Multiple hypothalamic circuits sense and regulate glucose levels

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Abstract

The hypothalamus monitors body energy status in part through specialized “glucose-sensing” neurons, which comprise both glucose-excited and glucose-inhibited cells. Here we discuss recent work on the elucidation of neurochemical identities and physiological significance of these hypothalamic cells, including caveats resulting from the currently imprecise functional and molecular definitions of glucose-sensing, and differences in glucose-sensing responses obtained with different experimental techniques. We discuss the recently observed “adaptive” glucose-sensing responses of orexin/hypocretin-containing neurons, that allows these cells to sense changes in glucose levels rather than its absolute concentration, as well as the glucose-sensing abilities of MCH, NPY and POMC-containing neurons, and the recent data on the role of ventromedial hypothalamic SF-1/glutamate-containing cells in glucose homeostasis. We propose a model where orexin/hypocretin and SF-1/glutamate neurons cooperate in stimulating the sympathetic outflow to the liver and pancreas to increase blood glucose, which in turn provides negative feedback inhibition to these cells. Orexin/hypocretin neurons also stimulate feeding and reward-seeking, and are activated by hunger and stress, thereby providing a potential link between glucose-sensing and goal-oriented behaviour. The cell-type specific neuromodulatory actions of glucose in several neurochemically distinct hypothalamic circuits are thus likely to be involved in coordinating “higher” brain function and behaviour with autonomic adjustments in blood glucose levels.
1. Introduction

About half a century ago, subgroups of hypothalamic neurons were found to show specialized excitatory or inhibitory firing responses to extracellular glucose, revealing a strategy for how the brain can directly monitor body energy status (3, 69, 70). Glucose-sensing in these “glucose-excited” and “glucose-inhibited” neurons was not a general energy-related response, because during examination of a large number of neurons in several brain areas, glucose-responding cells were observed only in the hypothalamus and brain stem, but not in other areas such as the thalamus or cortex (1, 60, 69, 70, 80, 83, 111). More recent work suggests that glucose-sensing neurons may also be found in substantia nigra (113). It is important to emphasize that the effects of glucose on at least some of the “glucose-sensing” neurons are fundamentally different for the general effects of glucose on neuronal firing due to energy availability. While it may be argued that “glucose-excited” neurons are simply a more sensitive version of non-glucose-sensing neurons (which would also be stimulated by glucose, especially if they are energy-depleted), “glucose-inhibited” neurons respond in the opposite way to that expected from the general stimulatory “fuel-injection” effects of glucose, and their operation is thus clearly different from a general energy-related effect. Glucose-sensing cells are also found outside the brain, in tissues such as the endocrine pancreas (glucose-excited β-cells and glucose-inhibited α-cells (7, 81)) and the gut (glucose-excited L-cells (79)), but in this review we will predominantly focus on a selection of recent studies of glucose-sensing in the hypothalamus. Our aim is not to provide a comprehensive overview of mechanisms of glucose-sensing in the brain and periphery, but to highlight key findings and caveats in the recent work linking glucose-sensing to specific neurochemically-defined hypothalamic neurons, and the implications of these findings for whole-body glucose homeostasis. For detailed discussions of the electrophysiological and molecular mechanisms of glucose-sensing in the brain, and in peripheral glucose-sensing cells of the pancreas and the intestine, the readers are referred to other recent reviews (e.g. (21, 40, 58, 63, 54, 81, 6, 102)). To put the subject into a more general physiological perspective, we will
begin with a brief overview of the nature and sources of glucose changes in the brain.

2. Physiological fluctuations in brain glucose levels

Simultaneous measurements of extracellular glucose levels in blood and brain show that brain [glucose] is generally lower than plasma [glucose], yet changes in blood [glucose] cause rapid parallel changes in brain [glucose] (83, 92). A thorough review of data on brain glucose levels (30) suggests that during euglycemia, brain glucose levels are around 0.7-2.5 mM, and a maximum of around 5 mM may be reached under severe plasma hyperglycemia. In turn, plasma hypoglycaemia can cause the brain glucose to fall to 0.2-0.5 mM. This triggers counter-regulatory responses, where pancreatic glucagon and adrenal catecholamine secretion, as well as hepatic glucose production, are stimulated through activation of sympathetic nerves. Apart from the counter-regulatory responses, which are orchestrated by glucosensors in both the brain and periphery (58, 85), hypoglycemia also induces feeding in mammals (glucoprivic feeding, (93)). Glucoprivic feeding has been recently suggested to involve glucose-sensing neurons in the ventromedial hypothalamus (VMH), since it was reduced by blockade of VMH glucokinase, a critical molecular component of some glucose-sensing neurons (31).

Although generally brain glucose levels are lower than those in the blood, it is commonly assumed that glucose concentrations can approach those in the blood in the vicinity of circumventricular organs, which areas of high-permeability in the blood-brain barrier, such as the median eminence in the hypothalamus (35). However, it has recently been shown that this does not necessarily apply to brain structures in close vicinity, such as the arcuate nucleus (ARC) (31), perhaps because of tanycyte barriers separating the ARC from the median eminence (64, 74).

Generally speaking, a meal will increase blood and thus also brain glucose, but the extent to which this happens depends on the food. The potency of foods to increase blood glucose is measured as the glycemic index (48) and a major factor
affecting this is the macronutrient composition of the food. In particular, meals high in protein or fat generally have a low glycemic index, i.e. they elevate blood glucose less than carbohydrate-rich meals. Another source of blood glucose is the endogenous production of glucose by the liver, which is also under hypothalamic control as reviewed below. Hepatic glucose production is controlled by pancreatic hormones insulin and glucagon as well as, but to a lesser extent, directly by autonomic innervation (77). Insulin and parasympathetic innervation increase hepatic glucose uptake and glycogen synthesis, whereas glucagon and sympathetic innervation promote glycogenolysis, gluconeogenesis, and glucose release. Blood glucose is also affected by glucose uptake into muscle and adipose tissue, both of which are increased by insulin and sympathetic innervation (67). Below, we will first discuss the neurochemical identities and functional features of glucose-sensing hypothalamic neurons, and then focus on recent studies suggesting that they are key regulators of sympathetic drive regulating uptake and release of glucose in peripheral tissues.

3. Glucose-sensing capabilities of neurochemically defined hypothalamic neurons

The lateral hypothalamus (LH)

Most recent work focused on LH cells that contain the peptide transmitters orexins/hypocretins, which are not expressed anywhere else in the brain (29, 86). Orexin/hypocretin-containing neurons project widely throughout the brain, with especially dense innervation of regions regulating arousal, metabolism and reward (75). Lack of orexins/hypocretins produces the symptoms of narcolepsy/cataplexy, hypophagia, hypoactivity and late-onset obesity (23, 42). Orexin/hypocretin neurons are more active during wakefulness and almost silent during slow-wave sleep (32, 52). Several lines of evidence indicate that the activity of orexins/hypocretin neurons promotes wakefulness, sympathetic outflow, exploratory locomotor activity, reward-seeking, and food consumption (13, 14, 44, 53, 91, 97, 99, 100, 109). Interestingly, recent studies also suggest
that underactivity and overactivity of the orexin/hypocretin system could be linked to depression and anxiety, respectively (16, 17, 47, 98).

Whole-animal studies looking at genetic markers of neuronal activation and orexin/hypocretin mRNA expression following in vivo manipulation of glucose levels concluded that orexin/hypocretin neurons are activated by systemic hypoglycaemia (86, 22, 61), and could thus be “glucose-inhibited”, especially considering that they are not directly modulated by insulin (110). During initial cellular-level investigations of the effects of glucose on rat LH neurons, it was found that orexin-A/hypocretin-1 was not present in glucose-inhibited neurons (55); and a more recent electrophysiological study also failed to elicit responses to glucose in rat orexin/hypocretin neurons (71). In contrast, at least three different groups independently reported acute inhibition of orexin/hypocretin neurons by glucose, using calcium imaging in rat orexin/hypocretin neurons (65), or whole-cell patch-clamp recordings from isolated mouse orexin/hypocretin cells (110), or mouse orexin/hypocretin cells in brain slices (41, 108).

The published discrepancies in the ability of glucose to inhibit orexin/hypocretin neurons could in theory be related to species differences (more responsive in the mouse, less responsive in the rat), which remains to be investigated in detail. If the rat orexin/hypocretin neurons are not electrically responsive to glucose, as suggested by the data in (71), then the calcium imaging data, which do show glucose-induced drops in calcium concentration in around 50% of rat orexin/hypocretin neurons (65), would suggest a possible dissociation between biochemical and electrical effects of glucose in these cells in the rat. As an alternative to the “species differences” explanation, the reported absence of acute glucose responses in orexin/hypocretin neurons in “cytosol-sparing” (cell-attached or perforated patch) recordings (71), but their presence in whole-cell recordings (19, 110), could in theory be explained by a currently unidentified cytosolic factor, which suppresses the glucose-sensing ability of these cells in in vitro preparations, but whose influence is removed in the whole-cell
The mechanism of glucose-induced inhibition of mouse orexin/hypocretin cells is not well understood, but involves activation of background K⁺ channels (reviewed in (20)). Interestingly, the glucose responses of orexin/hypocretin cells display a unique sugar selectivity, which suggests that the sensing pathway in orexin/hypocretin cells may be distinct from pathways involving glucose-binding proteins such as GLUT2, hSGLT3, and SGLT1 (39). The glucose responses of orexin/hypocretin neurons are also insensitive to glucokinase inhibitors, and cannot be mimicked by the intracellular ATP or extracellular lactate, suggesting that they do not require conventional glucose-metabolizing machinery (39, 40).

Interestingly, at physiological temperatures, about 70% of orexin/hypocretin exhibit only transient, or “adaptive”, responses to sustained physiological rises in glucose levels (Figure 1). Importantly, this allows orexin/hypocretin neurons to adjust their baseline potential to background glucose levels, and thus to continue to respond to changes in glucose levels independently of the glucose baseline (Figure 1). We propose that, by analogy with classical sensory organs such as the eye, this adaptation allows orexin/hypocretin neurons to adjust their glucose sensitivity to background glucose levels (108). In other words, the glucose dose-response curve of orexin/hypocretin cells is not fixed, but can slide along the glucose concentration axis depending on the glucose baseline. Analysis of membrane currents and membrane resistance during the glucose adaptation response of orexin/hypocretin cells suggests that the cellular mechanism of this adaptation involves a time-dependent closure of the hyperpolarizing ion channels originally opened by glucose, rather than opening of an additional population of depolarizing channels (108). How these channels become less active with time after they are opened by glucose is currently unknown, but this process is highly temperature sensitive (very slow or undetectable at room temperature, but prominent at 35 °C (108)). This steep temperature sensitivity is consistent with a process involving internalization/endocytosis of the glucose-activated channels and/or putative glucose receptors; this possibility, as well as
alternative explanations (e.g. phosphorylation-induced desensitization), remain to be examined.

Because orexin/hypocretin neurons are thought to stimulate wakefulness and signal reward deficiency, their inhibition by glucose may in theory be involved in anxiolytic, rewarding, and soporific effects of sugar ingestion (reviewed in (18)), as well as in the regulation of blood glucose levels (see section 5 below). In terms of arousal and reward, there is a theory that proposes a functional separation of the orexin/hypocretin system into a lateral population that regulates reward, and a medial population that regulates arousal (43). At least in the mouse, inhibition of orexin/hypocretin cells by glucose does not appear to follow such a clear topographic separation: the glucose responses are observed in most if not all orexin/hypocretin cells, across both lateral and medial parts of the lateral hypothalamic area, suggesting that glucose may affect both arousal and reward-related parts of the orexin/hypocretin system (108). However, analysis of the time-course of glucose responses in mouse hypothalamus suggests that the medial group of orexin/hypocretin cells contains a greater proportion of “adaptive” orexin/hypocretin neurons than the lateral group (108). It is thus tempting to speculate that “adaptive” and “nonadaptive” glucose-sensing responses may be differentially involved in arousal or reward.

Another population of widely-projecting lateral hypothalamic cells use the peptide transmitter melanin-concentrating hormone (MCH) (10). Although, like orexins/hypocretins, MCH is often considered an appetite-promoting transmitter, in many other respects, the physiological roles of MCH neurons appear to be the opposite of those of orexin/hypocretin cells. In mice, knockout of MCH increases energy expenditure and reduces body weight (88), and these characteristics are also seen in animals lacking the MCH receptor MCH1R (57). Central injection of MCH in rats increases the quantities of rapid-eye-movement, and especially slow wave, sleep (106), while deletion of MCH or MCH1R in mice leads to increased wheel running activity (115). These data suggest that endogenous MCH promotes sleep and suppresses locomotor activity and energy expenditure, i.e. the opposite of actions of orexins/hypocretins. It should be
noted that while MCH neurons are often referred to as “appetite-promoting”, their role in the control of food intake is not entirely clearcut. For example, in mice, knockout of MCH increases food intake during the day, but decreases it at night, with the net daily result being a reduction in food intake (88). Although most (57, 78, 82), but not all (76), studies show that brain injections of MCH increase food intake, mice lacking the MCH receptor actually exhibited increased food intake (24, 57). Feeding-related effects of MCH thus appear to be complex, and may be influenced by its possible roles in anxiety and depression (15, 37). A closely related issue (which remains to be resolved), is whether “MCH neurons” are a functionally homogenous population, or comprise sub-sets of cells with different profiles of ion channels and receptor expression, and differential projection patterns, giving rise to several different behavioural roles, as recently proposed for orexin/hypocretin neurons (43).

Examination of the effects of changes in glucose on the electrical excitability of MCH neurones using whole-cell recordings in mouse brain slices suggests that most MCH neurones are directly and dose-dependently depolarised and excited by glucose within the physiological concentration window (19). Based on the above-mentioned effects of MCH on locomotor activity and body energy balance, these data imply that glucose-induced excitation of MCH neurones may promote sleep and suppress energy expenditure. To the best of our knowledge, the glucose-sensing abilities of MCH neurons have not yet been examined in species other than mouse, or with different recording techniques.

*The arcuate nucleus of the hypothalamus (ARC)*

This hypothalamic region is currently probably the best understood in terms of the control of appetite and metabolism. The generally accepted model is that the ARC neuropeptide Y (NPY) neurons promote weight gain by stimulating appetite and suppressing energy expenditure, whereas ARC proopiomelanocortin (POMC) neurones cause weight loss by inhibiting feeding and stimulating energy expenditure (26, 87). A key feature of this model is that NPY and POMC neurons are oppositely regulated by signals of body energy status, such as leptin (26-28, 87, 105). Whether the NPY and POMC neatly correspond to “glucose-inhibited”
and “glucose-excited” neurons respectively (which would fit in nicely into the above model) is still debated. For example, Muroya and co-workers concluded that 94% of ARC cells that decreased their calcium levels in response to glucose were immunoreactive for NPY (66). Whole-cell patch-clamp recordings also indicated that a significant proportion (40%) of NPY neurons are glucose-inhibited (34). But, in contrast, perforated-patch recordings of Claret and colleagues failed to show inhibitory effects of glucose on NPY cells (25). These discrepancies appear to show a similar “methodology correlation” to discrepancies in the studies of orexin/hypocretin neurons noted above: in both NPY and orexin/hypocretin cells, glucose-induced inhibition appears to be readily observed in whole-cell recordings, but, paradoxically, not in the less invasive perforated-patch recordings. Interestingly, for arcuate glucose-excited POMC neurons, the differences in the literature appear to follow the opposite methodology correlation. Specifically, Fioramonti and colleagues did not observe any glucose responses in POMC neurons using the “cytosol-disrupting” whole-cell recordings (34). In contrast, using the “cytosol-preserving” loose-patch or perforated-patch recordings shows that the majority of POMC neurons are excited by glucose in the physiological concentration range (25, 46, 72). We would like to propose that the operation of glucose-excited neurons requires a highly diffusible cytosolic messenger (presumably ATP (59), but see (2)), and can thus be particularly easily disrupted, especially in small cells, by experimental techniques that “wash-out” the cytosol (such as the whole-cell recording with large-tipped pipettes). In contrast, glucose-inhibited neurons use a completely different (but as yet undetermined) intracellular signalling pathway (see (39, 40)), and we would like to speculate that this pathway is boosted in whole-cell recordings, possibly due to diffusion of a suppressor substance(s) away from cytosol into the pipette.

The ventromedial nucleus of the hypothalamus (VMH)
This hypothalamic area is probably the most studied in terms of brain glucose sensing, and has long been known to contain both glucose-excited and glucose-inhibited neurons. However, in terms of neurochemistry of glucose-sensing, the VMH is currently understood much less than the ARC and LH. Single cell gene
expression analysis suggests that some VMH neurons, including glucose-excited cells, are GABAergic; however, the GABAergic marker GAD is not expressed in a clear relationship to glucose-sensing capacity (49, 59). Recent data shows that most VMH neurons express a protein called steroidogenic factor (SF-1), and that SF-1-expressing neurons have key roles in glucose homeostasis (103, 114). However, how SF-1 expression relates to glucose-excited and glucose-inhibited neurons of the VMH is currently unclear, although there is some interesting recent data that indirectly suggest that some of the glucose-inhibited VMH neurons may express SF-1 and glutamate (see discussion at the end of section 4 below). Perhaps the most critical issue to resolve here is how gene expression and glucose-sensing identities of VMH neurons relate to their projection targets, since there is emerging evidence that different subregions of the VMH are differentially connected to other key feeding centres, such the ARC (96).

### 4. Need for better functional and molecular markers of “glucose-sensing”

After glucose-sensing has been linked to specific populations of vital neurons described above, more and more researchers have moved into this field, creating an increasing demand for clear criteria for classifying neurons as “glucose-excited” and “glucose-inhibited”. In terms of functional experiments, a key source of uncertainty stems from the fact that glucose is used as an energy fuel by all neurons, either directly, or indirectly by stimulating lactate production by astrocytes (71, 73). Thus, experimental alterations of glucose levels can often elicit general energy-related and/or neuroprotective responses that are unrelated to specific glucose-sensing. An example of this is silencing of neurons by very low glucose, which is a widespread response found in both glucose-sensing and non-glucose-sensing neurons, and often involving neuroprotective opening of ATP-inhibited K (K<sub>ATP</sub>) channels, and consequent hyperpolarization, induced by the fall in glucose (and thus cytosolic ATP) levels (60, 9). Although, in glucose-excited neurons, the hyperpolarization induced by 0 mM glucose is likely to be much faster than in non-glucose-sensing neurons, which has been effectively used by some researchers to identify glucose-sensing cells, this “speed” criterion can be very sensitive to experimental variables such as location of cells in the recording chamber and in the tissue (for brain slice recording).
these reasons, a more robust - and more physiological - criterion for classifying a glucose-excited neuron as “glucose-sensing” would perhaps be a test of whether its firing can be significantly affected by changes in extracellular glucose in the physiological glucose range in the brain, e.g. 1 to 2.5 mM (83), although there is currently no agreement on how large the effects on firing should be to pass this “qualifying test”. As mentioned in the introduction, the functional definition of glucose-inhibited neurons is much more clear, because their membrane potential responses to glucose occur in the opposite direction (hyperpolarization) from the general energy-related effects of glucose (depolarization).

In terms of molecular definitions of brain glucose-sensing cells, no molecules unique to glucose-sensing cells have yet been identified. Several markers of glucose-sensing ability have been proposed, including K-ATP channels, glucokinase, AMP kinase, and the GLUT2 transporter (25, 101, 111), but the correlation between the expression of these proteins and the brain location of glucose-sensing neurons is imperfect (5, 49, 50). For glucose-inhibited neurons, the need for molecular markers is particularly critical since even the final effector channels remain to be defined in exact molecular detail (20). It is probably accurate to say that it is currently unknown exactly what makes glucose-sensing neurons distinct from other neurons at the molecular level.

5. Hypothalamic glucose-sensing neurons as regulators of peripheral glucose handling

Although ARC neurons send signals to key central and peripheral regulators of energy balance (95, 62), the roles of glucose-sensing in the POMC and NPY cells in glucose homeostasis remain somewhat unclear, despite recent attempts to inactivate NPY/POMC glucose-sensing through targeted genetic manipulations (25, 72), but see discussion in (54, 40)). In orexin/hypocretin and MCH neurons, an equivalent analysis has not been performed due to the lack of knowledge of molecular components of glucose-sensing in these cells (19, 20, 38). However,
the original study by Yamanaka and colleagues that described the intrinsic inhibitory responses of orexin/hypocretin cells to glucose also observed a lack of fasting-induced stimulation of arousal in mice lacking orexins/hypocretins (110), suggesting that disinhibition of orexin/hypocretin cells by falling glucose may have a role in the initiation of foraging. In this section, we will not discuss the involvement of glucose-sensing neurons in behaviour and higher brain function further, but instead discuss recent findings on their interactions with peripheral tissues relevant to glucose homeostasis, focusing in particular on recent work on control of glucose homeostasis by LH orexin/hypocretin neurons and VMH SF-1 neurons.

As reviewed in (33, 104), orexin/hypocretin neurons can control basal metabolic rate in addition to alertness and reward-seeking. Intracerebroventricular (icv) administration of orexin-A/hypocretin-1 increases energy expenditure even in anaestheticized rats (107). A number of experiments (4, 89) showed that icv or intrathecal administration of orexin/hypocretin stimulates sympathetic outflow and increases plasma epinephrine and norepinephrine levels. Anatomical data employing pseudorabies virus trans-synaptic tract tracing (36, 51, 95), or electron microscopy and cholera toxin B subunit tracing (56), show that orexin/hypocretin cells project polysynaptically to various sympathetic outflow systems. Two recent papers have demonstrated the potential physiological significance of these findings in terms of glucose homeostasis.

The data of Yi and coworkers suggested that orexin/hypocretin neurons, especially those in the perifornical area, can stimulate endogenous glucose production and increase blood glucose through sympathetic nervous control (112). They retrodialyzed bicuculline into the LH and found that orexin/hypocretin neurons were specifically activated in the perifornical area. This led to stimulation of hepatic glucose production, which was inhibited by intracerebroventricular pretreatment with an orexin/hypocretin receptor antagonist. An increase in endogenous glucose production was also induced by intracerebroventricular injection of orexin-A/hypocretin-1, and this increase was inhibited by hepatic sympathetic denervation.
On the other hand, Shiuchi and colleagues (90) found no change in blood glucose, but increase in glucose turnover rate, upon direct infusion of orexin-A/hypocretin-1 into the medial hypothalamus. This was interpreted as simultaneous increase in glucose utilization and production, since it was also observed that glucose uptake by muscle was increased. Orexins/hypocretins promoted glucose uptake and insulin-dependent glycogen synthesis in skeletal muscle through action on beta-2-adrenoreceptors on nonmyocyte cells of skeletal muscle, suggesting that orexin/hypocretin cells could activate this mechanism through sympathetic nerves.

Based on these studies, it can be concluded that at least some of the orexin/hypocretin neurons controls key peripheral mediators of glucose homeostasis via the sympathetic nerves. It remains to be examined whether some subsets of orexin/hypocretin neurons, for example medial v lateral (43), are more important for this. We propose that these findings (90, 112) could mean that the activity of orexin/hypocretin neurons promotes shuttling of glucose from liver to muscle, as would be useful in foraging that is thought to be stimulated by the orexin/hypocretin system (110). Thus, it is possible that the glucose inhibition of orexin/hypocretin cells provides negative feedback in a circuit that consists of orexin/hypocretin cells, sympathetic nerves, the pancreas, and the liver (Figure 2). Since MCH cells decrease heart rate and thermogenesis via the sympathetic nervous system (8), and glucose increases their activity (19), they might also be involved in this putative feedback loop. It is noteworthy that MCH neurons also project polysynaptically via the sympathetic ganglia to the adrenal medulla, white and brown adipose tissue, and the liver (51, 68, 95). However, to the best of our knowledge, it is not yet known whether MCH neuron activity increases or decreases sympathetic outflow to these organs.

The VMH is especially important for the counter-regulatory response (84). Local glucoprivation in the VMH results in pancreatic glucagon secretion (12) and glucose infusion into the VMH suppresses glucagon secretion in response to falling blood glucose (11). In turn, the blunting of the counter-regulatory
response by repetitive hypoglycaemia is associated with an impairment of glucosensing in VMH glucose-inhibited neurons (94). Although the precise cellular mechanisms of the counter-regulatory response are not fully understood, and other factors, such as glucosensors in the portal vein, play a critical role (45), this evidence suggests that, like the orexin/hypocretin neurons, the VMH glucose-sensing neurons may control sympathetic outflow. As the glucose-sensing VMH cells are not currently neurochemically identified, it is not yet straightforward to demonstrate a connection to sympathetic innervation targets by tracing techniques. However, some hypotheses can be formulated from data looking at the expression of GAD-67 (glutamic acid decarboxylase, a GABAergic marker) in VMH neurons that are likely to be glucose-excited (59), and experiments looking at systemic effects of inactivation of glutamate release from VMH SF-1 neurons (103). The former study suggests that VMH glucose-excited neurons are GABAergic, whereas the latter shows that the counterregulatory release of glucagon upon fasting (which is stimulated by the sympathetic system) requires glutamate release from VMH SF-1 neurons. Therefore, we hypothesise that glucose-inhibited VMH neurons that increase sympathetic outflow and control glucagon secretion are glutamatergic (Figure 2), whereas at least some of VMH glucose-excited neurons are GABAergic (in line with the recent demonstration that glucose stimulates GABA release in the VMH, (116)).

6. Perspectives and significance
Many glucose-sensing neurons of the hypothalamus have now been assigned neurochemical identities, and are begging to be linked to specific “higher” and “lower” functions of the nervous system (Figure 2). Most, if not all, of the glucose-sensing neurons of the hypothalamus are well placed to affect peripheral glucose utilization in liver, muscle and adipose tissue through the autonomic nervous system (Figure 2). This might give a general rationale for their ability to sense changes in glucose levels as a feedback regulation of their control of these organs. Our hypothesis would predict that glucose-excited neurons inhibit sympathetic stimulation of key peripheral regulators of glucose homeostasis, whereas glucose-inhibited neurons would stimulate it. It remains a challenge to
find molecular markers and mechanisms that are unique to glucose-sensing cells. In our view, this is one of the key current obstacles in assessing the relative importance of the multiple circuits involved in hypothalamic glucose sensing, and in investigating how specific features of brain glucose-sensing cells, such as its adaptive time-course in some neurons (Figure 1), relate to the regulation of glucose homeostasis and behaviour.
**Figure legends**

**Figure 1.** Adaptive glucose-sensing in orexin/hypocretin neurons. Top, identification of an orexin/hypocretin neuron in a brain slice by post-recording immunocytochemistry. Middle, an orexin/hypocretin cell is transiently inhibited by glucose but then adapts its membrane potential back to baseline despite continuing presence of elevated glucose. Bottom, this adaptation allows an orexin/hypocretin cell to respond to a second change in glucose levels. Reproduced from Williams et al, 2008, with permission from the Proceedings of the National Academy of Sciences, USA.

**Figure 2.** Hypothetical model integrating the physiological roles of glucose-inhibited LH and VMH neurons (see text for details). Arrows indicate stimulation (or increased levels), t-bars show inhibition. Abbreviations: LH = lateral hypothalamus, VMH – ventromedial hypothalamus, LC = locus coeruleus, TMN = hypothalamic tuberomammillary nucleus, VTA = ventral tegmental area, CRF = corticotropin-releasing factor.
References


alertness, reward-seeking

VTA, LC, TMN, raphe, thalamus

stress, hunger (CRF, ghrelin)

HYPOTHALAMUS

LH orexin neurons

VMH SF-1/glutamate neurons

sympathetic outflow to liver and pancreas

blood glucose