GENETIC APPROACHES TO STUDYING ENERGY BALANCE: PERCEPTION AND INTEGRATION

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The homeostatic regulation of adiposity is a physiological concept that originated nearly 70 years ago, the genetic foundations of which have just begun to emerge. A key element of this concept is the existence of one or more humoral mediators, which circulate at levels that reflect body fat stores and which signal through neuronal receptors to elicit appropriate behavioural and metabolic responses over short time periods. Initial insights into adiposity regulation came from the positional cloning of mouse obesity mutations, but the field is now poised to address specific physiological questions using more sophisticated genetic approaches.

FORWARD GENETICS
A genetic analysis that proceeds from phenotype to genotype by positional cloning or candidate-gene analysis.

REVERSE GENETICS
Genetic analysis that proceeds from genotype to phenotype through gene-manipulation techniques.

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The past decade has seen a remarkable increase in our understanding of the molecular mechanisms that regulate energy homeostasis. Driven initially by the identification and analysis of previously existing mouse obesity mutations, such as *obese* (*ob*), *diabetes* (*db*), *agouti lethal yellow* (A^y) and *tubby*, this FORWARD GENETICS approach has been supplemented more recently by REVERSE GENETICS approaches, such as gene-inactivation and transgenic experiments.

The physiological characterization of mutant animals is central to both types of genetic approach. A genotype–phenotype correlation, regardless of how it is made, is only the first step in framing a mechanism-based hypothesis. We must ask not only what is the molecule and what does it do, but also how does it do it? Knockouts with no phenotype, obesity and diabetes genes with no apparent mechanism of action and physiological pathways with no molecules — once the bane of their respective investigators — are becoming less common as different approaches are brought to bear on a single biological question. In this sense, the 'whole' yielded by a multidisciplinary approach that combines genetics and physiology clearly exceeds the sum of its parts.

In recent years, our understanding of how information about body energy stores is communicated to the brain and subsequently integrated into behavioural and metabolic responses has greatly improved. Much of this progress is due to the identification of specific neurons in the ARCUATE NUCLEUS of the hypothalamus that act as sensors of whole-body energy status and that initiate downstream responses designed to maintain fuel stores at a constant level. Although many regions of the brain are involved in energy homeostasis, circuits that begin in the arcuate nucleus are some of the best understood at a molecular level. In this review, we highlight the roles of subsets of arcuate nucleus neurons in energy homeostasis, discuss downstream pathways that mediate the effects initiated by these cells and then consider how signals from the periphery are integrated in the arcuate nucleus. Throughout, we emphasize how studies that use forward genetics or reverse genetics approaches complement those that rely on more conventional physiological and pharmacological strategies, and discuss the extent to which pathways that are defined in rodents pertain to humans. Finally, we consider how this trio of approaches — genetics, physiology and pharmacology — can be applied most effectively to develop treatments for disorders of energy balance.

Historical perspective

In a leading hypothesis that guided the work of early neuroscientists, it was proposed that neuronal contributions to various bodily functions could be localized to discrete brain regions or 'centres'. Much of the data that

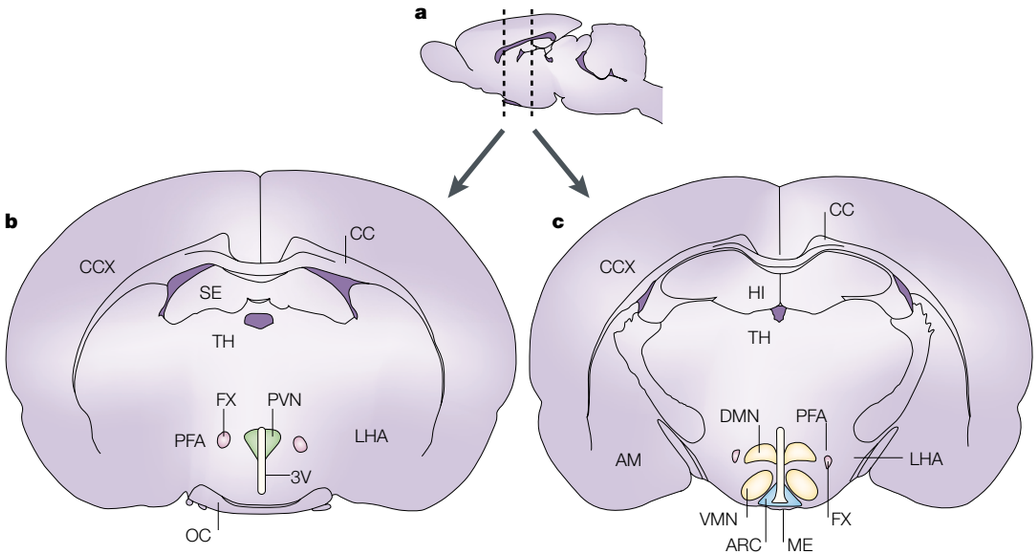

Figure 1 | **Main hypothalamic regions involved in regulation of food intake in the rat brain. a** | A longitudinal view of a rat brain, with olfactory bulb at the anterior end on the left and the caudal hindbrain at the posterior end on the right. **b**,**c** | Cross-sections of the brain (indicated by dashed lines in **a**). First-order neurons are those that respond to humoral signals. These are located in the arcuate nucleus (ARC, shown in turquoise) and project anteriorly to the paraventricular nucleus (PVN, shown in green), as well as to the perifornical area (PFA), which is adjacent to the fornix (FX, shown in pink), and to the lateral hypothalamic area (LHA). Other regions implicated in the control of food intake include the ventromedial nucleus (VMN) and dorsomedial nucleus (DMN). Abbreviations: AM, amygdala; CC, corpus callosum; OC, optic chiasm; SE, septum; TH, thalamus; 3V, third ventricle. Modified with permission from REF. 4 *Nature* © (2000) Macmillan Magazines Ltd.

support this hypothesis were based on lesioning studies, in which the role of a specific brain area was investigated by evaluating the consequences of its destruction. Among the most pronounced outcomes of such studies was the discovery, in the late 1940s, that bilateral lesions of the LATERAL HYPOTHALAMIC AREA (LHA) in rats cause anorexia and weight loss, whereas lesions of the hypothalamic ventromedial nucleus (VMN) (FIG. 1) cause profound obesity[1,2]. From these observations, a model emerged in which the LHA and VMN were believed to be the brain centres that control hunger and satiety, respectively; this model dominated the field of food-intake regulation for several decades.

Because brain lesioning studies are grounded in neuroanatomy, a positive outcome — for example, altered food-intake or body-weight regulation — is interpreted from the perspective of input to, output from or processing in the ablated region. However, few conclusions can be drawn from negative outcomes. The comparatively mild disorder of food intake and body weight that results from bilateral lesions of the hypothalamic arcuate nucleus exemplifies the inherent limitations of lesion-based studies of brain function[3]. On the basis of this unremarkable outcome, the arcuate nucleus was, for many years, assumed to be unimportant for the control of food intake. Eventually, however, it became evident that this brain area contains two discrete neuronal subsets, one that powerfully increases, and one that reduces, food intake[4]. Because electrolytic lesions destroy both sets of neurons, food intake is not markedly affected in lesioned animals, so revealing little about the contribution of these neuronal subsets to feeding behaviour. Because of this type of limitation, region-based models of brain function have given way to more recent molecular efforts to identify specific neuronal cell types that are involved in energy homeostasis, and to identify the molecules they contain, their projection fields and the factors that are involved in their regulation.

ARCUATE NUCLEUS
A region of the hypothalamus that lies at its most ventral portion and surrounds the third ventricle.

LATERAL HYPOTHALAMIC AREA (LHA). Hypothalamic region, adjacent to the perifornical area, that produces melanin concentrating hormone and hypocretin/orexin. Lesions of the LHA cause decreased feeding.

The wiring of the arcuate nucleus

Genetics and pharmacology sometimes disagree. The rapidly evolving story of the arcuate nucleus and food-intake regulation began with the discovery of neuropeptide Y (Npy) in 1982 (REF. 5) (BOX 1), which was identified owing to its similarity to pancreatic polypeptide and peptide YY. Its powerful stimulatory effects on food intake in rodents when centrally administered was a surprising result that was first reported by investigators studying the hypothalamic control of reproductive function[6]. Npy-producing neurons are found throughout the brain but, in the hypothalamus, are concentrated in the arcuate nucleus[4]. Mindful of the difference between what a gene product can do and what it normally does do, several groups investigated how changes in energy balance affect the activity of arcuate nucleus Npy neurons[7]. Although changes in neuropeptide mRNA levels are only indirectly related to changes in synaptic release, there is a striking parallel between stimuli that increase food intake in rodents and levels of arcuate nucleus *Npy* mRNA. For example, both the compensatory hyperphagia caused by previous food deprivation and the hyperphagia that accompanies uncontrolled diabetes cause an increase in arcuate nucleus *Npy* mRNA levels[8,9]. *Npy* mRNA levels were

NEUROPEPTIDES
Secreted peptides produced by neurons in the brain or spinal cord that are used for cell–cell communication, often as neurotransmitters.

PARACRINE
A form of cell–cell communication that depends on a secreted substance that acts over a short distance and does not enter the circulation.

INVERSE AGONIST
A ligand for a G-protein-coupled receptor that, on receptor binding, decreases the affinity of the ligand–receptor complex for the Gα protein subunit, thereby decreasing receptor activity.

HYPERPHAGIA
Increased feeding.

Box 1 | Molecular mediators of energy homeostasis

The signalling molecules discussed in this review generally fall into two main classes: those produced in the brain as NEUROPEPTIDES, which act as short-range components of neuronal circuits; and those produced in the periphery, which circulate in the bloodstream and provide feedback to the brain.

Neuropeptides

- ***Agouti protein.*** **A PARACRINE molecule that is normally produced in the skin, where it causes melanocytes to synthesize yellow pigment by binding to the melanocortin 1 receptor (Mc1r). Abnormal production of agouti protein in the brain can mimic the effects of agouti-related protein (Agrp), a neuropeptide that binds to Mc3r and Mc4r, and stimulates food intake and excess weight gain. (Agouti protein binds to Mc4r but not to Mc3r). Both agouti protein and Agrp are INVERSE AGONISTS and therefore inhibit melanocortin receptor function.**
- ***α-Melanocyte stimulating hormone (α-MSH).*** **A 13-amino-acid peptide produced by post-translational cleavage of proopiomelanocortin (Pomc); it is synthesized in the arcuate nucleus of the hypothalamus, the nucleus of the solitary tract in the hindbrain and the pituitary gland. α-MSH inhibits food intake and promotes weight loss by activating Mc3r and Mc4r in the brain. Measurements of *Pomc* mRNA levels are frequently used to identify and to characterize neurons that produce α-MSH.**
- ***Cocaine- and amphetamine-regulated transcript (Cart).*** **This encodes a neuropeptide, Cart42–89, that is expressed in many brain areas and is co-expressed with *Pomc* in the arcuate nucleus of the hypothalamus. Similar to α-MSH, Cart inhibits food intake and promotes weight loss. Pharmacological studies indicate that Cart might have additional functions in motor behaviour and/or stress; however, Cart is a misnomer, as physiological studies do not support the assumption that it is a downstream mediator of amphetamine exposure.**
- ***Hypocretin 1 and 2 (also known as orexin 1 and 2).*** **Neuropeptides that have similar effects and are produced from the same precursor gene in the lateral hypothalamus. A deficiency in hypocretin/orexin neurotransmission causes a sleep disorder, narcolepsy; however, some pharmacological studies have indicated that these molecules have a role in feeding (which accounts for the term 'orexin').**
- ***Melanin-concentrating hormone (Mch).*** **A cyclic neuropeptide produced in the lateral hypothalamus, but by different cells to those that produce hypocretins/orexins. Mch-producing neurons receive input directly from arcuate nucleus neurons, and Mch release stimulates food intake. Mch and α-MSH are structurally unrelated, but have opposing effects on feeding.**
- ***Neuropeptide Y (Npy).*** **This stimulates feeding and, in the arcuate nucleus of the hypothalamus, is expressed in the same neurons as *Agrp*. Unlike *Agrp*, *Npy* is expressed in other regions of the hypothalamus and other brain regions.**
- ***Thyrotropin-releasing hormone (Trh).*** **A three-amino-acid peptide produced by specialized cells in the paraventricular nucleus of the hypothalamus and transported through the local bloodstream to the pituitary gland, where it stimulates the release of thyroid stimulating hormone (Tsh). Tsh circulates through the body to cause the thyroid gland to release thyroxine, a small lipophilic hormone that acts on many tissues in the body.**

Peripheral molecules

- ***Leptin.*** **An endocrine hormone that is produced primarily by adipose tissues, the circulating levels of which are proportional to body fat stores. Deficiency of leptin in *obese* (*ob*/*ob*) mice is interpreted by the hypothalamus to indicate a state of reduced body fat, and therefore causes HYPERPHAGIA and reduced energy expenditure in a (paradoxical) effort to compensate for a perceived energy deficit.**
- ***Cholecystokinin (Cck).*** **This is released into the bloodstream from intestinal endocrine cells, mainly as 58- and 8-amino-acid forms, in response to a meal; it binds to receptors on the afferent vagus nerve and helps to terminate feeding. Cck8 is also produced in the brain, where it functions as a neuropeptide that controls behaviour.**
- ***Ghrelin.*** **A peptide released mainly from the stomach in a pattern opposite to that of Cck (ghrelin levels reach a peak before meal initiation). Named for its ability to stimulate growth hormone release, the ghrelin receptor (also known as the Ghs, or growth hormone secretagogue receptor) is expressed in the pituitary gland and arcuate nucleus of the hypothalamus, where its activation stimulates food intake.**
- ***Insulin.*** **A hormone released from the pancreas that regulates glucose homeostasis through its ability to stimulate glucose uptake, glycogen synthesis and other pathways of fuel storage in peripheral tissues. However, insulin also serves as a peripheral indicator of energy status and binds to receptors in the arcuate nucleus of the hypothalamus (see text).**

also elevated in the arcuate nucleus (but not in other brain regions) of *ob*/*ob* mice[10], which show marked hyperphagia when food is freely available. This observation gained further attention when the gene product that was mutated in *ob* was identified as leptin, a circulating protein that is produced mainly by adipose tissue, the levels of which in plasma reflect body fat stores[11–14] (BOX 1). Because a reduction in body fat stores and a concomitant reduction in circulating leptin were thought to be key events in the compensatory hyperphagia caused by previous food deprivation[15], Npy neurons in the arcuate nucleus were implicated as likely targets for leptin action on food intake (FIG. 2). Consistent with this idea, leptin administration to *ob*/*ob* mice causes both a reduction in food intake and a decrease in arcuate nucleus *Npy* mRNA levels[16].

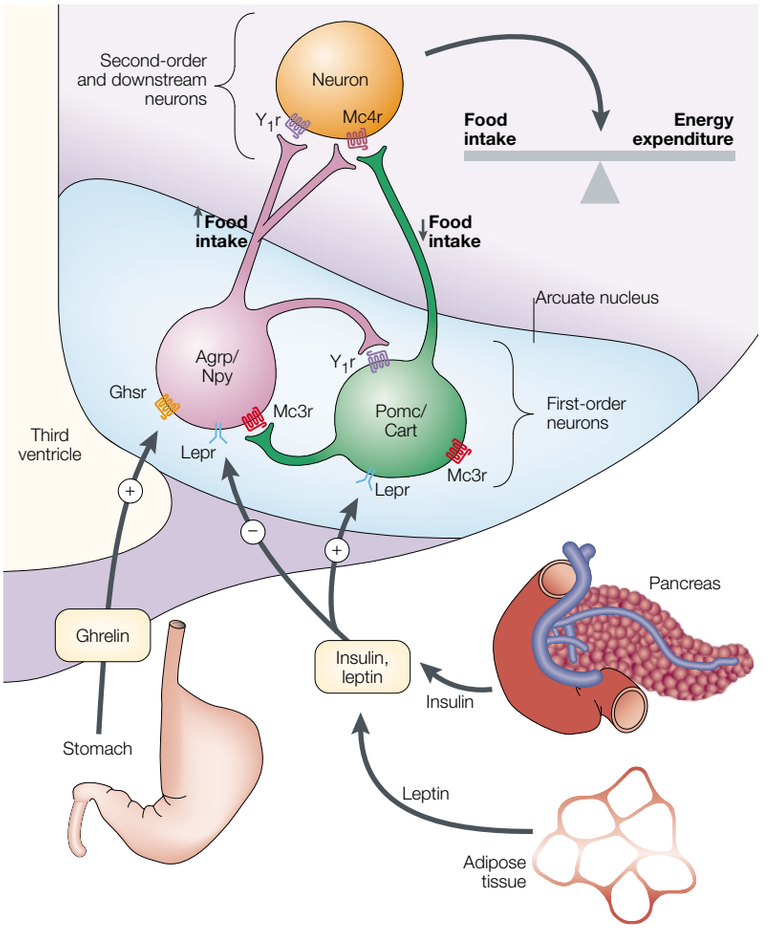

Figure 2 | **Control of energy homeostasis by arcuate nucleus neurons.** There are two sets of neurons in the arcuate nucleus — Agrp/Npy and Pomc/Cart neurons — that are regulated by circulating hormones. Agrp (agouti-related protein) and Npy (neuropeptide Y) are neuropeptides that stimulate food intake and decrease energy expenditure, whereas α-melanocyte stimulating hormone (a post-translational derivative of proopiomelanocortin, Pomc) and Cart (cocaine- and amphetamine-regulated transcript) are neuropeptides that inhibit food intake and increase energy expenditure. Insulin and leptin are hormones that circulate in proportion to body adipose stores; they inhibit Agrp/Npy neurons and stimulate adjacent Pomc/Cart neurons. Lower insulin and leptin levels are therefore predicted to activate Agrp/Npy neurons, while inhibiting Pomc/Cart neurons. Ghrelin is a circulating peptide secreted from the stomach that can activate Agrp/Npy neurons, thereby stimulating food intake; this provides a potential molecular mechanism for integrating long-term energy balance signals with short-term meal pattern signals. Ghsr, growth hormone secretagogue receptor; Lepr, leptin receptor; Mc3r/Mc4r, melanocortin 3/4 receptor; Y_1r, neuropeptide Y1 receptor.

These and other observations provide an abundance of physiological and pharmacological evidence for Npy as a hypothalamic mediator of feeding behaviour that is induced by changes in leptin signalling[4]. Doubts about this hypothesis were raised, however, by a genetic experiment: mice that were rendered *Npy* deficient by gene targeting were found to consume normal amounts of food and show normal patterns of weight gain[17]. Moreover, the food-intake response to fasting seems to be intact in *Npy*-deficient mice, although some data report a mild impairment[18]. Doubly mutant *ob*/*ob*; $Npy^{-/-}$ mice also develop obesity, although it is less severe than in the single mutant *ob*/*ob* animals. Nevertheless, the phenotype of *Npy*-deficient mice, at least with regard to feeding behaviour in animals that produce and respond to leptin normally, is considerably less marked than that predicted. This observation prompts concern about the relationship between genetic and physiological approaches in studying feeding behaviour. According to one view, Npy is not a crucial regulator of feeding behaviour[19]; conversely, perhaps Npy is so important that its lifelong absence induces compensatory mechanisms. Ironically, the resolution of this controversy might best be achieved by collaboration between the proponents of the two viewpoints. Do *Npy*-knockout mice have subtle physiological or neuroanatomical abnormalities that point to alterations in brain circuitry? Can genetic approaches be applied to investigate how adult animals are affected by the acute loss of Npy signalling using, for example, inducible methods[20] of gene inactivation?

Although answers to the latter question are not yet available, recent studies have shed some light on the former. Even though the absence of Npy does not impair the hyperphagic response to fasting, *Npy*-deficient animals fail to develop hyperphagia in response to uncontrolled diabetes[21]. So, if compensation for lifelong *Npy* deficiency does occur, it seems to be limited to perturbations in energy homeostasis that are caused by caloric restriction. Notably, attempts to identify anatomical or physiological signs of compensation have been unsuccessful: in general, *Npy*-deficient mice have normal levels of other neuropeptides in the brain, and respond normally to several ANORECTIC or OREXIGENIC agents (including Npy)[18,22,23]. Of course, the absence of evidence for compensation is exactly that, and a definitive answer about a role of Npy in energy homeostasis will require our understanding of other aspects of arcuate nucleus circuitry.

Genetics and pharmacology together. Originally recognized as an endocrine hormone released from the pituitary gland, α-melanocyte stimulating hormone (α-MSH, see BOX 1) was not known to be a neuropeptide that regulates energy homeostasis until some 40 years after its discovery[24]. α-MSH is one of several peptides that are produced by post-translational cleavage of proopiomelanocortin (Pomc), named because it also gives rise to adrenocorticotropic hormone (ACTH) and β-endorphin. α-MSH and ACTH, collectively known as melanocortins, activate the same family of receptors. Ironically, a genetic clue to the role of melanocortins in body-weight regulation was described almost 100 years ago; mice that carry an unusual dominant mutation of the agouti coat colour gene known as A^y, develop yellow hair colour, obesity and increased body length[25]. In hindsight, it all makes sense: agouti protein is a paracrine signalling molecule, the actions of which are normally limited to the skin, where it signals through the melanocortin 1 receptor (Mc1r), which is expressed on melanocytes to control hair colour. In A^y mice, however, agouti protein is ubiquitously expressed owing to the regulatory nature of the mutation, which allows it to signal through Mc4r, which is expressed in the brain[26] (FIG. 3). However, unlike α-MSH, which activates melanocortin receptors

ANORECTIC
The quality of inhibiting food intake.

OREXIGENIC
The quality of stimulating food intake.

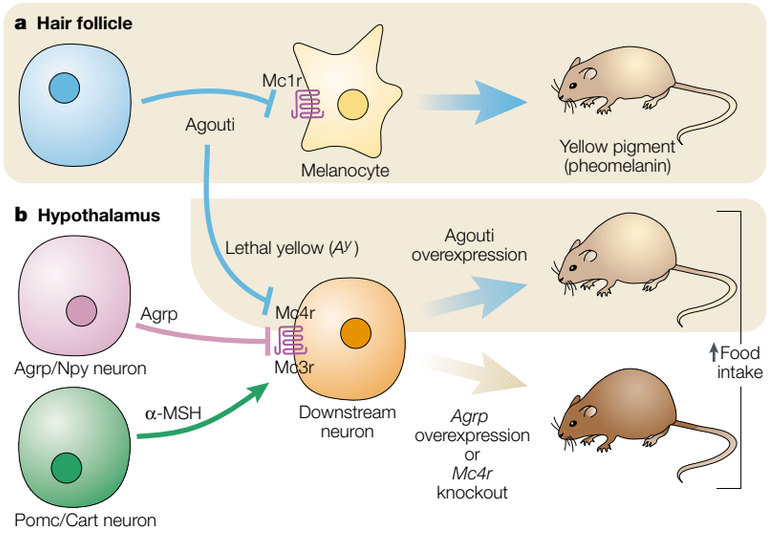

Figure 3 | **Effect of endogenous melanocortin antagonists on pigmentation and body weight. a** | Agouti protein is a paracrine signalling molecule that is produced in hair follicles and that inhibits the melanocortin 1 receptor (Mc1r), which causes hair follicle melanocytes to produce yellow pigment (pheomelanin). **b** | In mice that carry the *Aʸ* mutation, agouti protein is expressed ubiquitously, and can mimic the ability of Agrp (agouti-related protein) to inhibit the Mc4r in the brain. Agrp, but not agouti protein, also inhibits the Mc3r. Cart, cocaine- and amphetamine-regulated transcript; α-MSH, α-melanocyte stimulating hormone; Npy, neuropeptide Y; Pomc, proopiomelanocortin.

and thereby stimulates adenylate cyclase and increases intracellular concentrations of cAMP, Agouti protein is a melanocortin receptor antagonist (or, more accurately, an inverse agonist) that decreases intracellular cAMP levels. So, a yellow coat and obesity in *Aʸ* mice are caused, respectively, by inhibition of Mc1r and Mc4r function. The *Aʸ* mouse model took on new significance after the discovery of Agouti-related protein (Agrp). Identified on the basis of its sequence similarity to Agouti protein[27,28], Agrp is also an inverse agonist of melanocortin receptors (mainly the Mc3r and Mc4r), but is normally expressed in the hypothalamus instead of the skin (FIG. 3).

Data from genetics and pharmacological studies came together under the melanocortin umbrella when *Mc4r* was inactivated by gene targeting[29], and mutant mice were found to develop an obesity–overgrowth syndrome similar to that caused by overexpression of Agouti protein or Agrp. At the same time, central nervous system (CNS) administration of synthetic melanocortin receptor agonists was found to cause decreased feeding and weight loss in mice[30]. Together, these results indicated that Mc4r signalling is an important regulator of feeding and weight gain. Emphasizing the clinical relevance of this work, *MC4R* mutations are the most common cause of monogenic obesity in humans, with an estimated prevalence rate of 4% among individuals with severe childhood-onset obesity[31,32].

This umbrella broadened to include neuroanatomy when *Agrp* and *Npy* were found to be expressed in the same neurons in the arcuate nucleus[33]; although Npy neurons are present throughout the CNS, those that express *Agrp* (known as Agrp/Npy neurons) are found only in the arcuate nucleus. Similar to the regulation of Npy (and opposite to that of Pomc), expression of *Agrp* in the arcuate nucleus increases in response to fasting or genetic leptin deficiency. These neurons therefore seem to be uniquely able to increase food intake, as they can both activate Npy receptors and inhibit melanocortin receptors. Furthermore, Pomc, the precursor that gives rise to α-MSH, was found to be expressed in the same neurons[2] as Cart (cocaine- and amphetamine-regulated transcript, see BOX 1), a neuropeptide that has powerful anorexigenic effects similar to those of the melanocortin agonists[34].

These observations provide the foundation for a modern view of how the hypothalamus senses and responds to changes in energy balance. It seems that two adjacent groups of cells in the arcuate nucleus, the Agrp/Npy neurons and the Pomc/Cart neurons, act as the primary site in the brain for receiving the humoral signals that reflect body fat stores. They then transduce those signals into behavioural and metabolic responses that attempt to maintain body fat stores at a constant level (FIG. 2).

This view of the arcuate nucleus raises an intriguing physiological question: why should there be two groups of neuronal sensors rather than one? One possible explanation is that one or both groups are hard-wired to sense deviations from normal in a single direction, such that the Agrp/Npy neurons primarily sense energy deficits, whereas the Pomc/Cart neurons primarily sense energy excess. To the extent that neuropeptide mRNA levels indicate the magnitude of the neuronal response, this explanation seems to apply to the Agrp/Npy neurons, as *Npy* mRNA levels are upregulated by starvation but are not affected by overfeeding[35]. However, *Pomc* mRNA levels in rodents are downregulated by starvation[36] and are upregulated by overfeeding[37]; so Pomc, and perhaps Cart, respond to both deficits and excesses of energy. This set of observations has implications for understanding why it is easier to gain than to lose weight. This is because the response to energy excess might be limited primarily to increased neuropeptide production by the Pomc/Cart cells, whereas energy deficits both downregulate neuropeptide production by the Pomc/Cart cells and stimulate neuropeptide production by Agrp/Npy cells. In some ways, this hypothesis shares features with the satiety and feeding centre ideas that guided physiologists 40 years ago. An important difference, however, is that more genetic tools exist now with which to investigate these ideas. For example, it could be predicted that animals in which Pomc/Cart neurons had been ablated by expression of a toxic transgene, as has been done for orexin/hypocretin neurons[38], would respond to negative but not to positive changes in energy balance.

Another deviation from the historical concept of functional hypothalamic 'centres' is that physiological hypotheses can now be guided by molecular and neuroanatomical data. In particular, the arcuate nucleus circuit is more complex than two independent sensors with reciprocal effects and parallel projections;

neuroanatomical studies show that there are projections between Agrp/Npy and Pomc/Cart neurons in the arcuate nucleus[39,40]. Underscoring the potential significance of these intra-arcuate connections is the observation of melanocortin receptor specialization: *Mc4r* is widely expressed throughout the brain and spinal cord[41], but expression of *Mc3r* is primarily limited to the arcuate nucleus and other regions of the hypothalamus[39] (FIG. 2).

The potential role for crosstalk between Agrp/Npy and Pomc/Cart neurons is apparent from the phenotype of *Mc3r*-deficient mice, which was recently described independently by two groups[42,43]. Although the body weight of *Mc3r*-knockout animals is grossly normal, they have defects in energy expenditure, body composition and nutrient partitioning: mutant animals have increased adiposity, reduced lean body mass and gain more weight per calorie consumed than non-mutant animals. However, it is not at all clear how this phenotype is explained from a molecular perspective, as current knowledge of the arcuate nucleus circuit (FIG. 2) does not clarify how energy expenditure regulation is accomplished independently of energy intake. To answer this question, we need a better understanding of arcuate nucleus circuity, not only a descriptive map of projections and mRNA populations, but also a functional map that details whether receptors function presynaptically or postsynaptically, and how electrical activity changes in response to receptor activation.

One technological advance brought to this area of study has been the creation of mice that express green fluorescent protein under the control of the *Pomc* promoter[40]. These mice allowed Cowley *et al.*[40] to study the electrical activity of cells that they could identify as being arcuate nucleus Pomc neurons in a brain slice preparation. Using this approach, they found that the activity of Pomc neurons increased 2–10 min after exposure to leptin. The biochemical mechanism by which leptin exerts this effect is complex, but importantly, it is reduced by Mc3r activation, which provides a potential mechanism for negative autoregulation (FIG. 2). These observations not only provide insight into the wiring of the circuit, but also underscore that the regulation of arcuate nucleus neurons involves effects that are both short term, presumably mediated by post-translational modulation of ion channel activity, and long term, presumably mediated by changes in gene expression (see below).

Downstream of the arcuate nucleus

As described above, neurons in the arcuate nucleus are the primary neuronal sensor for altered energy stores, using information from both humoral signals and AFFERENT NEURONS to provide an integrated measurement of energy deficits or surpluses. How the brain responds to that information is more complex. Nonetheless, two general principles have emerged: first, the response is always homeostatic in both its direction and magnitude; and second, alterations in both intake and expenditure are used to help balance the energy equation.

Projections from the arcuate nucleus to the lateral hypothalamus synapse on neurons that express melanin-concentrating hormone (Mch, see BOX 1), which has been shown previously to be a key endogenous stimulator of food intake[44]. This type of projection — arcuate nucleus to lateral hypothalamus — might therefore be a key component of the circuitry that translates changes in the fuel gauge into changes at the fuel pump[2]. By contrast, CNS regulation of energy expenditure has, historically, been a black box, in part because SYMPATHETIC outflow — one of the main routes by which the brain determines how much energy the body spends on THERMOGENESIS, cardiac output and heat loss through radiation — is mediated by a complex network of neurons, the anatomical connections of which are incompletely understood. In the hypothalamus, however, recent studies indicate a molecular circuit by which changes in the fuel gauge are linked to changes at the gas pedal.

The thyroid axis and energy expenditure. The hallmarks of reduced thyroid activity — decreased oxygen consumption and a general decrease in cellular metabolism — form an adaptive response to food deprivation by slowing the rate at which body energy stores are depleted. This set of responses involves altered output from neurons in the paraventricular nucleus of the hypothalamus that produce thyrotropin-releasing hormone (Trh, see BOX 1) (FIG. 4a). Together with neurons that control the GONADOTROPHIC, SOMATOTROPHIC and ADRENAL AXES, these neurons transfer their products to the pituitary gland through release into the pituitary portal circulation (FIG. 4a).

Several years ago, Lechan and colleagues found that the effects of starvation on reducing *Trh* expression in rodents are blunted by the systemic administration of leptin[45]; furthermore, neither fasting nor leptin affected *Trh* expression in animals treated with monosodium glutamate (MSG), which is a neurotoxin that targets the arcuate nucleus[46]. This finding indicates that both the effect of starvation on *Trh* expression and the ability of leptin to block this effect require input from neurons in the arcuate nucleus. Indeed, both the Agrp/Npy and the Pomc/Cart neurons project to paraventricular nucleus neurons that express *Trh*[47–49]. Furthermore, centrally administered α-MSH or Agrp in rodents causes the expected decrease or increase, respectively, in circulating levels of thyroid-stimulating hormone β-subunit (Tshb). In addition, the stimulatory effect of leptin on Trh production in isolated hypothalamus preparations can be blocked by Mc4r antagonists[50]. The effect of fasting to downregulate hypothalamic melanocortin signalling, therefore, is implicated in starvation-induced hypothyroidism.

Given this persuasive combination of anatomical and physiological evidence for melanocortinergic regulation of Trh production, it might seem surprising that humans and mice with genetic defects in melanocortin signalling have apparently normal thyroid function. Humans with heterozygous or homozygous *MC4R* deficiency show no evidence of hypothyroidism[31,32,51].

AFFERENT NEURON
A neuron that provides input, usually from axonal projections, to other neurons or brain regions.

SYMPATHETIC NERVOUS SYSTEM
The division of the autonomic nervous system that stimulates heart rate, blood pressure and heat production.

THERMOGENESIS
The production of heat that occurs when the body burns calories, such as during exercise.

GONADOTROPHIC AXIS
The system whereby the hypothalamus causes release of hormones from the pituitary gland that control reproductive function.

SOMATOTROPHIC AXIS
The system by which the hypothalamus causes release of growth hormone from the pituitary gland.

ADRENAL GLAND
An organ above the kidney that synthesizes certain steroid hormones and releases adrenaline. The portion of the adrenal gland that synthesizes cortisol (corticosterone in rodents) is stimulated by adrenocorticotrophic hormone released from the pituitary gland.

THYROXINE
The main circulating hormone produced by the thyroid gland that stimulates metabolic rate.

CRE–*LOXP*
A site-specific recombination system. Two short DNA sequences (*lox* sites) are engineered to flank the target DNA. Activation of the Cre-recombinase enzyme catalyses recombination between the *lox* sites, leading to excision of the intervening sequence.

STRIATUM
The portion of the basal ganglia that includes the caudate nucleus, putamen and nucleus accumbens.

BASAL GANGLIA
A group of interconnected nuclei in the forebrain and midbrain that includes the striatum (putamen and caudate nucleus), globus pallidus, subthalamic nucleus, ventral tegmental area and substantia nigra.

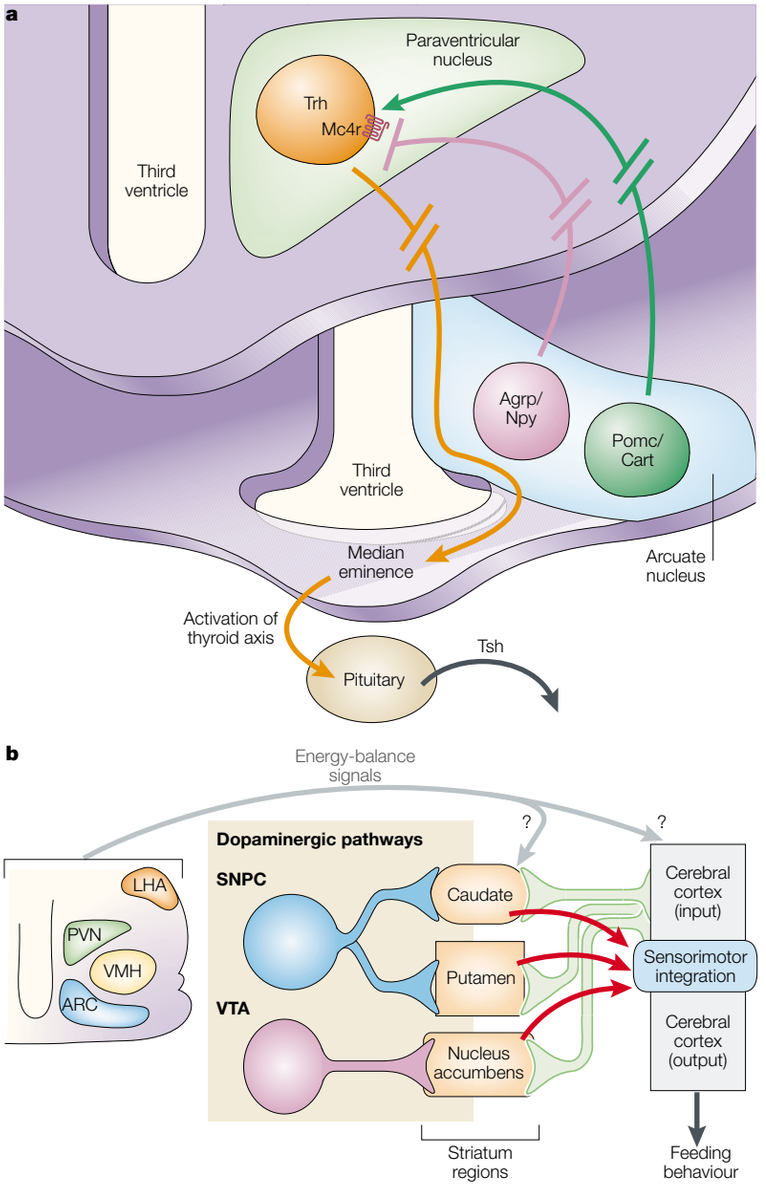

Figure 4 | **Control of energy balance downstream of the arcuate nucleus. a** | A model of melanocortin regulation of the thyroid axis. Activation of melanocortin 4 receptor (Mc4r) on Trh (thyrotropin-releasing hormone)-producing neurons in the hypothalamic paraventricular nucleus occurs when α-melanocyte stimulating hormone (α-MSH) is released from axon terminals that project from Pomc/Cart neurons in the arcuate nucleus. This mechanism might have a key role in the ability of leptin to increase hypothalamic Trh production. During fasting, low levels of leptin inhibit α-MSH release while increasing agouti-related protein (Agrp) release, a combination that inhibits Trh neurons and might mediate fasting-induced hypothyroidism. **b** | Model of dopaminergic regulation of food intake. Three areas of the striatum — the caudate nucleus, the putamen and the nucleus accumbens — receive afferent projections from dopaminergic neurons in the substantia nigra pars compacta (SNPC) or the ventral tegmental area (VTA). In general, pathways from the SNPC modulate motor activity, and pathways from the VTA modulate behaviours that depend on motivation and reward; modulation is thought to involve reciprocal connections between the cortex and striatum that allow sensorimotor information to be integrated. Dopamine-deficient animals can be rescued from starvation by dopamine production in the caudate and putamen[55,56,62,63], but the circuitry whereby dopaminergic systems that are involved in feeding behaviour might be linked to hypothalamic systems that sense changes in energy balance has not been identified. ARC, arcuate nucleus; Cart, cocaine- and amphetamine-regulated transcript; LHA, lateral hypothalamic area; Npy, neuropeptide Y; Pomc, proopiomelanocortin; PVN, paraventricular nucleus; Tsh, thyroid-stimulating hormone; Trh, thyrotropin-releasing hormone; VMH, ventromedial hypothalamic nucleus.

Surprisingly, detailed thyroid function studies have not been reported in *Mc4r*-knockout mice or in A^y mice (in which Mc4r function is inhibited by the ectopic expression of Agouti). In *Mc3r*-knockout animals, in which increased energy efficiency is one of the most striking features of the mutant phenotype (as described above), there is a small reduction in THYROXINE (BOX 1) levels that is not statistically significant[43].

What, then, do we make of this apparent discrepancy between physiology and genetics? Perhaps melanocortinergic regulation of central thyroid function mediates the normal metabolic response to starvation, but 'developmental compensation' preserves thyroid function in individuals or animals with *Mc3r* or *Mc4r* mutations. Conversely, perhaps the potential for melanocortinergic regulation of thyroid function is just that, and the circuit normally contributes very little to overall regulation of the energy expenditure. Critical evaluation of these hypotheses is possible with modern reagents; indeed, it would be interesting to compare central thyroid function in animals with inherited defects in melanocortin signalling to those in which the same genetic defect is induced in mature animals using CRE–*LOXP* technology[20]. In addition, some clues as to the effect of melanocortinergic regulation of the thyroid axis on overall energy expenditure are apparent in the metabolic phenotypes that are caused by defects in leptin signalling. Although the details vary, in general, both rodents and humans with defects in leptin or the leptin receptor are more profoundly hypometabolic than are individuals with defects in melanocortin signalling, and, in some individuals, this might involve hypothyroidism[31,32,52,53]. So, to the extent that central regulation of the thyroid axis by leptin is mediated by melanocortinergic neurons, hypothyroidism caused by a deficiency of leptin signalling but not by a deficiency of melanocortin signalling might reflect melanocortin-independent regulation of thyroid function by leptin[54]. Regardless of these conundrums and considerations, geneticists and physiologists agree that the CNS melanocortinergic system has a key role in regulating energy expenditure.

Why we eat: new roles for old neurotransmitters. At one level, feeding is a simple but delicate act of coordination: brainstem nuclei, orofacial musculature and the oesophagus work in concert to ensure that nutrients are safely delivered to their intended location. Above the brainstem, however, feeding is much more than nutrient delivery: the motivation to eat, the sensation of eating and the pleasure that is associated with consumption of a palatable meal all depend on higher brain functions that must be integrated with the hypothalamic fuel gauge and neuromuscular machinery.

At least some of this integration probably takes place in the STRIATUM (FIG. 4b). The caudate nucleus and the putamen (often referred to jointly as the caudate–putamen) are BASAL GANGLIA structures that are perhaps best known for their role in movement disorders. Parkinsonian symptoms, such as akinesia and rigidity, are not caused by damage to the basal ganglia itself, but by loss of its

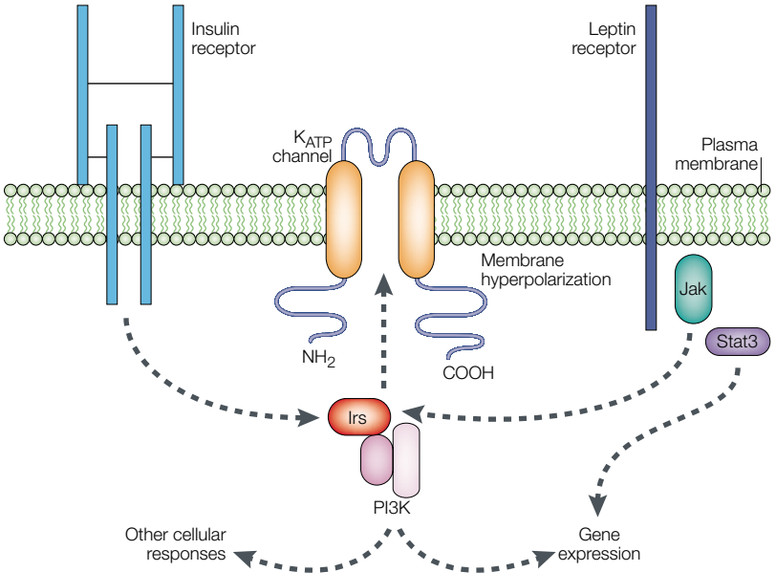

Figure 5 | **A potential mechanism for integrating insulin and leptin signalling in hypothalamic neurons.** Insulin receptor activation leads to tyrosine phosphorylation of a family of insulin receptor substrate (Irs) proteins. Tyrosine-phosphorylated Irs proteins activate phosphatidylinositol 3-kinase (PI3K), which activates ATP-sensitive potassium (K_{ATP}) channels in the neuronal plasma membrane. Leptin also activates the Irs–PI3K pathway, potentially through activation of the tyrosine kinase, Jak2. This effect is proposed to hyperpolarize the neuron, providing a plausible mechanism whereby leptin and insulin inhibit agouti-related protein (Agrp)/neuropeptide Y (Npy) neurons in the arcuate nucleus. Leptin-mediated Jak activation also stimulates signalling by the transcription factor, Stat3, which is proposed to mediate the effects of leptin on gene expression.

DOPAMINERGIC input from neurons in the SUBSTANTIA NIGRA. Patients with Parkinson disease often develop a wasting syndrome that has been ascribed to motor problems, but recent studies from Palmiter and colleagues[55,56] indicate a different explanation: affected individuals might be able to eat but lose their motivation to do so.

Individual knockouts of the dopamine D1A, D2, D3 and D4 receptors cause various behavioural deficits in mice but do not seriously impair feeding[57–61]. However, seven years ago, Zhou and Palmiter described the construction of so-called dopamine-deficient mice[62], in which gene-targeting technology and the enzymology of CATECHOLAMINE biosynthesis were combined to inactivate tyrosine hydroxylase selectively in dopaminergic neurons. Mutant animals had a syndrome of hypoactivity and reduced feeding that could be rescued by providing them with the dopamine precursor L-DOPA; however, the two behaviours — locomotion and feeding — did not always change in parallel, which indicates that they were mediated by different pathways[63]. A plausible hypothesis at the time was that dopamine loss from nerve terminals in the caudate–putamen might account for the movement disorder, as in Parkinson disease, and that loss of dopamine from a different brain region might account for the APHAGIA. Indeed, a likely candidate for this 'other site' was the nucleus accumbens, which receives dopaminergic input from the VENTRAL TEGMENTAL AREA and is strongly implicated in behaviours that depend on the motivation and reward that are associated with a pleasurable activity.

To investigate the role of dopamine in movement and feeding, Szczypka *et al.*[55] turned not to Cre–*loxP* technology but to gene therapy. They injected a viral vector into discrete brain regions that enabled neurons near the injection site to produce dopamine, independent of whether those neurons were dopaminergic to begin with. Remarkably, the injection of this vector into the caudate–putamen restored feeding behaviour but not locomotor activity[56], which showed that the two pathways were distinct, but not in the way that was expected. Part of the explanation might be that striatal dopamine is required to integrate cortical sensory cues with several types of motivational signal. For example, hunger, palatability or conditioned reward can vary independently, but all contribute to the motivation to eat. Because dopamine-deficient animals will die from starvation if left untreated, the caudate–putamen is potentially where hypothalamic signals that measure energy balance are integrated (FIG. 4b).

According to this hypothesis, energy homeostasis requires the ability to sense changes in energy balance, to initiate appropriate compensatory signals and to translate those signals into a motivated behaviour. It is the last component that is defective in dopamine-deficient mice, and that can be rescued by restoring dopamine production to the caudate–putamen. More crudely, perhaps, do dopamine-deficient mice know that they are hungry but just do not care? If so, the hypothalamic energy homeostasis circuit should be intact in dopamine-deficient animals. In addition, feeding that is motivated by reward should be rescued by restoring dopamine production to the nucleus accumbens but not to the caudate–putamen. However, this seems not to be the case, as Szczypka *et al.*[56] found that a preference for sucrose or a palatable diet was rescued by restoring dopamine production to either area. Motivation and reward are more complicated than starvation, and can be broken down into several components, some of which depend on striatal dopamine and some of which might not[64]. In addition, it is not clear how information about energy balance might be transmitted to the caudate–putamen, which has prominent inputs from the cortex and substantia nigra, but not from the hypothalamus (FIG. 4b).

Identifying this sort of functional circuitry might benefit from new approaches in which TRANSNEURONAL TRACING or neuronal imaging is combined with reverse genetics. Manipulating gene expression in a temporally and/or spatially restricted manner is also likely to have a central role in answering some of these outstanding questions.

Acetylcholine is another well-studied neurotransmitter that has recently been implicated in energy homeostasis. Unlike individual dopamine receptor knockouts, which have no energy balance defects, mice deficient for the M3 muscarinic receptor gene (*Chrm3*) have growth retardation and reduced body weight[65]. As *Chrm3* is expressed widely throughout the body, and is involved in exocrine and gastrointestinal tissue movements, the growth retardation in these mice was initially ascribed to hypophagia owing to reduced secretion from the salivary gland. More recently, however, growth retardation in

DOPAMINE
An important neurotransmitter produced by the substantia nigra.

SUBSTANTIA NIGRA
A region of the ventral midbrain that contains pigment and sends afferent dopamine-releasing neurons to the striatum.

CATECHOLAMINE
A neurotransmitter that is derived from tyrosine, such as dopamine, adrenaline or noradrenaline.

APHAGIA
Failure to eat.

VENTRAL TEGMENTAL AREA
A region of the ventral midbrain that sends afferent dopamine-releasing neurons to the nucleus accumbens.

TRANSNEURONAL TRACING
An experimental approach for mapping neuronal circuits.

Chrm3-knockout mice has been shown to be caused by central hypophagia, as reduced feeding and weight gain were observed on both standard pellet food and wet mash food[66]. Direct evidence of a muscarinic circuit in the hypothalamus came from physiological experiments in which *Chrm3*-knockout mice received brain injections of Agrp or Mch. Mutant mice failed to respond to injection of Agrp, but responded normally to injection of Mch, which implicates Chrm3 in the circuitry between the arcuate nucleus and the lateral hypothalamus[66]. Indeed, M3 muscarinic receptors were found to be expressed on neurons that also express *Mch* mRNA in the lateral hypothalamus; these neurons also express *Mc4r*, and respond directly to melanocortin stimulation. So, rather than acting as an intermediary link in the chain and a potential roadblock between the arcuate nucleus and the lateral hypothalamus, acetylcholine (and Chrm3) more probably has a permissive role, which allows lateral hypothalamic neurons to respond to melanocortinergic stimuli.

Signal integration

As described above, neuronal subsets that contain Pomc/Cart and Agrp/Npy in the arcuate nucleus are 'first-order' neurons that initiate and orchestrate downstream responses. How do those first-order neurons receive and integrate signals from the periphery? In addition to the adiposity signal provided by leptin, insulin also seems to have a crucial role. Moreover, long-term signals for body fat stores must be integrated with short-term gut-related signals for meal termination: we stop eating not only because our body fat stores have been repleted, but also because we are full! Recent work has begun to clarify how some of this integration might occur.

Insulin: new roles and genetic opportunities. In addition to its central role in the control of blood glucose levels, Woods and Porte proposed some 20 years before the discovery of leptin that the pancreatic hormone, insulin, is an afferent signal to the brain that couples changes of body adiposity to compensatory changes of food intake[67]. This hypothesis is supported by evidence that, similar to leptin, insulin circulates in plasma at concentrations that are proportional to body fat content, that insulin receptors are present in the brain and that intracerebroventricular (icv) insulin infusion in rodents and in other mammals reduces food intake and body weight[7]. The finding that neuron-specific deletion of insulin receptors causes weight gain in mice provides further support for this hypothesis[68]. Because insulin receptors are concentrated in the arcuate nucleus[69,70], and as icv-administered insulin blocks fasting-induced increases of *Agrp*/*Npy* mRNA in this brain area[71], both insulin and leptin are proposed to provide inhibitory input to arcuate nucleus Agrp/Npy neurons in proportion to body fat stores.

Clarifying the mechanisms by which the effects of insulin and leptin are integrated with each other, and exert opposing effects on Agrp/Npy and Pomc/Cart neurons in the arcuate nucleus, are important and active areas of research. At first glance, there seems no obvious point at which their signalling might converge. The insulin receptor is a heterodimeric receptor tyrosine kinase, and phosphorylation of insulin receptor substrate (Irs) proteins alters cellular metabolism through phosphatidylinositol 3-kinase (PI3K)-dependent pathways, whereas the leptin receptor (Lepr) is a class I cytokine receptor that modulates gene expression through the Jak–Stat pathway, Stat3 in particular[72–74].

Five years ago, however, Spanswick *et al.*[75] used rat brain slice cultures to study the effects of leptin on electrical activity, and reported that leptin causes a population of hypothalamic neurons in the arcuate and ventromedial nuclei to HYPERPOLARIZE through the activation of an ATP-sensitive potassium channel. Remarkably, the same population of neurons that was hyperpolarized by leptin was also hyperpolarized by insulin, which indicates how the acute cellular effects of insulin and leptin might converge (FIG. 5). Therefore, an intracellular signalling pathway might exist in which activation of PI3K is the target not only of insulin signalling through Irs proteins, but also of leptin signalling (FIG. 5). In support of this model, the ability of insulin to regulate the firing of certain arcuate nucleus neurons is blocked by inhibitors of PI3K[76]. More recently, Niswender *et al.*[77] have shown that leptin administration to rodents *in vivo* activates the Irs–PI3K pathway in the mediobasal hypothalamus, and that icv infusion of a PI3K inhibitor blocks the ability of leptin to reduce food intake for up to 24 hours. Although much work remains to be done, these data indicate that intracellular leptin signalling might involve both the Jak–Stat and the PI3K pathways, with the former mediating effects on gene expression and the latter mediating more acute neuronal responses.

These observations set the stage for genetic engineering experiments to investigate the extent to which the two hormones, leptin and insulin, signal through the same downstream mechanism at the level of individual neurons. They also prepare the way to determine which aspects of leptin signalling are mediated by changes in gene expression and which are mediated by changes in electrical activity. For example, Cre–*loxP* technology could be used to remove the leptin or insulin receptor from specific arcuate nucleus neurons, and compare the phenotypes of single and double mutant animals. In addition, because the biochemical mechanism of leptin-induced PI3K activation is likely to depend on Jak but not on Stat, animals that are engineered to lack Stat3 function in arcuate nucleus neurons should reveal phenotypes that depend on the ability of leptin to modulate transcription but not electrical activity. Finally, reduced PI3K signalling is a key factor that underlies insulin resistance in peripheral tissues, which indicates an intriguing parallel between type II diabetes and obesity. Perhaps the mechanisms that are responsible for insulin resistance in peripheral tissues are mirrored in the brain, where resistance to both leptin and insulin at the level of PI3K might contribute to both obesity and diabetes[78]. Understanding the mechanisms of resistance in this setting could provide therapeutic targets for treating both diseases.

HYPERPOLARIZATION
A decrease in the electrical potential across the plasma membrane from its resting value, usually associated with reduced neuronal firing.

DAUER
Juvenile nematode in which development is arrested during unsuitable conditions and resumes when conditions improve.

Integrating long-term and short-term signals. If energy intake is adjusted to changing energy needs, the frequency with which meals are taken, the size of individual meals, or both, must be regulated by signals that are related to changing body fuel stores. However, neither meal size nor frequency is likely to be controlled directly by leptin or insulin, as their circulating levels do not vary in a manner that can explain meal-to-meal changes in food intake[79,80]. In addition, mechanisms that regulate feeding patterns show remarkable diversity across mammalian species. Whereas humans typically consume three meals a day, for example, rodents eat many more, relatively smaller meals.

Nonetheless, physiological studies indicate that the effects of adiposity stores on feeding patterns are likely to involve signals that regulate meal termination rather than meal initiation. For example, the anorexic effect of leptin is characterized by the consumption of meals that are smaller in size, with no change in meal frequency[81], whereas Npy exerts the opposite effects on meal intake[82]. One of several candidate signals to control meal termination, the effects of which might be integrated with those of leptin and/or insulin, is cholecystokinin (Cck, see BOX 1). Named for its effects on gallbladder and pancreatic secretion, Cck is released from the duodenum and small intestine during feeding, can act on afferent fibres of the vagus nerve to inhibit feeding and is therefore called a 'satiety signal'[83].

The physiological role of satiety signals has been more difficult to pin down using genetic approaches, partly because of multiple, redundant satiety signals, and partly because the ones that are known (such as Cck) are involved in other aspects of gastrointestinal physiology and behaviour[84,85]. For example, mice that carry a targeted mutation of the Cck type A receptor (*Cckar*) gene fail to respond to the satiety effects of Cck, but have normal levels of food intake and body weight[86]. Conversely, double mutant *Cckar* and *Cckbr*-null mice show reduced body weight, increased energy expenditure and compensatory (presumably) hyperphagia[87]. However, many pharmacological and genetic studies implicate Cckbr in anxiety- and reward-related behaviours[88], and therefore increased energy expenditure in *Cckbr*-knockout mice might reflect a secondary response. This apparent paradox is a reminder that changes in body weight, growth or adiposity can be caused by various abnormalities, only some of which might represent primary defects in energy homeostasis.

What are the anatomical and molecular substrates for integrating meal termination signals with energy homeostasis signals? The nucleus of the solitary tract (NTS) is an important area in the brainstem and processes satiety-related information. So, adiposity-related humoral signals that act in the arcuate nucleus might generate signals that are transmitted through descending projections to hindbrain areas, such as the NTS. In this way, the response of NTS neurons to satiety signals can be augmented or attenuated according to the nutritional state of the animal. Support for this model stems from the findings that centrally administered leptin or insulin enhances the satiety effect of Cck[89,90], and conversely, that fasting-induced leptin deficiency blunts the satiety response to Cck[91]. Recent evidence, however, indicates that leptin can act directly in the NTS to reduce food intake and body weight[92]. The NTS, therefore, contains neurons that not only respond to input from satiety signals, but also might be first-order neurons (analogous to those in the arcuate nucleus) that respond directly to leptin.

To investigate the contribution of the brainstem to the response to satiety signals, independent of input from higher brain areas, Grill and Smith developed a method for surgically isolating the forebrain from the hindbrain in rats[93]. Although such 'decerebrate' animals have profound neurological deficits, they nonetheless show intact satiety responses to Cck, but are unable to increase food intake in response to energy deprivation[94]. These observations indicate that, although the forebrain areas are not required for the ability to sense and respond to satiety signals, they might have a key role in adaptive changes in energy intake when body fat stores are threatened.

Ghrelin. Although Cck has been implicated in meal termination, recent studies indicate that another gut peptide, ghrelin, might have a reciprocal role. Originally discovered as a substance that stimulates growth hormone secretion through receptors in the pituitary gland[95], ghrelin is synthesized and stored mainly in the stomach and has recently been found to have potent orexigenic effects that are mediated by receptors in the hypothalamus[96]. In humans, ghrelin circulates at levels that peak just before meal onset and drop abruptly thereafter[97], and intravenous infusion of ghrelin substantially increases food intake at a single meal[98]. Ghrelin might, therefore, be a gut peptide that contributes to the perception of hunger and triggers meal initiation. Short-term signals, such as

Box 2 | Model organisms for studies of energy homeostasis

Genetic and physiological studies are generally carried out in laboratory mice and rats, respectively, and the two together provide a powerful approach. Mice provided the substrates for the positional cloning of leptin and the identification of the melanocortin pathway, whereas studies in rats provided the groundwork for understanding how dopaminergic signalling influences feeding. To some extent, the strengths of one organism have been used to launch comparable efforts in the other; for example, targeted injections into specific brain nuclei are now carried out in mice. Similarly, the availability of the rat genome sequence should help to dissect the genetics of rat models of obesity and diabetes. What has been missing from the body-weight regulation genetic toolbox, however, is an invertebrate model organism, in which the power of saturation mutagenesis and enhancer–suppressor screens can be brought to bear on this field. Several years ago, the genetic basis of social feeding behaviour in *Caenorhabditis elegans* was traced to a seven-transmembrane-domain protein with some sequence similarity to the neuropeptide Y (Npy) receptor family[99], but the ligand has not been identified, and the relevance of social feeding behaviour to energy balance is not yet clear. More promising, however, are studies of ageing, DAUER formation and longevity in *C. elegans*, all of which are affected by neuronally expressed genes that are homologous to components of the insulin signalling pathway[100,101]. Finally, recent work on insulin signalling in *Drosophila* indicates a remarkable parallel between mammals and invertebrates[102], in which specialized insulin-producing cells in the central nervous system have a key role in carbohydrate homeostasis.

TONIC SUPPRESSION
The steady-state inhibition of a process that is regulated physiologically by disinhibition.

ghrelin and Cck, might therefore exert opposing effects on meal-to-meal regulation of energy intake through mechanisms that depend on neuronal circuits that are responsive to input from long-term adiposity signals, such as leptin and insulin. Interestingly, ghrelin seems to stimulate the activation of Agrp/Npy neurons in the arcuate nucleus (FIG. 2). This indicates that ghrelin might trigger meal onset, at least in part, by functionally antagonizing the TONIC SUPPRESSION of Agrp/Npy neurons by insulin and leptin.

Concluding remarks

In many ways, our current understanding of bodyweight regulation is similar to that first articulated in 1930 by the physiologist Walter Cannon, when he coined the term 'homeostasis'. A big difference now, of course, is that genetic approaches have helped to define molecular components that make up the homeostatic circuits. Most of these components have been identified through the positional cloning of previously existing mouse obesity mutations. It seems likely that the continued application of forward genetics — large-scale ethylnitrosourea (ENU) mutagenesis projects and the development of sensitized screens — as well as the use of other model organisms (BOX 2) will continue to bring molecular insights into this physiological process. The genome, however, is finite, and a point in the not-too-distant future can easily be envisaged when, for example, every neuropeptide and G-protein-coupled receptor will have been characterized with regard to its pattern of expression and knockout phenotype. Our experience so far indicates that this type of information will provide a starting point, but that hypotheses that are grounded in physiology and make use of genetic engineering will be needed to reach a deeper understanding of the dynamics and relationships of homeostatic circuits.

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