Rodríguez-Cuenca, R.M. Deoliveira, T. Castañeda, R.
603 Datta, J.Z. Dong, M. Culler, M.W. Sleeman, C. V Álvarez, R. Gallego, C.J. Lelliott, D.
604 Carling, M.H. Tschöp, C. Diéguez, A. Vidal-Puig, Hypothalamic Fatty Acid Metabolism
605 Mediates the Orexigenic Action of Ghrelin, Cell Metab. 7 (2008) 389–399.
606 doi:10.1016/j.cmet.2008.03.006.
607 [50] Y. Minokoshi, T. Alquier, N. Furukawa, Y.-B. Kim, A. Lee, B. Xue, J. Mu, F. Foufelle, P.
608 Ferré, M.J. Birnbaum, B.J. Stuck, B.B. Kahn, AMP-kinase regulates food intake by
609 responding to hormonal and nutrient signals in the hypothalamus, Nature. 428 (2004) 569.
610 doi:org/10.1038/nature02440.

611 [51] F.P.K. Thomas M. Loftus, Donna E. Jaworsky, Gojeb L. Frehywot, Craig A. Townsend,
612 Gabriele V. Ronnett, M. Daniel Lane, Reduced Food Intake and Body Weight in Mice
613 Treated with Fatty Acid Synthase Inhibitors, Science (80-.). 288 (2000) 2379–2382.
614 doi:10.1126/science.288.5475.2379.
615 [52] D.S. Paula Mera , Joan Francesc Mir , Gemma Fabriàs, Josefina Casas, Ana S. H. Costa,
616 Maria Ida Malandrino, José-Antonio Fernández-López, Xavier Remesar, Su Gao, Shigeru
617 Chohnan, Maria Sol Rodríguez-Peña, Harald Petry, Guillermina Asins, Fausto G. Hegardt,
618 Lau, Long-Term Increased Carnitine Palmitoyltransferase 1A Expression in Ventromedial
619 Hypotalamus Causes Hyperphagia and Alters the Hypothalamic Lipidomic Profile, PLoS
620 One. (2004). doi:10.1371/journal.pone.0097195.
621 [53] R. Nogueiras, P. Wiedmer, D. Perez-Tilve, C. Veyrat-Durebex, J.M. Keogh, G.M. Sutton,
622 P.T. Pfluger, T.R. Castaneda, S. Neschen, S.M. Hofmann, P.N. Howles, D.A. Morgan, S.C.
623 Benoit, I. Szanto, B. Schrott, A. Schürmann, H.G. Joost, C. Hammond, D.Y. Hui, S.C.
624 Woods, K. Rahmouni, A.A. Butler, I.S. Farooqi, S. O'Rahilly, F. Rohner-Jeanrenaud, M.H.
625 Tschöp, The central melanocortin system directly controls peripheral lipid metabolism, J.
626 Clin. Invest. 117 (2007) 3475–3488. doi:10.1172/JCI31743.
627 [54] P.D. Raposinho, R.B. White, M.L. Aubert, The melanocortin agonist Melanotan-II reduces
628 the orexigenic and adipogenic effects of neuropeptide Y (NPY) but does not affect the NPY-
629 driven suppressive effects on the gonadotropic and somatotropic axes in the male rat., J.
630 Neuroendocrinol. 15 (2003) 173–81.
631 [55] J.P. Cavalcanti-de-Albuquerque, M.R. Zimmer, J. Bober, M.O. Dietrich, Rapid shift in
632 substrate utilization driven by hypothalamic Agrp neurons, BioRxiv. (2016).
633 doi:org/10.1101/086348.

[56] A. Reichenbach, R. Stark, M. Mequinion, R.R.G. Denis, J.F. Goularte, R.E. Clarke, S.H. Lockie, M.B. Lemus, G.M. Kowalski, C.R. Bruce, C. Huang, R.B. Schittenhelm, R.L. Mynatt, B.J. Oldfield, M.J. Watt, S. Luquet, Z.B. Andrews, AgRP Neurons Require Carnitine Acetyltransferase to Regulate Metabolic Flexibility and Peripheral Nutrient Partitioning, Cell Rep. 22 (2018) 1745–1759. doi:10.1016/j.celrep.2018.01.067.

[57] N.J. Rothwell, M.J. Stock, A role for brown adipose tissue in diet-induced thermogenesis, Nature. 281 (1979) 31. doi:org/10.1038/281031a0.

[58] M.D. Marian Apfelbaum, M.D. Jean Bostsarron, M.D. Dimitri Lacatis, Effect of caloric restriction and excessive caloric intake on energy expenditure, Am. J. Clin. Nutr. 24 (1971) 1405–1409.

[59] X. Yang, H.-B. Ruan, Neuronal Control of Adaptive Thermogenesis, Front. Endocrinol. (Lausanne). 6 (2015) 1–9. doi:10.3389/fendo.2015.00149.

[60] Y.H. Yasuda T, Masaki T, Kakuma T, Hypothalamic melanocortin system regulates sympathetic nerve activity in brown adipose tissue, Exp Biol Med. 229(3):235 (2004).

[61] H. Bin Ruan, M.O. Dietrich, Z.W. Liu, M.R. Zimmer, M.D. Li, J.P. Singh, K. Zhang, R. Yin, J. Wu, T.L. Horvath, X. Yang, O-GlcNAc transferase enables AgRP neurons to suppress browning of white fat, Cell. 159 (2014) 306–317. doi:10.1016/j.cell.2014.09.010.

[62] C.S. Wu, O.Y.N. Bongmba, J. Yue, J.H. Lee, L. Lin, K. Saito, G. Pradhan, D.P. Li, H.L. Pan, A. Xu, S. Guo, Y. Xu, Y. Sun, Suppression of GHS-R in AgRP neurons mitigates diet-induced obesity by activating thermogenesis, Int. J. Mol. Sci. 18 (2017). doi:10.3390/ijms18040832.

[63] G.J. Morton, D.E. Cummings, D.G. Baskin, G.S. Barsh, M.W. Schwartz, Central nervous system control of food intake and body weight, Nature. 443 (2006) 289.

657 doi:org/10.1038/nature05026.
658 [64] J.W. Sohn, Network of hypothalamic neurons that control appetite, BMB Rep. 48 (2015)
659 229–233. doi:10.5483/BMBRep.2015.48.4.272.
660 [65] S.M. Sternson, Let them eat fat, Nature. 477 (2011) 166. doi:org/10.1038/477166a.
661 [66] R.D. Cone, Anatomy and regulation of the central melanocortin system, Nat. Neurosci. 8
662 (2005) 571. doi:org/10.1038/nn1455.
663 [67] R.M. Leibowitz SF, Roossin P, Chronic norepinephrine injection into the hypothalamic
664 paraventricular nucleus produces hyperphagia and increased body weight in the rat.,
665 Pharmacol Biochem Behav. 21(5):801- (1984).
666 [68] Y. Aponte, D. Atasoy, S.M. Sternson, AGRP neurons are sufficient to orchestrate feeding
667 behavior rapidly and without training, Nat. Neurosci. 14 (2011) 351.
668 doi:org/10.1038/nn.2739.
669 [69] J.J. Warren, D. Blanchette, D. V Dawson, A. Teresa, K.R. Phipps, D. Starr, D.R. Drake, HHS
670 Public Access, 44 (2017) 319–335. doi:10.1111/cdoe.12200.Factors.
671 [70] K. Tan, Z.A. Knight, J.M. Friedman, Ablation of AgRP neurons impairs adaption to
672 restricted feeding, Mol. Metab. 3 (2014) 694–704. doi:10.1016/j.molmet.2014.07.002.
673 [71] R.D.P. Serge Luquet, Francisco A. Perez, Thomas S. Hnasko, NPY/AgRP Neurons Are
674 Essential for Feeding in Adult Mice but Can Be Ablated in Neonates, Science (80-.). 310
675 (2005) 683–685. doi:10.1126/science.1115524.
676 [72] J.C. Erickson, K.E. Clegg, R.D. Palmiter, Sensitivity to leptin and susceptibility to seizures of
677 mice lacking neuropeptide Y, Nature. 381 (1996) 415. doi:org/10.1038/381415a0.
678 [73] S. Qian, H. Chen, D. Weingarth, E. Myrna, D.E. Novi, X. Guan, H. Yu, Y. Feng, E. Frazier,
679 A. Chen, E. Ramon, L.P. Shearman, S. Gopal-truter, D.J. Macneil, L.H.T. Van Der Ploeg, J.

680 Donald, M.E. Trumbauer, Z. Shen, R.E. Camacho, D.J. Marsh, Neither Agouti-Related
681 Protein nor Neuropeptide Y Is Critically Required for the Regulation of Energy Homeostasis
682 in Mice, Society. 22 (2002) 5027–5035. doi:10.1128/MCB.22.14.5027.
683 [74] Q. Wu, M.P. Boyle, R.D. Palmiter, Loss of GABAergic Signaling by AgRP Neurons to the
684 Parabrachial Nucleus Leads to Starvation, Cell. 137 (2009) 1225–1234.
685 doi:10.1016/j.cell.2009.04.022.
686 [75] Joel K. Elmquist Roberto Coppari Nina Balthasar Masumi Ichinose Bradford B. Lowell,
687 Identifying hypothalamic pathways controlling food intake, body weight, and glucose
688 homeostasis, J. Comp. Neurol. 493 (n.d.) 63–71. doi:org/10.1002/cne.20786.
689 [76] D. Atasoy, J.N. Betley, H.H. Su, S.M. Sternson, Deconstruction of a neural circuit for hunger,
690 Nature. 488 (2012) 172. doi:org/10.1038/nature11270.
691 [77] P. J. Verhulst A. Lintermans S. Janssen D. Loeckx U. Himmelreich J. Buyse J. Tack I.
692 Depoortere, GPR39, a Receptor of the Ghrelin Receptor Family, Plays a Role in the
693 Regulation of Glucose Homeostasis in a Mouse Model of Early Onset Diet-Induced Obesity,
694 J. Neuroendocrinol. 23 (2011) 490–500. doi:10.1111/j.1365-28262011.02132.x.
695 [78] Y. Sun, P. Wang, H. Zheng, R.G. Smith, Ghrelin stimulation of growth hormone release and
696 appetite is mediated through the growth hormone secretagogue receptor., Proc. Natl. Acad.
697 Sci. U. S. A. 101 (2004) 4679–84. doi:10.1073/pnas.0305930101.
698 [79] D. Chen, S., Chen, H., Zhou, J., Pradhan, G., Sun, Y., Pan, H. and Li, Ghrelin receptors
699 mediate ghrelin-induced excitation of agouti-related protein/neuropeptide Y but not pro-
700 opiomelanocortin neurons, J. Neurochem. 4 (2017). doi:org/10.1111/jnc.14080.
701 [80] M. Goto, H. Arima, M. Watanabe, M. Hayashi, R. Banno, I. Sato, H. Nagasaki, Y. Oiso,
702 Ghrelin increases neuropeptide Y and agouti-related peptide gene expression in the arcuate

703 nucleus in rat hypothalamic organotypic cultures, Endocrinology. 147 (2006) 5102–5109.
704 doi:10.1210/en.2006-0104.
705 [81] R. Lage, C. Diéguez, A. Vidal-Puig, M. López, AMPK: a metabolic gauge regulating whole-
706 body energy homeostasis, Trends Mol. Med. 14 (2008) 539–549.
707 doi:10.1016/j.molmed.2008.09.007.
708 [82] Z.B. Andrews, Z.-W. Liu, N. Walllingford, D.M. Erion, E. Borok, J.M. Friedman, M.H.
709 Tschöp, M. Shanabrough, G. Cline, G.I. Shulman, A. Coppola, X.-B. Gao, T.L. Horvath, S.
710 Diano, UCP2 mediates ghrelin's action on NPY/AgRP neurons by lowering free radicals,
711 Nature. 454 (2008) 846. doi:org/10.1038/nature07181.
712 [83] M.O. Dietrich, C. Antunes, G. Geliang, Z. Liu, Y. Nie, A.W. Xu, D.O. Souza, Q. Gao, S.
713 Diano, B. Gao, T.L. Horvath, Agrp neurons mediate Sirt's action on the melanocortin system
714 and energy balace: roles for Sirt1 in neuronal firing and synaptic plasticity, 30 (2011) 11815–
715 11825. doi:10.1523/JNEUROSCI.2234-10.2010.Agrp.
716 [84] D.A. Velásquez, G. Martínez, A. Romero, M.J. Vázquez, K.D. Boit, I.G. Dopeso-Reyes, M.
717 López, A. Vidal, R. Nogueiras, C. Diéguez, The Central Sirtuin 1/p53 Pathway Is Essential
718 for the Orexigenic Action of Ghrelin, Diabetes. 60 (2011) 1177 LP-1185.
719 doi:org/10.2337/db10-0802.
720 [85] R. Nogueiras, M. López, R. Lage, D. Perez-Tilve, P. Pfluger, H. Mendieta-Zerón, M. Sakkou,
721 P. Wiedmer, S.C. Benoit, R. Datta, J.Z. Dong, M. Culler, M. Sleeman, A. Vidal-Puig, T.
722 Horvath, M. Treier, C. Diéguez, M.H. Tschöp, Bsx, a novel hypothalamic factor linking
723 feeding with locomotor activity, is regulated by energy availability, Endocrinology. 149
724 (2008) 3009–3015. doi:10.1210/en.2007-1684.
725 [86] A. Brunet, L.B. Sweeney, J.F. Sturgill, K.F. Chua, P.L. Greer, Y. Lin, H. Tran, S.E. Ross, R.

726 Mostoslavsky, H.Y. Cohen, L.S. Hu, H.-L. Cheng, M.P. Jedrychowski, S.P. Gygi, D.A.
727 Sinclair, F.W. Alt, M.E. Greenberg, Stress-Dependent Regulation of FOXO Transcription
728 Factors by the SIRT1 Deacetylase, Science (80-.). 303 (2004) 2011 LP-2015.
729 doi:10.1126/science.1094647.
730 [87] P.S. Pardo, J.S. Mohamed, M.A. Lopez, A.M. Boriek, Induction of Sirt1 by mechanical
731 stretch of skeletal muscle through the early response factor EGR1 triggers an antioxidative
732 response, J. Biol. Chem. 286 (2011) 2559–2566. doi:10.1074/jbc.M110.149153.
733 [88] M.O. Dietrich, J. Bober, J.G. Ferreira, L.A. Tellez, Y.S. Mineur, D.O. Souza, X.-B. Gao,
734 M.R. Picciotto, I. Araújo, Z.-W. Liu, T.L. Horvath, AgRP neurons regulate development of
735 dopamine neuronal plasticity and nonfood-associated behaviors, Nat. Neurosci. 15 (2012)
736 1108. doi:org/10.1038/nn.3147.
737 [89] M. Tang-Christensen, N. Vrang, S. Ortmann, M. Bidlingmaier, T.L. Horvath, M. Tschöp,
738 Central administration of ghrelin and agouti-related protein (83-132) increases food intake
739 and decreases spontaneous locomotor activity in rats, Endocrinology. 145 (2004) 4645–4652.
740 doi:10.1210/en.2004-0529.
741 [90] A. Lira, M. Zhou, N. Castanon, M.S. Ansorge, J.A. Gordon, J.H. Francis, M. Bradley-Moore,
742 J. Lira, M.D. Underwood, V. Arango, H.F. Kung, M.A. Hofer, R. Hen, J.A. Gingrich, Altered
743 depression-related behaviors and functional changes in the dorsal raphe nucleus of serotonin
744 transporter-deficient mice, Biol. Psychiatry. 54 (2003) 960–971. doi:10.1016/S0006-
745 3223(03)00696-6.
746 [91] M.O. Dietrich, M.R. Zimmer, J. Bober, T.L. Horvath, Hypothalamic Agrp Neurons Drive
747 Stereotypic Behaviors beyond Feeding, Cell. 160 (2015) 1222–1232.
748 doi:10.1016/j.cell.2015.02.024.

749 [92] A. Sahay, K.N. Scobie, A.S. Hill, C.M. O'Carroll, M.A. Kheirbek, N.S. Burghardt, A.A.
750 Fenton, A. Dranovsky, R. Hen, Increasing adult hippocampal neurogenesis is sufficient to
751 improve pattern separation, Nature. 472 (2011) 466. doi:org/10.1038/nature09817.
752 [93] L. Paeger, I. Karakasilioti, J. Altmüller, P. Frommolt, J. Brüning, P. Kloppenburg,
753 Antagonistic modulation of NPY/AgRP and POMC neurons in the arcuate nucleus by
754 noradrenalin, Elife. 6 (2017) 1–18. doi:10.7554/eLife.25770.
755 [94] K.E. Claflin, J.A. Sandgren, A.M. Lambertz, B.J. Weidemann, N.K. Littlejohn, C.M.L.
756 Burnett, N.A. Pearson, D.A. Morgan, K.N. Gibson-corley, K. Rahmouni, J.L. Grobe,
757 Angiotensin AT 1A receptors on leptin receptor – expressing cells control resting
758 metabolism, 127 (2017) 1–11. doi:10.1172/JCI88641.
759 [95] K. Nakajima, Z. Cui, C. Li, J. Meister, Y. Cui, O. Fu, A.S. Smith, S. Jain, B.B. Lowell, M.J.
760 Krashes, J. Wess, Gs-coupled GPCR signalling in AgRP neurons triggers sustained increase
761 in food intake, Nat. Commun. 7 (2016) 10268. doi:org/10.1038/ncomms10268.
762 [96] J.N. Betley, S. Xu, Z.F.H. Cao, R. Gong, C.J. Magnus, Y. Yu, S.M. Sternson, Neurons for
763 hunger and thirst transmit a negative-valence teaching signal, Nature. 521 (2015) 180.
764 http://dx.doi.org/10.1038/nature14416.
765 [97] D. Atasoy, S.M. Sternson, Chemogenetic Tools for Causal Cellular and Neuronal Biology,
766 Physiol. Rev. 98 (2017) 391–418. doi:10.1152/physrev.00009.2017.
767 [98] Y. Anraku, H. Kuwahara, Y. Fukusato, A. Mizoguchi, T. Ishii, K. Nitta, Y. Matsumoto, K.
768 Toh, K. Miyata, S. Uchida, K. Nishina, K. Osada, K. Itaka, N. Nishiyama, H. Mizusawa, T.
769 Yamasoba, T. Yokota, K. Kataoka, Glycaemic control boosts glucosylated nanocarrier
770 crossing the BBB into the brain, Nat. Commun. 8 (2017) 1001. doi:10.1038/s41467-017-
771 00952-3.

772 [99] L. Yang, Y. Qi, Y. Yang, Astrocytes Control Food Intake by Inhibiting AGRP Neuron
773 Activity via Adenosine A1 Receptors, Cell Rep. 11 (2015) 798–807.
774 doi:10.1016/j.celrep.2015.04.002.
775 [100] B. Liu, Tiemin & Kong, Dong & Shah, Bhavik & Ye, Chian & Koda, Shuichi & Saunders,
776 Arpiar & Ding, Jun & Yang, Zongfang & L Sabatini, Bernardo & B Lowell, Fasting
777 Activation of AgRP Neurons Requires NMDA Receptors and Involves Spinogenesis and
778 Increased Excitatory Tone, Neuron. 73. 511-22 (2012). doi:10.1016/j.neuron.2011.11.027.
779 [101] A.H. Shibata M., Banno R., Sugiyama M., Tominaga T., Onoue T., Tsunekawa T., Azuma
780 Y., Hagiwara D., Lu W., Ito Y., Goto M., Suga H., Sugimura Y., Oiso Y., AgRP Neuron-
781 Specific Deletion of Glucocorticoid Receptor Leads to Increased Energy Expenditure and
782 Decreased Body Weight in Female Mice on a High-Fat Diet, Endocrinology. 157 (2016)
783 1457–1466. doi:org/10.1210/en.2015-1430.
784 [102] D.O. and C.B. Andrew C. Shin, Nika Filatova, Claudia Lindtner , Tiffany Chi, Seta Degann,
785 Insulin receptor signaling in POMC, but not AgRP, neurons controls adipose tissue insulin
786 action, Diabetes. 66 (2017) 1560 LP-1571. doi:10.2337/db16-1238.
787 [103] O.K. Egan, M.A. Inglis, G.M. Anderson, Leptin Signaling in AgRP Neurons Modulates
788 Puberty Onset and Adult Fertility in Mice, J. Neurosci. 37 (2017) 3875 LP-3886.
789 doi:10.1523/JNEUROSCI.3138-16.2017.
790 [104] Y. Kuperman, M. Weiss, J. Dine, K. Staikin, O. Golani, A. Ramot, T. Nahum, C. Kühne, Y.
791 Shemesh, W. Wurst, A. Harmelin, J.M. Deussing, M. Eder, A. Chen, CRFR1 in AgRP
792 Neurons Modulates Sympathetic Nervous System Activity to Adapt to Cold Stress and
793 Fasting, Cell Metab. 23 (2016) 1185–1199. doi:10.1016/j.cmet.2016.04.017.
794 [105] S.M. Steculorum, L. Paeger, S. Bremser, N. Evers, Y. Hinze, M. Idzko, P. Kloppenburg, J.C.

795 Brüning, Hypothalamic UDP Increases in Obesity and Promotes Feeding via P2Y6-
796 Dependent Activation of AgRP Neurons, Cell. 162 (2015) 1404–1417.
797 doi:10.1016/j.cell.2015.08.032.
798 [106] J. Wu, C. Zhu, L. Yang, Z. Wang, L. Wang, S. Wang, P. Gao, Y. Zhang, Q. Jiang, X. Zhu, G.
799 Shu, N-Oleoylglycine-Induced Hyperphagia Is Associated with the Activation of Agouti-
800 Related Protein (AgRP) Neuron by Cannabinoid Receptor Type 1 (CB1R), J. Agric. Food
801 Chem. 65 (2017) 1051–1057. doi:10.1021/acs.jafc.6b05281.
802 [107] J. Xu, A.M. Bernstein, A. Wong, X.-H. Lu, S. Khoja, X.W. Yang, D.L. Davies, P. Micevych,
803 M. V Sofroniew, B.S. Khakh, P2X4 Receptor Reporter Mice: Sparse Brain Expression and
804 Feeding-Related Presynaptic Facilitation in the Arcuate Nucleus, J. Neurosci. 36 (2016) 8902
805 LP-8920. doi:10.1523/JNEUROSCI.1496-16.2016.
806 [108] H. Ren, J.R. Cook, N. Kon, D. Accili, Gpr17 in AgRP Neurons Regulates Feeding and
807 Sensitivity to Insulin and Leptin, Diabetes. 64 (2015) 3670 LP-3679. doi:10.2337/db15-0390.
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809

Figure legends

Figure 1: Melanocortin system. AgRP/NPY and POMC/CART neurons are located at the base of the hypothalamus in close proximity to fenestrated capillaries. POMC neurons exert an anorexigenic function and increase energy expenditure through the expression of the post-transcriptional product of the POMC gene, including α-melanocyte-stimulating hormone (α-MSH), β-MSH, Ɣ-MSH that binds to the melanocortin receptor (MCR), an anorexigenic target. NPY/AgRP neurons increase food intake and decrease energy expenditure through the release of neuropeptide Y (NPY), γ- amino-butyric acid (GABA), and agouti-related protein (AgRP). NPY act through NPY1R and NPY5R, while AgRP antagonize α-MSH action and GABA is an inhibitory neurotransmitter that exerts orexigenic action through GABAergic-mediated inhibition of POMC neurons.

Figure 2: Glucose and insulin pathways´connection between the CNS and peripheral system..1) Meal consumption increases glucose levels, which depolarize POMC neurons due to the increase in the ATP/ADP ratio that closes the KATP channel. 2) In AgRP neurons, the increase in glucose level causes AKT phosphorylation that activates neuronal nitric oxide synthase (nNOS) and produces nitric oxide (NO). NO binds to soluble guanylyl cyclase (sGC) increasing cyclic guanosine monophosphate (cGMP) levels. cGMP activates protein kinase GII (PKGII) that phosphorylates the cystic fibrosis transmembrane conductance regulator (CFTR), increasing conductance to the chloride channel and hyperpolarizing the neuron. 3) The insulin produced as a result of an increase in glucose level inactivates AgRP neurons as a feedback mechanism. Insulin acts on AgRP neurons through insulin receptor (IR). Insulin receptor substrate (IRS) proteins bind to phosphorylated residues on the IR to activate phosphoinositide 3 kinase (PI3K) that produces phosphatidylinositol 3 phosphate (PIP3) from

833 phosphatidylinositol 2 phosphate (PIP2). PIP3 activates KATP, resulting in hyperpolarization and
834 decreasing the firing rate. Phosphoinositide-dependent kinase 1 (PDK1) binds to PIP3 and activates
835 AKT, which enters the nucleus and leads to the exclusion of the forkhead box O (FOXO) 1, which
836 decreases AgRP transcription. 4) The inhibition of AgRP neurons increases the expression of hepatic
837 IL-6 through liver innervation. IL-6 activates Janus kinase/signal transducers and activators of the
838 transcription (Jack-STAT) pathway that inhibits the expression of glucose-6-phosphatase (G6Pase),
839 thereby suppressing hepatic gluconeogenesis. The insulin signaling pathway has also been described
840 in POMC neurons, but the exclusion of FOXO1 from the nucleus promotes POMC transcriptional
841 activation.
842
843 **Figure 3: Ghrelin signaling pathway in AgRP neurons**. Ghrelin activates the growth hormone
844 secretagogue receptor 1a (GHSR1a), a G couple receptor that interacts with Gq protein. The G alpha
845 subunit recruits phospholipase C (PLCß) that produces inosito-1,4,5-triphosphate (IP3), which
846 increases the Ca^{++} level and interacts with calmodulin (CaM) to activate CaM-dependent protein
847 kinase kinases (CaMKK). This enzyme phosphorylates AMPK, which phosphorylates acetyl-CoA
848 carboxylase (ACC). ACC activation causes a reduction in malonyl-CoA and disinhibition of carnitine
849 palmitoyl transferase 1 (CPT1). The CPT1A enzyme increases long chain fatty acid (LCFA) transport
850 into mitochondria for oxidation. Increased fatty-acid oxidation results in an increase of reactive
851 oxygen species (ROS) and subsequent uncoupling protein-2 (UCP2) activity, which helps to buffer
852 intracellular ROS. UCP2 acts as a critical mitochondrial biogenic signal that further enhances the
853 bioenergetic capacity of AgRP neurons and ensures efficient transcription of AgRP and NPY genes
854 through the transcriptional factors BSX with FoxO1 and pCREB. The Sirtuin 1/p53 pathway is
855 essential for the orexigenic action of ghrelin and is involved in AMPK activation.

856

857 **Table 1. Receptors expressed in AgRP neurons**

858

Receptor	Type	Ligand	Function	Reference
Adenosine A1 receptors (A1R)	G protein-coupled receptors	Adenosine	Astrocytes inhibit AgRP neuron activity via A1R	[99]
Angiotensin AT1A receptors	G protein-coupled receptors	Angiotensin	Contribute to the control of resting metabolic rate (RMR)	[94]
NMDA receptors (NMDARs)	Ionotropic glutamate receptors	Glutamate	Control of body weight, body fat, food intake, glucose homeostasis.	[20,100]
Glucocorticoid receptors	Nuclear receptor	Glucocorticoids	Control of body weight and energy expenditure	[101]
Insulin receptor	Tyrosine kinase receptors	Insulin	Control of body weight and food intake	[35,102]
Leptin receptor LepR	Type I cytokine receptor	Leptin	Control of food intake	[103]
α2A – adrenergic receptors	G protein-coupled receptors	Noradrenalin	Feeding and food intake	[93]
Corticotropin-releasing factor receptor type 1 (CRFR1)	G protein-coupled receptors	Corticotropin-releasing hormone (CRH)	Protects the organism from hypothermia and hypoglycemia	[104]
Growth hormone secretagogue receptor (GHS-R1A)	G protein-coupled receptors	Ghrelin	Food intake	[80]
Pyrimidinergic receptor P2Y6	G protein-coupled receptors	Uridine-diphosphate	Food intake and systemic insulin sensitivity in obesity	[41,105]
Cannabinoid receptor type 1 (CB1R)	G protein-coupled receptors	N-oleoylglycine (OLGly)	Food intake	[106]
Purinergic receptor P2X4	ATP-gated cation channels	ATP	Food intake	[107]

Uracil nucleotide/cysteinyl leukotriene receptor GPR17	G protein-coupled receptors	Purinergic and cysteinyl-leukotriene ligands Unknown endogenous ligand	Energy balance and glucose homeostasis	[108]
Polyunsaturated fatty acid (PUFA) receptors GPR40	G protein-coupled receptors	Polyunsaturated fatty acid	Body weight mass regulation	[44]

859

Figure 1

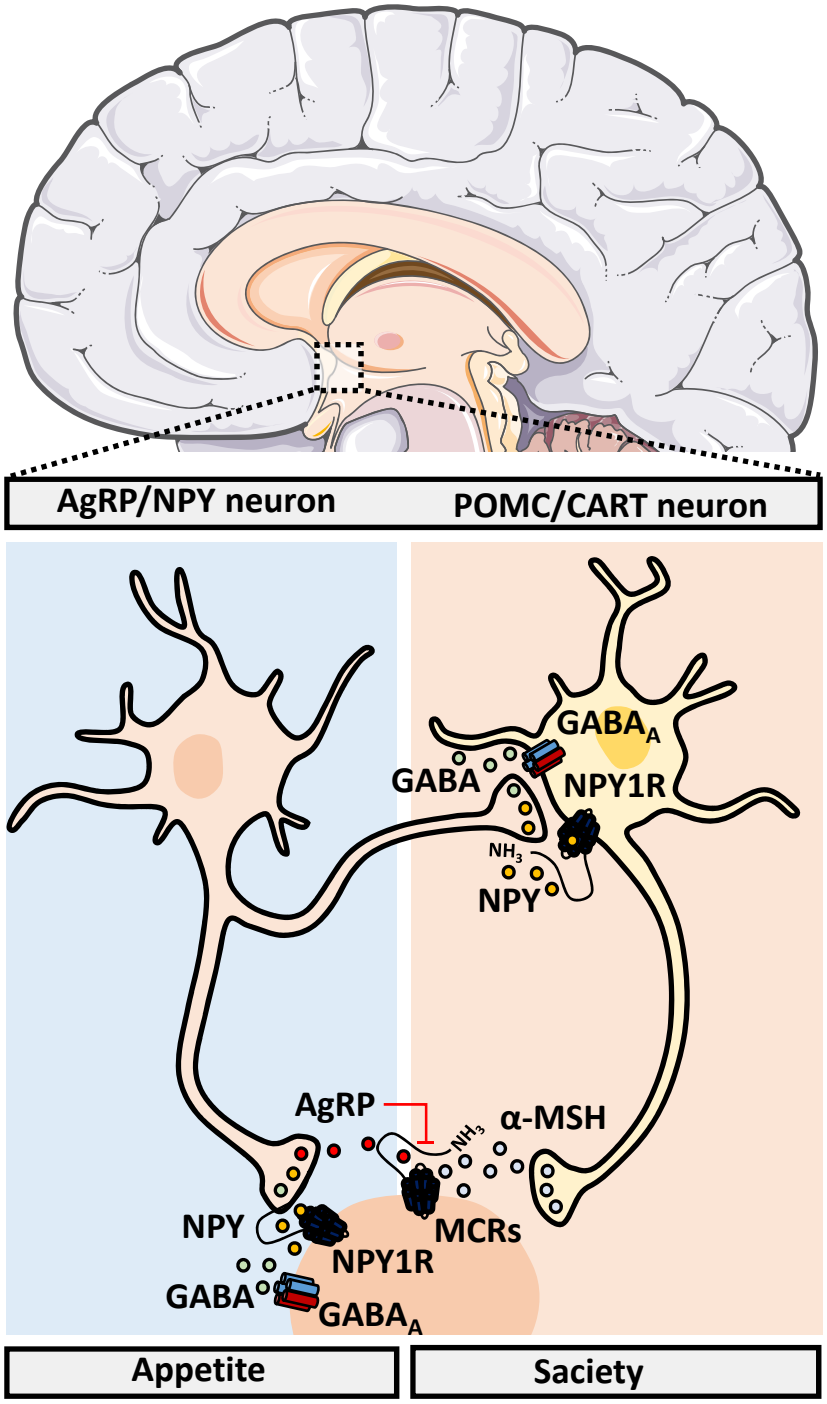

AgRP/NPY neuron
POMC/CART neuron
GABA$_A$
GABA
NPY1R
NH$_3$
NPY
AgRP
α-MSH
NH$_3$
NPY
MCRs
NPY1R
GABA
GABA$_A$
Appetite
Saciety

Figure 2

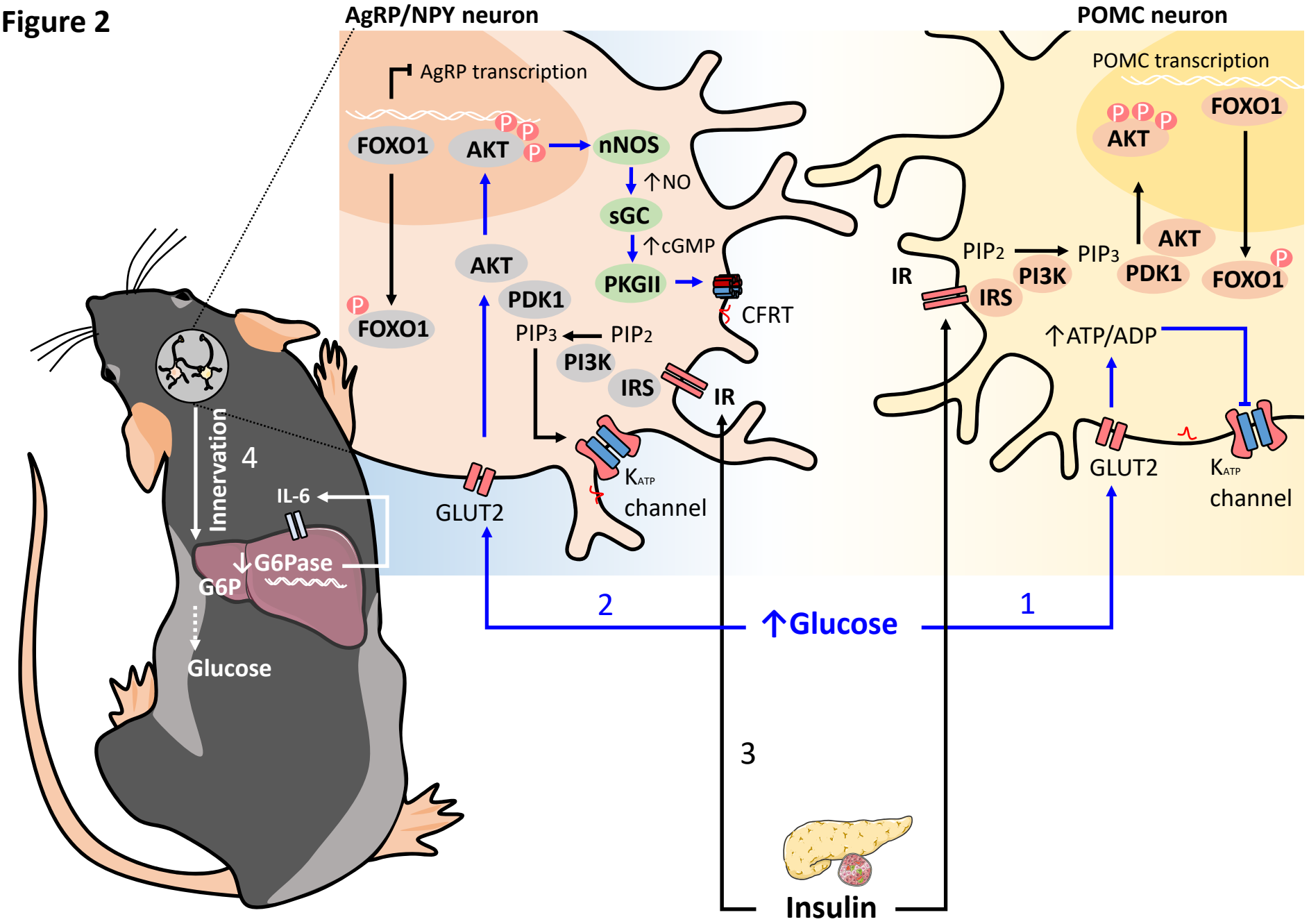
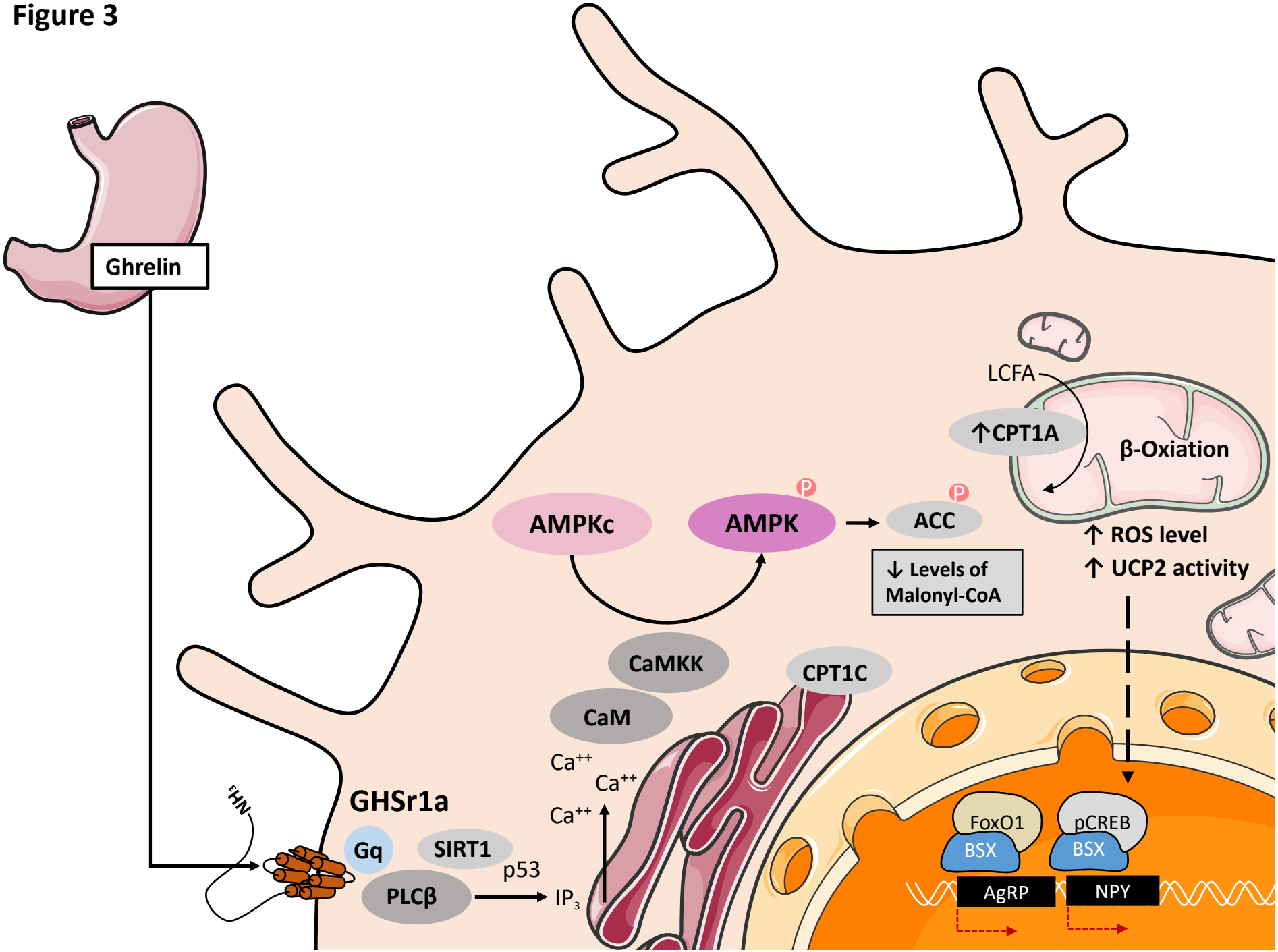

AgRP/NPY neuron
POMC neuron
AgRP transcription
POMC transcription
FOXO1
AKT
nNOS
↑NO
sGC
↑cGMP
PKGII
CFRT
PDK1
PIP3
PIP2
PI3K
IRS
IR
GLUT2
KATP channel
↑ATP/ADP
Innervation
4
IL-6
G6Pase
G6P
Glucose
2
↑Glucose
1
3
Insulin

Figure 3


Ghrelin
LCFA
↑CPT1A
β-Oxiation
AMPKc
AMPK
ACC
↓ Levels of Malonyl-CoA
↑ ROS level
↑ UCP2 activity
CaMKK
CaM
CPT1C
Ca++
Ca++
Ca++
GHSr1a
NH2
Gq
SIRT1
p53
PLCβ
IP3
FoxO1
BSX
pCREB
BSX
AgRP
NPY