Published in final edited form as:

Physiol Behav. 2013 September 10; 121: 117–124. doi:10.1016/j.physbeh.2013.03.023.

Lateral hypothalamus as a sensor-regulator in respiratory and metabolic control

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Abstract

Physiological fluctuations in the levels of hormones, nutrients, and gasses are sensed in parallel by interacting control systems distributed throughout the brain and body. We discuss the logic of this arrangement and the definitions of “sensing”; and then focus on lateral hypothalamic (LH) control of energy balance and respiration. LH neurons control diverse behavioral and autonomic processes by projecting throughout the neuraxis. Three recently characterized types of LH cells are discussed here. LH orexin/hypocretin (ORX) neurons fire predominantly during wakefulness and are thought to promote reward-seeking, arousal, obesity resistance, and adaptive thermogenesis. Bidirectional control of ORX cells by extracellular macronutrients may add a new regulatory loop to these processes. ORX neurons also stimulate breathing and are activated by acid/CO₂ *in vivo* and *in vitro*. LH melanin-concentrating hormone (MCH) neurons fire mostly during sleep, promote physical inactivity, weight gain, and may impair glucose tolerance. Reported stimulation of MCH neurons by glucose may thus modulate energy homeostasis. Leptin receptor (LepR) neurons of the LH are distinct from ORX and MCH neurons, and may suppress feeding and locomotion by signaling to the mesolimbic dopamine system and local ORX neurons. Integration within the ORX–MCH–LepR microcircuit is suggested by anatomical and behavioral data, but requires clarification with direct assays of functional connectivity. Further studies of how LH circuits counteract evolutionarily-relevant environmental fluctuations will provide key information about the logic and fragilities of brain controllers of healthy homeostasis.

Keywords

hypothalamus; sensing; control; CO₂; glucose; leptin; orexin; MCH; Neurotensin

1 Introduction

The brain is in a constant dialog with the internal environment of the body, forming a control system that maintains homeostasis. This review discusses possible cellular correlates of this control, with a specific focus on the lateral hypothalamus (LH), the classical “hunger and

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wakefulness” brain center [1–3]. The modern understanding is that the LH is not cellularly homogeneous, but contains several functionally and molecularly distinct classes of widely-projecting neurons. These LH cells control a vast array of vital physiological processes, including food intake, locomotor activity, wakefulness, sleep, blood pressure, reward-seeking, and breathing [4–6]. In turn, the activity of LH neurons is regulated by an equally numerous array of neural, endocrine, and metabolic inputs [7–12].

Here, we focus on three chemical signals that directly regulate the activity of LH neurons: macronutrients, leptin, and acid/CO₂. We will specifically discuss the effects of these signals on three distinct types of LH neurons, as recently defined by non-overlapping expression of orexin/hypocretin (ORX), melanin-concentrating hormone (MCH), and leptin receptor (LepR). Other types of LH neurons have also been described, *e.g.* GAD65 neurons and NPY neurons, which are both distinct from ORX and MCH cells [13,14]. However, it is currently unclear whether or not they overlap with LH LepR neurons, thus they will not be considered separately in the review. The important reciprocal interactions between LH and other brain circuits are also not discussed here in detail but are covered in other recent reviews (*e.g.* [15,16]).

Because a key focus of this review is on sensing of the internal state, we will briefly discuss general reasons for distributed (brain and peripheral) sensing of the internal environment, and revisit basic definitions of sensors. We will then discuss recent data on the ORX, MCH, and LepR neurons of the LH within a “sensor-regulator” framework.

2 Parallel and distributed sensing of vital variables inside and outside the brain

Experimental evidence for specialized brain sensors of basic physiological variables such as glucose existed for many decades [17]. Yet until recently, the action of nutrients and hormones on peripheral tissues was considered sufficient for stabilizing vital parameters such as blood glucose levels [15]. Indeed, sensors for many basic physiological variables are found in the periphery, *e.g.* the endocrine pancreas for glucose, and the carotid body for O₂/CO₂. *Via* neural and hormonal responses, such peripheral sensors form classical feedback loops safeguarding internal levels of gasses and glucose from perturbations. Why are brain sensors of the same variables required?

Recent experiments suggest that without inputs from the brain, peripheral control loops are insufficient to safeguard body homeostasis. Disrupting brain sensing of macronutrients such as glucose leads to metabolic disturbances, including defects in pancreatic glucose-stimulated insulin secretion (*e.g.* [18–20]). For hormones, the effects of deletion of receptors for insulin and leptin from specific populations of hypothalamic neurons demonstrate that hormone sensing by brain circuits is essential for peripheral glucose homeostasis and normal body weight [21–23]. For gasses, deleting hypothalamus-specific transmitters disrupts modulation of respiration by CO₂ [24,25]. Thus a substantial body of evidence now indicates that brain sensors of homeostasis-related signals are required for normal health.

Placing direct sensors of internal variables within the brain has logical advantages for preparing for the future, for reducing reliance on individual links, and for monitoring the function of peripheral tissues. A key limitation of peripheral organs is that, beyond the anticipatory actions of intrinsic circadian clocks [26], they are largely reactive control systems that produce corrective actions after a change has occurred (*e.g.* release of insulin triggered by a rise in glucose). In contrast, a key function of the brain is to estimate the future from the present, and prepare the body for a change before it occurs (*e.g.* salivation at the sight of food). Such predictive actions need to occur in proportion to internal needs in order to be energy-efficient. It would thus be useful for the brain to adjust its predictive actions based on internal levels of vital variables.

Direct brain sensing of signals such as glucose also reduces ambiguity. For example, elevated blood insulin can signal either elevated glucose or an insulinoma. Measuring blood glucose would help the brain resolve this ambiguity, and also inform the brain about pancreatic input–output function. This logic may explain why the brain itself contains sensors for both “primary” (nutrients, gasses) and “secondary” (hormonal) signals. Finally, placing sensors in parallel (Fig. 1B) protects the system against collapse or “input blindness” that would be caused by failure in one sensor in a serial system (Fig. 1A). Indeed, there is now considerable experimental support for multiple distributed sensors for glucose [27], pH/CO₂ [28], and hormones such as leptin and ghrelin [29,30]. In contrast, serial sensing systems such as those discussed in early models of brain leptin sensing [31], and depicted in Fig. 1A, are not supported by current experimental evidence.

3 Defining “sensing”: ubiquitous v specialized responses to vital variables

Extreme changes in life-supporting contents of the extracellular fluid can, by definition, have disruptive effects on all neuronal functions, and trigger general neuroprotective responses. We draw a distinction these non-specific effects from specialized “sensing” responses discussed here, even though the two types of responses can share molecular machinery. This section is intended as a clarification of this distinction.

Maintaining vital variables such as glucose and pH within a narrow physiological range (≈ 0.7 – 2.5 mM for brain glucose, ≈ 6.9 – 7.4 for brain pH, [32–35]) is crucial for normal neuronal activity. Outside these physiological ranges, neuronal function is reduced in ubiquitous and stereotyped ways. Such “life-preserving” effects differ from the more specialized sensing responses within physiological ranges. For the purposes of this review, we define “sensing” responses as steep changes in firing rate caused by normal physiological variations in the levels of the sensed parameter. For glucose, such specialized sensing responses probably occur above 0.2 mM (see below), and are most steep in the physiological range of glucose levels (≈ 4 – 7 mM for the plasma, ≈ 1 – 2.5 mM for the brain, [33]). Below 0.2 mM glucose, ubiquitous glucose-entry pathways stop being saturated and thus changes in [glucose] influence cellular function [36]. This is where general neuroprotective “shut down” responses are seen (Fig. 2).

A famous example of a protein involved in both general and specific responses is the ATP-inhibited K channel (K-ATP). K-ATP channels have a dual role in responses of different excitable cells to glucose. The first role is general, and involves hyperpolarization caused by opening of K-ATP channels following a fall in cytosolic ATP/ADP ratio [36,37]. This ubiquitous neuroprotective response occurs in both glucosensing and non-glucosensing neurons, as well as in other cells, when extracellular glucose becomes unphysiologically low (reviewed in detail in [36]). This response is functionally distinct from the physiological glucosensing capability that may or may not be present in the same cell [36]. For example, K-ATP channels in ORX neurons open, and hyperpolarize/inhibit the cell, when extracellular glucose is reduced to 0.1 mM, a pathologically low concentration that would produce loss of consciousness [36]. This does not mean that ORX neurons will be “glucose-excited” in the physiological glucose range. Indeed, rises in [glucose] >0.2 mM have been reported to hyperpolarize and inhibit ORX cells in several independent studies [38–40]. This illustrates the presence of a general neuroprotective response, and of a specific glucosensing response, in the same cell. The two responses are clearly distinct in both direction (glucose-induced depolarization *v* hyperpolarization) and concentration-dependence.

The second function of K-ATP channel – the specialized glucosensing response – is similar to the first, since it also involves K-ATP channel closure by a rise in ATP/ADP ratio. However, it is also very different because it occurs within the physiological range of glucose levels and requires additional molecules. This specialized glucosensing response occurs only in a few “glucose-excited” cell types, such as the pancreatic β -cell. Here, the presence of additional molecules (*e.g.* glucokinase for the β -cell) extends K-ATP channel's dependence on extracellular glucose to physiological concentrations of glucose [41]. Without such additional molecules, K-ATP channels are maximally closed above ≈ 0.2 mM extracellular glucose (due to saturation of glucose entry, see [36]). This illustrates that the general and the specialized glucose responses can share molecular machinery (*e.g.* K-ATP channel), but glucosensing in the physiological glucose range requires additional components. The distinction between specialized and general glucose responses is summarized in Fig. 2.

Although less researched, the same may hold for other vital variables such as extracellular pH. For example, as reviewed by Chesler [32], extracellular acidosis has been widely observed to diminish neuronal activity. Presumably this serves a general feedback-neuroprotective function, since neuronal activity generally increases extracellular acidity [32]. In contrast, specialized chemosensory neurons steeply increase their firing rate upon extracellular acidification in the physiological range [42].

4 Overview of ORX, MCH, and LepR neurons of the LH

4.1 ORX and MCH neurons

Anatomical and physiological properties of ORX and MCH neurons of the LH have been the subject of several recent reviews [10,43,44]. Only some key points are briefly summarized here. In the brain, ORX and MCH neurons are found only in the LH, but their axons extend throughout the brain, and have largely overlapping projection fields. ORX and MCH neuropeptides are used as defining markers of ORX and MCH cells, but both cell types express numerous transmitters. ORX cells express glutamatergic markers, and can affect

their projection targets on a millisecond time-scale *via* fast AMPA-receptor-mediated glutamate transmission [45]. Dynorphin and NARP are also expressed in ORX cells, and are lost concurrently with ORX in human narcoleptics, indicating that loss of ORX neurons (rather than only ORX peptides) is a cause of human narcolepsy [46]. In turn, MCH neurons contain transmitters such as GABA, nesfatin, and CART, which may have critical roles in glucose homeostasis [47].

ORX and MCH cells are physiologically antagonistic in at least some key respects. ORX cells promote wakefulness [48], while MCH cells may promote sleep [49]. Loss of ORX cells causes obesity [50], while loss of MCH cells causes leanness [51]. ORX has been linked to anxiety [52], while MCH is thought to be anxiolytic [53]. ORX peptides are predominantly neuroexcitatory [54], while MCH is considered to be an inhibitory peptide [55]. ORX cells fire action potentials mostly during wakefulness (especially active wakefulness), while MCH neurons fire predominantly during sleep (especially REM sleep) [56–58]. In terms of autonomic actions, ORX neurons may activate the sympathetic system to drive glucose release from the liver and glucose uptake by muscle, and may promote brown-adipose tissue thermogenesis and increased cardiovascular function [59]. In contrast, *via* the parasympathetic system, MCH neurons may promote bradycardia and increase fat deposition in the white adipose tissue [60,61]. The metabolic and respiratory functions of ORX and MCH are discussed more closely in Section 5.

Considering the diverse roles of ORX and MCH neurons, a key question is whether ORX and/or MCH neurons are subdivided into functionally specialized subpopulations. For ORX neurons, there is evidence of such subdivisions at the level of inputs [62,63] and electrophysiological properties [40,45]. Whether such different groups of ORX neurons have distinct projection targets is currently unclear. For example, in the mouse, ORX cells in different areas of the LH are similarly likely to project to the ventral tegmental area (VTA, a “reward” center) or the locus coeruleus (an “arousal center”) [64].

4.2 LH LepR neurons

Diverse types of leptin receptor-expressing (LepR) neurons are distributed throughout the brain [65], and their relative roles have been intensely researched during the past decade. Recent studies of the fluorescently-tagged LepR neurons in the LH indicate that LH LepR cells do not contain ORX or MCH, and thus represent a distinct group of LH neurons. Anterograde tracing between LHA and VTA suggests that LH LepR neurons project to VTA, but not to striatum or nucleus accumbens [11]. Interestingly, retrograde tracing with fluorogold beads injected into the VTA suggests that VTA receives inputs from LH LepR neurons, but not from the “classical” LepR neurons in the hypothalamic arcuate nucleus [11]. Infusion of leptin selectively into the LH reduces food intake and body weight, and increases VTA TH gene expression and mesolimbic DA content [11]. Thus, LH LepR cells are a distinct population of anorexigenic neurons that may signal adiposity levels to the VTA.

5 LH neurons and macronutrients

5.1 Glucose sensing by LH neurons

Historical *in vivo* electrophysiology experiments show that the LH contains both glucose-excited and glucose-inhibited neurons [17]. After ORX and MCH neurons of the LH were discovered, their electrophysiological glucose responses have been characterized *in vitro* [38,66], and, in the case of MCH neurons, the metabolic significance of these responses in the whole-animal has also been investigated [67]. The same physiological shifts in extracellular glucose concentration alter the firing of ORX and MCH cells in different directions: ORX cells are glucose-inhibited and MCH cells are glucose-excited [66]. These responses occur irrespective of whether or not the changes in [glucose] are osmotically compensated.

5.1.1 Glucose sensing by MCH neurons—Glucose-induced excitation of MCH cells has recently been shown to follow the classical (β -cell like) mechanism involving glucose-dependent closure of Kir6.2/SUR1-containing ATP-inhibited K^+ (K-ATP) channels [67]. Expression of ATP-resistant (and thus overactive) K-ATP channels selectively in MCH neurons, chronically from birth, ablates MCH cell glucosensing and impairs systemic glucose tolerance [67]. This suggests that glucose excitation of MCH cells may improve glucose tolerance. However, the acute *v* chronic roles of MCH neurons in regulating glucose fluxes are not clear at present. While glucose tolerance worsens if MCH cells are made chronically glucose-insensitive from birth [67], it improves if MCH cells are destroyed in the adult [47]. In turn, MCH overexpression leads to obesity and insulin resistance [68], and increases insulin levels [69]. It is possible that, like ORX neurons (see below), MCH cells could have multiple and differentially-modulated effects on glucose release and uptake, a key subject for further investigation. Glucose actions of MCH in the context of other functions of MCH neurons (*e.g.* hypoactivity, anxiolytic effects) also remain to be explored.

5.1.2 Glucose sensing by ORX neurons—In contrast to MCH cells, ORX cells are activated following *in vivo* hypoglycemia [70,71], and are electrically inhibited by D-glucose, mannose, and 2-deoxyglucose (but not galactose, L-glucose, alpha-methyl-D-glucoside, or fructose) *in vitro* [72]. Glucose-induced hyperpolarization of ORX cells involves background K^+ currents [73]. A similar biophysical mechanism for glucose-induced inhibition has been reported in glucose-inhibited invertebrate neurons [74]. Background channels could be encoded by members of “KCNK” gene family (also known as “K2P” or “tandem-pore” channels), which codes for at least 15 ion channel subunits in mammals, as well as by other genes (discussed in [75]). So far, when some (5 out of 15) of the KCNK have been knocked out from birth, ORX glucosensing persisted, suggesting that other candidate subunits, both within and outside KCNK family, are able to drive glucose-induced hyperpolarization of ORX cells [39,75,76].

In the mouse, ORX neurons are electrophysiologically dichotomous in both their electrical properties and glucose responses [40,45]. Some ORX cells adaptively reduce their glucose-stimulated K^+ conductance when glucose levels are stable, thereby maintaining excitability and glucose-trend sensitivity at different glucose baselines [40]. Such stimulus adaptations

are very common in the classical external sensory systems [77], and may serve a number of functions, such as tuning sensor sensitivity to stimulus baseline. Other ORX neurons respond to sustained change in [glucose] with sustained hyperpolarization, which means that their activity may track absolute levels of glucose rather than just changes in those levels [40]. Projections from sustained and adaptive ORX glucosensors converge on areas regulating arousal and reward [64].

Emerging evidence suggests that the sensitivity of ORX neurons to glucose varies based on the physiological composition of the extracellular space, allowing them to act as “conditional glucosensors”. ORX neurons clearly act as glucosensors at a physiological extracellular pH of ≈ 7.25 [76,73] (and as pH/CO₂ sensors in physiological [glucose] [76], see Section 6.2). However, ORX glucosensing is dose-dependently prevented by lactate, pyruvate, ATP [78], reduced extracellular pH [73], and (through pH-independent effects) by nutritionally-relevant mixtures of dietary amino acids [79]. This suggests that ORX cells may reduce their glucose sensitivity in favor of integrating other signals, when energy levels are high and/or when CO₂ levels are high. The dependence of ORX glucosensing on the levels of other energy substrates and on pH, as well the general responses of ORX cells to unphysiologically low glucose (Fig. 2) should be considered when interpreting the direction of ORX cell glucose responses [80,81].

The system-level implications of glucose-induced inhibition of ORX neurons remain to be clarified. The hypotheses that it could be involved in after-meal sleepiness [38] and/or in a feedback loop controlling peripheral glucose production and utilization [27], should be examined directly. Genetic tools for manipulating adult ORX neurons would help to explore these issues.

5.2 Amino acid sensing by LH neurons

5.2.1 Background: physiological levels of extracellular AAs and their brain actions—Ingested protein and AAs elevate AA concentrations in plasma [82,83] and the brain [84–87] on a time-scale of tens of minutes. Although to the best of our knowledge, similar direct measurements have not been made during starvation and dietary AA deficiencies, these conditions may also elevate brain AA levels. During starvation, tissue proteins are broken down for energy substrates, in particular to fuel hepatic gluconeogenesis [88]. This induces a rise in plasma AAs after around 3–4 days of starvation in rodents and humans [89,90]. Essential AA (eAA) deficiency leads to a rapid increase in protein degradation in order to replenish the missing eAA [91]. Several plasma eAAs (leucine, isoleucine, valine, tyrosine, phenylalanine, histidine, tryptophan and methionine) as well as the nonessential AAs (nAAs) glutamine, proline and alanine can suppress hepatic autophagy (large scale protein degradation in liver) to supply other tissues with AAs [91–93].

Increased dietary protein content increases satiety and decreases food intake in both the short and long-term [94–96]. However, diet deficient in an eAA suppresses food intake [97–100], presumably in order to locate diets more complete in eAAs. In terms of arousal, in humans, protein may be more effective than carbohydrate in increasing arousal and attention, although to the best of our knowledge the relative roles of eAAs and nAAs have

not been examined. For example, protein-rich meals have been reported to be more effective at promoting cognitive arousal than isocaloric carbohydrate-rich meals [101,102].

Sensing of dietary protein is likely to occur directly in the brain, because vagotomy, which ablates communication from gut nutrient sensors, does not fully suppress satiety induced by high-protein diet [103]. Furthermore, i.c.v. injections of leucine or an AA mixture decrease food intake [104]. As reviewed thoroughly by Gietzen et al., considerable evidence points to direct sensing of dietary AAs in the anterior piriform cortex [105]. In addition, eAA-deficient diet increases c-fos expression in dorsomedial hypothalamus [106], and cutting fibers running anteriorly from dorsomedial hypothalamus increases intake of an eAA-deficient diet [107]. The essential AA leucine is sensed by neurons in the arcuate nucleus of the hypothalamus [108], and a further study showed c-fos activation in ARC POMC neurons, as well as in paraventricular nucleus oxytocin neurons and in the nucleus of the solitary tract, in response to leucine infusion near the ARC [87]. However, it is unclear how the leucine concentrations that reached putative sensor neurons in these studies compared to physiological baseline or postprandial values [84,87,109].

5.2.2 AA sensing by LH ORX cells—ORX cells in the LH are excited by physiological AA mixtures *in vitro* and *in vivo*, and AA gavage increases ORX receptor-dependent locomotion [79]. These effects are likely to be independent of pH effects described above, since physiological AA mixtures do not significantly change the pH of physiologically-buffered extracellular solutions used to investigate ORX cell activity (Mahesh Karnani, unpublished observations). Pharmacological and biophysical evidence suggests that stimulation of ORX neurons by dietary AAs involves a dual mechanism comprising activation of electrogenic (depolarizing) “system-A” transporters and closure of K-ATP channels [79]. Consistent with the substrate profile of system-A AA transporters [110,111], nAAs elicited larger responses than eAAs [79]. This may seem counterintuitive, since in terms of regulation of behavior, eAA levels may seem more critical to detect, because unlike nAAs, they cannot be made in the body and so have to be obtained through foraging. However, a rise in nAA levels in the brain may in fact indicate a fall in eAA levels in the blood, because essential and nAAs compete with each other for entry across the blood–brain barrier [112]. It is thus possible that increased excitation of ORX neurons by nAAs signals a fall in blood levels of eAAs, thereby (hypothetically) triggering a foraging or metabolic response required to counteract eAA deficiency.

Although AA sensing by ORX cells occurs *in vitro* and *in vivo*, its physiological role(s) on the whole-animal level remain to be determined. At the cellular level, it seems likely that ORX neurons are not “energy-meters” but may sense macronutrient balance, since some energy sources (glucose) are inhibitory and others (AAs, lactate) are excitatory. Interestingly, these opposing effects are reminiscent of human studies suggesting that protein-rich meals are more effective at promoting cognitive arousal than isocaloric carbohydrate-rich meals [101,102]. Furthermore, at the cellular level, the extracellular levels of AAs may be a permissive factor in determining whether ORX cells respond to glucose, since AAs suppress ORX glucose responses [79].

6 LH neurons, respiration, and CO₂

6.1 Historical links between the LH and respiratory control

Classical lesion studies identified the LH not only as a key source of wakefulness and hunger signals, but also highlighted the importance of the LH in the regulation of breathing. In particular, the LH was historically proposed to be a source of respiration-facilitating impulses by Redgate and Gellhorn in the 1950s [6], who made electrolytic lesions in cat LH while monitoring the rate and depth of respiration. LH lesions produced rapid reductions in the rate and depth of respiration, while inhibition of the LH by barbiturates also reduced respiration [6]. This is supported by recent data revealing an anatomical ‘hotspot’ for breathing stimulation very close to the LH area where ORX neurons are found [113]. Importantly, it has been recently shown that focal microdialysis of CO₂ into the LH increases breathing during wakefulness, suggesting that the LH is a central chemoreceptor site [114].

6.2 ORX neurons, breathing, and CO₂

Analyses of *in vivo* phenotypes of ORX deficient mice, *in vivo* pharmacological evidence, and *in vitro* electrophysiological recordings from ORX neurons all suggest a role for ORX neurons in adjusting breathing to CO₂ levels, especially during wakefulness [28]. Antagonism of ORX receptors inhibits CO₂-induced ventilation [115], and ORX knockout can reduce CO₂-induced ventilation by up to 50% [116]. At the cellular level, ORX neurons sense basic signals that are regulated by breathing, such as H⁺ and CO₂, which in the body are always linked by the reaction $\text{H}^+ + \text{HCO}_3^- \leftrightarrow \text{CO}_2 + \text{H}_2\text{O}$ [32]. *In vivo*, elevated CO₂ stimulates the expression of the activation marker c-fos in ORX neurons [117]. *In vitro*, electrophysiological recordings of the membrane potential of ORX neurons in mouse brain slices show that the firing rate of some ORX cells is sensitive to the ambient levels of H⁺ and CO₂ [12].

Brain interstitial pH is around 7.25 [34], but it can fluctuate between 6.9 and 7.4, for example during hypo or hyperventilation respectively [118]. Physiological acidosis (*e.g.* pH = 7) excites ORX neurons, while physiological alkalosis (*e.g.* pH = 7.4) causes electrical silencing (Fig. 3B). These responses resemble those of known chemosensory neurons, such as those in medullary raphe, in both direction (acidification is excitatory, alkalization is inhibitory) and sensitivity (~100% change in firing rate per 0.1 unit change in pH_e). These effects appear to be mediated, at least in part, by acid-induced inhibition of background K⁺ currents in the ORX cell membrane [12]. Although these currents functionally resemble TASK-like tandem-pore K⁺ channels proposed to mediate pH-sensing in certain classical chemosensory neurons such as retrotrapezoid nucleus, their molecular identity remains to be determined, since acid-sensing in ORX neurons – and in retrotrapezoid nucleus neurons – persists in TASK1/3 knockout mice [76,119]. Recent data suggest that other acid-sensing channels, such as ASICs, may contribute to regulation of breathing by ORX neurons [120], but it remains to be investigated whether they are responsible for electrophysiological effects of pH changes on ORX cell firing.

Overall, these data strongly suggest that, during wakefulness, ORX neurons regulate breathing in response to changes in CO₂ levels. Whether the respiratory-related ORX neurons are a distinct population from “metabolic” and “arousal” ORX neurons remains to be clarified. The intriguing possibility that MCH and/or LepR neurons of the LH are also involved in respiratory control also deserves to be investigated.

7 LH neurons and leptin

7.1 Leptin directly regulates “non-ORX non-MCH” neurons in the LH

Early models of leptin action on the hypothalamus envisioned a serial arrangement where first-order leptin sensor neurons in the arcuate nucleus then synapsed onto second-order neurons in the LH and other areas [31]. It subsequently became clear, however, that LepR-expressing neurons are much more widely distributed throughout the brain [29]. The relative roles of LepR neurons in different brain areas are still being investigated. Neurochemically-speaking, the main antiobesity effects of leptin are thought to be mediated predominantly by GABAergic rather than glutamatergic neurons [22].

Recent data suggest that LH LepR neurons are important regulators of body energy status, and link anorexigenic leptin action to the mesolimbic DA system [121,122]. LH LepR neurons are neurochemically distinct from ORX and MCH cells, as indicated by lack of co-localization between ORX/MCH and GFP in LepR-GFP mice, and by lack of pSTAT3 (a marker of LepR activation) in ORX/MCH neurons [11]. Most LH LepR cells express GAD1, suggesting that they are inhibitory GABAergic neurons [11].

When applied by bath to LH brain slices, leptin rapidly depolarizes $\approx 35\%$ but hyperpolarizes $\approx 20\%$ of LH LepR neurons [11]. Both effects are thought to be postsynaptic, thus suggesting functionally distinct subpopulations among LH LepR cells [11]. Interestingly, leptin-induced c-fos immunoreactivity is not seen in VTA-projecting LH LepR neurons, suggesting that VTA-projecting LH LepR cells are not leptin-excited [11] (but see below). How the rapid (minutes) electrophysiological effects relate to the action of leptin on feeding is unclear, because the effects of intra-LH leptin infusion on feeding take several hours to develop [11]. This could suggest that the action of leptin of LH LepR cells is not a “stop-go” feeding signal but has a more permissive role, perhaps requiring a coincidence of some later signal(s) to elicit feeding. The role of the $\approx 50\%$ of LH LepR cells that did not display rapid membrane potential responses to leptin also remains to be clarified.

7.2 Some LH LepR neurons express neurotensin

In addition to their electrophysiological and projection diversity, LH LepR cells are heterogeneous neurochemically. Approximately 60% of them express the neuropeptide neurotensin (NTS) [122]. NTS-neurons are found throughout the brain, but LepRs are only present in LH NTS neurons [122]. Knocking out LepR in NTS neurons should thus affect only LH LepR-NTS cells [122]. The deletion of LepR from NTS cells in mice caused early-onset obesity, increased feeding, decreased locomotor activity, and reduced the weight-loss effects of exogenous leptin [122]. Electrophysiologically, leptin induces a rapid direct depolarization of LepR-expressing LH NTS cells [122]. Thus the action of leptin on LH

NTS cells likely causes inhibition in their postsynaptic targets, such as ORX cells and the VTA [122], since all LH LepR cells are thought to be GABAergic [11].

The actions of leptin on leptin-excited LH LepR-NTS neurons [122], and perhaps on leptin-inhibited LH LepR-nonNTS neurons [11], thus play a key role in antiobesity effects of leptin. The underlying circuits may involve projections from LH LepR cells to the VTA and to local ORX neurons. However, many aspects remain to be clarified, in particular there are some discrepancies between electrophysiological and genetic data. For example, LH LepR-NTS neurons are directly activated by leptin *in vitro* and project to the VTA [122], yet *in vivo* leptin does not induce c-fos in VTA-projecting LH LepR neurons, suggesting that most VTA-projecting LH LepR cells are not leptin-activated [11]. It is also unclear how the antiobesity effects of leptin-excited LepR-NTS cells could be explained by their GABAergic (inhibitory) innervation of ORX cells [122], although – paradoxically – leptin increases ORX mRNA expression [122]. These ambiguities could reflect the unclear functional implications of some genetic measurements (see below), or could be related to projections of LH LepR neurons outside the LH and VTA [11].

8 Overview and concluding remarks

Several studies suggest that LH-specific sensing of glucose, leptin, and CO₂ is vital for normal respiratory and metabolic function *in vivo* [67], but the broader physiological implications of these findings are not yet fully understood. For example, is there a significant cross-talk between the different signals, whereby a respiratory signal could influence metabolic function, *e.g.* as speculated in [123]? Some answers will undoubtedly arise from a better understanding of the LH as a functionally interconnected microcircuit. Gene-expression related methods, such as measurements of mRNA or c-fos may need to be complemented by more direct and “real-time” methods. This is because genetic changes may not always translate linearly into circuit function, especially at the level of release and action of transmitters from neurons. Moreover, gene expression is slow, while important interactions between LH neurons, the external world, and other brain circuits can occur at a very fast (seconds/milliseconds) timescale [58,124]. Direct measurements of functional connections between LH neurons and their targets with fast tools such as optogenetics and electrophysiology are well placed to fill in these gaps. Continuing investigations into these issues are important, because the emerging controllability of LH by factors linked to diet and respiration may suggest new strategies for improving whole-body energy balance.

Acknowledgments

This article is based on a lecture during the 2012 Annual Meeting of the Society for the Study of Ingestive Behavior, Zurich, Switzerland, July 10–14, 2012, made possible in part by generous donations from Novo Nordisk A/S, Research Diets, Inc., Sanofi, Inc., and TSE, Inc.

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Highlights

- LH regulates respiration via ORX cells.
- LH regulates energy balance via ORX, MCH and LepR cells.
- ORX, MHC, and LepR cells sense and integrate key feedback signals.

Fig. 1.

Model system architectures for brain sensor-regulators. A) In sequential/serial sensing models, primary stimuli such as nutrients are sensed mostly outside the brain, and then this information is relayed *via* hormonal and neural signals to a sequence of brain centers. B) In parallel/distributed models, stimuli are directly sensed in parallel by several brain sensors distributed throughout diverse and mutually interconnected brain circuits.

Fig. 2.

Relationship between glucose and neuronal function. Most neurons reduce their activity (*e.g.* *via* K-ATP channel opening) when glucose levels drop below a critical value (≈ 0.2 mM, see text for detail). Above this life-threatening range, most cells are protected from glucose fluctuations due to saturation of glucose entry transporters. However, a smaller number of specialized “glucosensor” excitable cells continue to be steeply modulated by glucose in the physiological concentration range, allowing appropriate defenses to be initiated before [glucose] becomes life-threateningly low.

A) Glucose responses of ORX neurons

B) Acid/CO₂ responses of ORX neurons

Fig. 3.

Glucose and CO₂ responses of ORX neurons. A) ORX neurons can respond in a sustained elevation in extracellular glucose levels with either sustained (left) or transient (right) membrane hyperpolarization. Whole-cell patch-clamp recordings from acute brain slices from young mice. B) ORX cells are excited by extracellular acidification. The membrane potential and firing rate of mouse ORX neurons can reversibly modulated by changes in extracellular CO₂ and pH levels.

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