The hypothalamus and metabolism: integrating signals to control energy and glucose homeostasis
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Molecules acting in the central nervous system play a critical role in the control of both energy and glucose homeostasis. The hypothalamus consists of a highly diverse collection of interconnected neurons and supporting glial cells that allow this region of the brain to sense and respond to a diverse range of hormonal and metabolic signals. We review recent advances in our understanding of the anatomical architecture and molecular mechanisms within the hypothalamus and how these facilitate the orchestration of systemic metabolic processes.

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Introduction
The brain is crucial in regulating metabolism. From reports describing the effects of lesioning discrete anatomical regions, through to studies of human monogenic disorders and genetically engineered murine models, the primacy of the central nervous system in controlling energy and glucose homeostasis remains clear [1]. In addition, on-going associations from GWAS continue to highlight a neuronal component to body-weight regulation [2].

Within the brain, a number of anatomical regions are recognised to play a role in metabolic homeostasis [3–4]. However, the hypothalamus in particular is critical in sensing and integrating signals from the periphery and effecting appropriate physiological changes to maintain homeostasis.

Here, we review emerging data on anatomical considerations that make the hypothalamus so well adapted to this role. We will also focus on studies of the leptin-melanocortin pathway, exploring how different physiological functions are subserved by different hypothalamic populations. We discuss developments in our understanding of the cross talk between the hypothalamus and peripheral organs in the control of glucose homeostasis and highlight data on more novel circulating metabolic signals potentially acting through the hypothalamus.

Tanyocytes and the blood–brain barrier
In order to access neuronal populations, circulating molecules have to negotiate past the blood–brain barrier (BBB). The BBB is composed of tight junctions between the endothelial cells lining brain microvasculature and serves to regulate access of circulating factors into the brain parenchyma. However, there are discrete regions of the brain known as the circumventricular organs (CVO), in which the brain endothelium is fenestrated. This includes the median eminence (ME) of the basal hypothalamus, situated ventral to the 3rd ventricle and adjacent to the arcuate nucleus (ARC). The ME also contains tanyocytes, a population of highly polarised glial cells that line the 3rd ventricle and send long projections down into the capillary network lying deeper within the ME. Investigators in Lille have previously shown that there is regional variation in the distribution of tight junction proteins in this area and have postulated that the ME may acts as a privileged site for peripheral molecules to specifically target the ARC [5–6]. Data now emerging from this group indicate that within the ME there is a degree of previously unappreciated plasticity in the interface between brain and circulation. Using in vivo imaging to detect fluorescently labelled ligand, Schaeffer et al. demonstrated that circulating ghrelin (a stomach derived orexigenic protein) rapidly bound neurons in the vicinity of fenestrated capillaries, with the amount of ghrelin bound to NPY neurons directly influenced by nutritional state [7]. In particular, fasting significantly increased the number of NPY fluorescent-ghrelin labelled neurons, with this labelling falling away with re-feeding.

Langlet et al. reported that fasting both increased the tight junction barrier of tanyocytes lining the third ventricle and increased fenestration of the microvasculature running from the ME into the ARC. This gliovascular
reorganisation was mimicked by 2-deoxyglucose-induced glucoprivation and was duly reversed by raising blood glucose levels, with VEGF-A produced in tanycytes appearing critical in modulating these barrier properties [8**].

A potential role for tanycytes in brain glucose sensing has also been investigated by Lanfray et al. who have identified that regulation of feeding by glucose may be mediated through glial production of endozeptines, a family of peptides produced specifically in glial cells, which are known to bind benzodiazepine receptors [9]. The endozeptine octadecaneuropeptide (ODN) has a potent suppressive effect upon appetite when given centrally and, intriguingly, Lanfray and colleagues showed that central administration of an ODN agonist suppressed the hyperphagic response induced by glucoprivation, whilst central administration of an ODN antagonist suppressed the anorexigenic effect of glucose.

Tanycytes may also have other specialised roles. Lee et al. reported that ME tanycytes have a neurogenic capacity with disruption of these cells by CT guided irradiation leading to changes in body weight, energy expenditure and activity [10]. Studies by Haan et al. have also implicated hypothalamic tanycytes as a population of progenitor cells, being able to populate appetite-regulating centres such as the ARC with neurons through postnatal life and into adulthood [11].

**Leptin action within the hypothalamus**

The hypothalamic arcuate nucleus contains two populations of cells that are the best characterised leptin-responsive neurons in the brain [12]. A long-standing working model states that leptin inhibits orexigenic AgRP/NPY neurons and excites anorexigenic POMC neurons, with both sets of neurons projecting further to second-order neuronal populations, both within and beyond the hypothalamus. However, whilst these two neuronal populations undoubtedly play pivotal roles in energy homeostasis, many studies over the last decade, driven by advances in Cre-lox technology, have unsurprisingly revealed far more complexity to the action of leptin within the hypothalamus, both in terms of site of action and ascribed role [13*]. Thus, compared to mice globally lacking leptin receptors, the obesity phenotype in mice lacking leptin receptors from POMC neurons alone is much less pronounced [14]. Similarly, body weight is increased by Lepr deletion from AgRP neurons, with simultaneous deletion in both POMC and AgRP neurons having an incremental effect on obesity [15]. The non-redundant role of leptin receptors elsewhere in the hypothalamus is highlighted by the findings that selective deletion of Leprb in SF1 (steroidogenic factor 1) neurons of the VMH also produces modest obesity [16].

Hypothalamic leptin receptors may also have a role in glucose homeostasis. db/db animals globally lacking functional leptin receptors are hyperphagic, obese and have elevated glucose and insulin levels. Several groups have demonstrated in db/db animals that selective re-expression of the leptin receptor in just one side of the arcuate or within arcuate POMC neurons produces robust improvements in the glucose/insulin profile, while body weight is only modestly reduced [17–18] Similar results have been reported by Berglund and colleagues using a model where endogenous LEPR expression was prevented by a LoxP-flanked transcription blocker (loxTB), but could be reactivated by Cre recombines. Re-expression of LEPR only in POMC neurons in the ARC had only a modest effect on energy balance with the authors arguing that the normalisation of blood glucose and reduction in hepatic insulin resistance was independent of changes in body weight [19].

POMC neurons also express insulin receptors and several recent studies have focused on dissecting the interplay of leptin and insulin on these neurons. Williams et al. have indicated that leptin-induced c-fos activity within ARC POMC neurons defines a separate population from POMC neurons that express insulin receptors, with the acute responses to leptin and insulin largely segregated in distinct subpopulations of POMC cells [20]. Intriguingly Hill et al. showed that mice lacking both leptin and insulin receptors in POMC neurons display systemic insulin resistance, which is distinct from the single deletion of either receptor, indicating that direct action of both insulin and leptin on POMC neurons may be required to maintain normal glucose homeostasis [21].

**Serotonin signalling within the hypothalamus and the control of appetite**

The effects of serotonin (5-hydroxytryptamine, 5-HT) on physiology are mediated by multiple serotonin receptors (5-HTR). The last five years have seen an increased understanding in the role of the 5-hydroxytryptamine 2C receptors (5-HT2CRs) within the hypothalamus and how they can influence melanocortin tone. Xu et al. generated mice with global 5-HT2CR deficiency and mice with 5-HT2CRs re-expressed only on POMC neurons [22*]. The global null mice developed hyperphagia and obesity, but both were normalised with selective re-expression on POMC neurons only. 5-HT2CRs expressing POMC neurons have also been implicated as being relevant regulators of insulin sensitivity and hepatic glucose homeostasis independent; at least in part, of their role in appetitive behaviour [23]. More mechanistic data on how this receptor functions has recently been uncovered, with the putative transient receptor potential C (TRPC) channels reported to mediate the activation of a subpopulation of POMC neurons by mGPP (a 5-HT(2C)R agonist) [24].

This increase in functional knowledge has driven a renewed interest in the development of pharmacological
agents targeting central serotonin receptors, which have long been known to reduce food intake. For example, Doslikova and colleagues have combined pharmacological studies with electrophysiological analysis to uncover a functional heterogeneity of ARC POMC neurons. They defined distinct subpopulations of POMC cells activated by 5-HT2CRs but disinhibited through a 5-HT1BR-mediated suppression of local inhibitory inputs, thereby suggesting that a combination of a 5-HT2CR agonist with a 5-HT1BR agonist may be a powerful therapeutic strategy to treat obesity [25].

Other groups have explored the possibility of a direct link between the serotonin and leptin signalling pathways. In 2009, Yadav et al. reported that inactivation of the leptin receptor in serotonergic neurons was able to fully recapitulate the phenotype seen in dlh/db mice, implicating a serotonin-dependent mechanism in the leptin regulation of bone mass, appetite and energy expenditure [26]. However, others have questioned the validity of these findings. Lam et al. for example, showed that although some leptin receptor (LepRb) neurons do lie close to 5-HT neurons in the dorsal raphe (DR), 5-HT neurons do not co-express LepRb, making it less likely that leptin directly influence 5-HT neurons [27].

**Cellular mechanisms within POMC neurons**

A number of cellular mechanisms have been identified that, when disrupted, can interfere with POMC neuronal function. For example, hypoxia-inducible factor (HIF), a nuclear transcription factor that responds to environmental and pathological hypoxia, was found to directly control the transcription of the POMC gene, with disruption of HIF in POMC neurons impairing hypothalamic glucose sensing and causing energy imbalance to promote obesity development [28]. Two studies have recently implicated autophagy, an important intracellular mechanism for the degradation of damaged proteins and organelles, as critical to the correct functioning of a POMC neuron. One study showed that selective loss of autophagy in POMC neurons decreases α-melanocyte-stimulating hormone (MSH) levels, promoting adiposity, impairing lipolysis and altering glucose homeostasis [29]. The second study deleted Atg7, an important autophagy gene, in POMC neurons, and found this disrupted the maturation of POMC-containing axonal projections, thereby causing higher post-weaning body weight, increased adiposity and glucose intolerance [30]. Both studies implicated hypothalamic autophagy deficiency in the pathogenesis of obesity.

**Hypothalamic melanocortin receptors**

A number of recent reports have revealed ever more complexity within central melanocortin receptors. Melanocortin Receptor Accessory Protein 2 (MRAP2) is a small, single-pass transmembrane protein that has previously been shown to interact with melanocortin receptors in vivo [31]. Although it was recognised to be highly expressed within hypothalamic regions enriched with MC4R, its role in vivo was unclear. Now two contemporaneous reports — one based around murine models, another using genetically modified zebrafish — have indicated that MRAP2 may play a critical role in modulating melanocortin tone within the hypothalamus. Asai et al. reported that loss of Mrap2 in mice, either globally or only within the brain, resulted in the development of severe obesity at a young age [32]. Although it remains to be determined whether this was driven by an increase in food intake and/or a decrease in energy expenditure, it appears, in part at least, to be due to a reduction in melanocortin 4 receptor activity. Asai and colleagues also uncovered potentially pathogenic MRAP2 genetic variants in a cohort of obese humans, suggesting the gene may also contribute to body-weight regulation in humans. Sebag et al. studied zebrafish to highlight an intriguing role for MRAP in growth and development [33]. They reported that this protein exists in two different paralogs, MRAP2a and MRAP2b. Expressed in the larval, MRAP2a blocked the function of MC4R, thus stimulating growth during larval development. In contrast MRAP2b was expressed later in development, appearing to enhance responsiveness to α-MSH once the zebrafish began feeding and thereby adding another regulatory step in the feeding pathway.

Thus far, the MC3R has remained the ‘lesser sibling’ of the two central melanocortin receptors, at least with respect to their role in the control of food intake. However, Renquist and colleagues have reported that the Mc3r−/− mouse exhibits defective fasting-induced refeeding, white adipose tissue lipolysis, liver triglyceride accumulation, and regulation of the adipostatic and hypothalamic-adrenal-pituitary axes [34]. These data indicate that MC3R may be required for communicating nutritional status to both central and peripheral tissues involved in nutrient partitioning.

Finally, the role of melanocortin 4 receptor signalling in the response to two different variations of bariatric surgery has recently been studied. Bariatric surgical procedures such as Roux-en-Y gastric bypass (RYGB) and Vertical Sleeve Gastroectomy (VSG) are effective long-term therapy for the treatment of severe obesity. Such procedures likely bring about weight loss through multiple physiological mechanisms, involving changes in food intake, food preference and energy expenditure. Hatoum et al. reported that while Mc4r+/− mice remained fully responsive to RYGB, Mc4r−/− mice lost substantially less weight after surgery than wild-type animals, indicating that MC4R signalling is necessary for the weight loss effects of RYGB [35]. In contrast, Mul and colleagues reported that both Mc4r+/− and Mc4r−/− rats were fully responsive to VSG [36]. The reasons underlying the differences between these two studies are not
clear, but it may be that different surgical procedures, as well as species differences between mice and rats, all contribute to influence the interactions between genotype and bariatric surgery outcome.

**Cross talk between the hypothalamus and peripheral organs controlling glucose homeostasis**

**Glucose**

Subgroups of ‘glucose sensing’ neurons within the lateral, arcuate, and ventromedial hypothalamic regions are well recognised to display specific excitatory or inhibitory electrical responses to changes in extracellular glucose levels [37].

Glucose sensing on POMC neurons has been reported to have a role in the physiological control of systemic blood glucose [38]. Parton et al. disrupted ATP-sensitive potassium (KATP) channels in glucose excited POMC neurons and found that whole-body response to a systemic glucose challenge was significantly impaired. This group also reported that an alteration of UCP2-mediated glucose sensing in these same neurons could be demonstrated in obese mice on a high-fat diet, suggesting that loss of glucose sensing by POMC neurons may have a role in the development of type 2 diabetes.

Osundiji et al. provided evidence that hypothalamic glucose sensors may play a role in controlling insulin secretion from the pancreas. As well as reporting that activation of hypothalamic glucose sensing neurons by ICV infusion of glucose improves insulin secretion during a glucose tolerance test, they demonstrated that pharmacological inhibition of hypothalamic glucose sensing significantly impaired first-phase insulin secretion following an intravenous glucose bolus [39*].

**Insulin**

Insulin receptors are widely distributed in the brain and are highly expressed within the ARC. Chronic ICV infusion of insulin can reduce food intake [40], neuron-specific loss of insulin receptors throughout the brain brings about a modest increase in body fat [41], and specific loss of insulin receptors within hypothalamic nuclei adjacent to the 3rd ventricle causes hyperphagia [42]. Despite these data, the physiological relevance and role of hypothalamic insulin sensing remains to be fully determined. One particularly contentious area relates to the role of CNS insulin signalling in the regulation of hepatic glucose metabolism. Multiple studies based in rodent models suggest that central insulin signalling modifies neural output to the liver and is required for the rapid suppression of hepatic glucose production [43].

However, work done by Cherrington and others (reviewed in [44**]) makes a compelling case that although the brains of larger animals can sense changes in plasma insulin levels and effect hepatic gene transcription, in terms of changes in hepatic glucose flux, this central effect is subsumed under by the more dominant direct hepatic effect of insulin. For example, in a study in conscious dogs in which an acute physiological rise in insulin was brought about by hepatic portal vein insulin, Ramnanan et al. reported that concomitant inhibition of hypothalamic insulin action did not alter the effects of the hormone on hepatic glucose flux [45*].

It may be that this central insulin signalling has more of a role in the longer term, setting a basal tone for hepatic glucose metabolism, with chronic hypothalamic insulin resistance contributing, at least in part, to hepatic insulin resistance. Other data do speak to a putative link between the hypothalamus and hepatic glucose metabolism. For example, Coomans et al. studied the metabolism of mice that had undergone bilateral supra-chiasmatic lesioning, and argued that damage to a discrete hypothalamic region engenders a magnitude of insulin resistance in the liver far in excess of that expected with the modest concomitant weight gain [46]. It may also be that signals other than glucose and insulin are involved. Su et al. have recently suggested the elevation in circulating levels of leucine postprandially are sensed by the brain through the metabolism of leucine to acetyl-CoA and malonyl-CoA in the mediobasal hypothalamus, and thereby able to lower plasma glucose levels through inhibition of liver glucose output.

**Thyroid action in the hypothalamus**

Thyroid hormones can also influence feeding behaviour. Tri-iodothyronine (T3) is able to increase food intake by acting within the brain [47], but as this hormone has cellular effects on many other tissues, the degree to which these CNS actions contribute to global energy balance has often been contentious. However, López and colleagues have confirmed the effect of thyroid hormones within the hypothalamus to be critical in mediating their physiological and pathophysiological effects on energy balance [48**]. Combining stereotactic delivery of hormones, pharmacological agents and adenoviral vectors to discrete regions of the hypothalamus, together with detailed whole-body metabolic phenotyping, their results demonstrated that thyroid-hormone-induced modulation of AMPK activity and lipid metabolism in the hypothalamus is an important regulator of energy homeostasis. T3 induced a hyperphagia response driven by changes in the levels of orexigenic and anorexigenic hypothalamic neuropeptides, as well as marked upregulation of de novo lipogenesis specifically in the hypothalamus and activation of BAT via the sympathetic nervous system.

More recently, the same group have extended their analysis of hypothalamic thyroid action and implicated mammalian target of rapamycin (mTOR) signalling as having a role in thyroid induced feeding behaviour [49].
They reported that hyperthyroid rats have an up-regulation of the hypothalamic mammalian target of rapamycin (mTOR) signalling pathway. Further, both central administration of T3 and genetic activation of thyroid hormone signalling in the ARC recapitulated the effects of hyperthyroidism effects upon feeding and the mTOR pathway. Finally, central inhibition of mTOR signalling with rapamycin was effective in reducing the hyperphagia.

**Amino acid sensing in the hypothalamus**

Two recent studies have provided more detail as to how the sensing of amino acids in the hypothalamus may influence orexigenic and anorexigenic pathways controlling energy balance. Karnani et al. showed that orexin/hypocretin neurons are activated by dietary amino acids (AAs), through a dual mechanism involving inhibition of KATP channels and activation of system-A amino acid transporters, implying that orx/hct cells sense macronutrient balance in extracellular fluid [50]. In another study, our group has described a role for FTO (Fat mass and Obesity Associated), a gene strongly associated with human obesity, in the coupling of amino acid levels to mammalian Target Of Rapamycin Complex 1 (mTORC1) signalling, suggesting that FTO may influence body composition through playing a role in cellular nutrient sensing [51].

**Fibroblast growth factors and potential central actions**

**FGF21**

Fibroblast growth factor-21 (FGF21) is a peptide hormone secreted by the liver and other tissues that is increasingly recognised to be a key physiological regulator of the adaptive starvation response [32]. Amongst the many metabolic effects ascribed to it, FGF21 induces hepatic fatty acid oxidation and ketogenesis, increases insulin sensitivity and inhibits somatic growth [52]. Circulating FGF21 can enter the brain and some of its diverse effects may, in part, be mediated centrally [53]. Sarruf et al. infused recombinant human FGF21 into the lateral ventricles of obese rats at a dose that did not appear to cross back into the peripheral circulation. This intervention increased both food intake and energy expenditure but had no effect on total body weight or fat content. Despite this, there was a significant improvement of insulin sensitivity by increased insulin-mediated suppression of endogenous glucose production. However, a full understanding of the site and receptor through which these actions are mediated remains to be determined.

**FGF19**

Fibroblast growth factor-19 (FGF19) is a hormone produced in the distal ileum and secreted into the circulation after a meal. While it is recognised to control the enterohepatic circulation of bile acids, FGF15/19 also regulates hepatic protein and glycogen metabolism in an insulin-independent manner [54**]. Recent data have emerged to indicate that, like many other gut derived hormones, FGF19 may also have an effect upon metabolism via the central nervous system [55]. Ryan et al. reported that not only are FGF-receptors 1 and 4 present in the rodent hypothalamus, but also that high fat feeding significantly reduced their expression levels. ICV administration of FGF19 reduced food intake and body weight while similar administration of an FGF-receptor inhibitor increased food intake and impaired glucose tolerance.

**Conclusions**

The importance of the role of the hypothalamus in controlling metabolism remains undiminished. During a time when many investigators have, quite appropriately, moved away from a purely ‘hypothalmo-centric’ view of the pathways governing energy homeostasis to concentrate on the brainstem, cortex and reward centres, so our understanding of the intrinsic apparatus of the hypothalamus has increased. Perhaps not surprisingly, there remains a high degree of intricacy, both within and between neuronal populations. However, as the design of successful management strategies for the treatment of metabolic disorders is dependent upon a better understanding of the fundamental mechanisms of appetite and energy balance, there remains an imperative to continue to explore these systems. The major pathways within the brain that control food intake are highly conserved amongst mammals, with naturally occurring or genetically modified, murine models closely matching the phenotype of many of the human monogenic obesity disorders. As an increasing number of novel genetic loci continue to be identified, model organisms will continue to play a major role in unravelling the complex and intricate circuitry involved.

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**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

5. Langlet F, Mullier A, Bouret SG, Prevot V, Dehouck B: Tanyocyte-like cells form a blood–cerebrospinal fluid barrier in the
Combining in vivo multiphoton microscopy with fluorescently labeled ligands, this paper demonstrates that circulating ghrelin, a potent orexigenic hormone, rapidly binds neurons in the vicinity of fenestrated capillaries and that the number of labelled cell bodies varies with feeding status.


Data presented in these studies indicate that a decrease in blood glucose levels during fasting alters the structural organization of this blood-hypothalamic barrier, thereby improving access of metabolic substrates to the arcuate nucleus. These changes are driven by increased VEGF-A expression in tanyctyes, with the neutralization of VEGF signaling blocking this remodelling and impairing the physiological response to refeeding.


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concludes that CNS insulin action is not essential for the rapid suppression of glucose production caused by the hormone.

45. Ramnathan CJ, Kraft G, Smith MS, Farmer B, Neal D, Williams PE, Lautz M, Farmer T, Donahue EP, Cherngton AD, Edgerton DS: Interaction between the central and peripheral effects of insulin in controlling hepatic glucose metabolism in the conscious dog. *Diabetes* 2013, 62:74-84. A study to assess the role of central insulin action in the response of the liver to normal physiological hyperinsulinemia, as may be seen following a meal. Clear data indicate that insulin acting within the brain is not a determinant of the rapid inhibition of hepatic glucose metabolism caused by physiological hyperinsulinemia.


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