

MC4R neurons weigh in differently

Allison W Xu & Gregory S Barsh

Food intake and energy expenditure were thought to be simultaneously controlled by the same group of neurons, but now new evidence shows that they are independently regulated by an anatomically distinct set of melanocortin neurons.

It's that time of year again—when holiday feasts are closely followed by health club fanaticism, a seasonal reminder that energy is, indeed, balanced. Losing weight is not just a test of our willpower, but a fight against an incredibly robust and resilient biological system that has evolved over thousands of years to ensure the maintenance of a steady body weight. Despite considerable daily variation in both energy intake and expenditure, most animals (including most humans) maintain a steady body weight for prolonged periods of time. This is because a highly regulated process of energy balance precisely matches what goes in (food intake) with what goes out (energy expenditure due to basal metabolic rate and physical activity). One of the key molecules in this balancing act is a receptor for hypothalamic neuropeptides, the melanocortin 4 receptor (MC4R). Loss-of-function mutations of this receptor cause profound obesity in both rodents and humans^{1,2}. The MC4R is expressed in many regions of the brain, and it was previously thought that the same MC4R-expressing neurons control both sides of the energy balance equation. Now, a study by Balthasar *et al.* in a recent issue of *Cell* shows that food intake and energy expenditure are controlled by different brain areas³. Thus, genetic components controlling both sides of the energy balance equation can now be dissected into anatomically distinct entities.

The authors used gene targeting to generate an *Mc4r*-knockout allele that could be reactivated by Cre recombinase. The reactivated *Mc4r* allele was under the control of the endogenous MC4R regulatory sequences and could therefore be expected to be active only in native MC4R-expressing neurons. The authors used this 'Cre-activating' allele to selectively restore MC4R function in specific brain regions. It

Allison Xu is at the Diabetes Center and the Department of Anatomy at the University of California San Francisco, HSW 1002, 513 Parnassus Avenue, San Francisco, California 94143-0534, USA. Gregory Barsh is in the Department of Genetics and Pediatrics, Stanford University School of Medicine, Stanford, California 94305, USA. e-mail: axu@diabetes.ucsf.edu or gbarsh@cmgm.stanford.edu

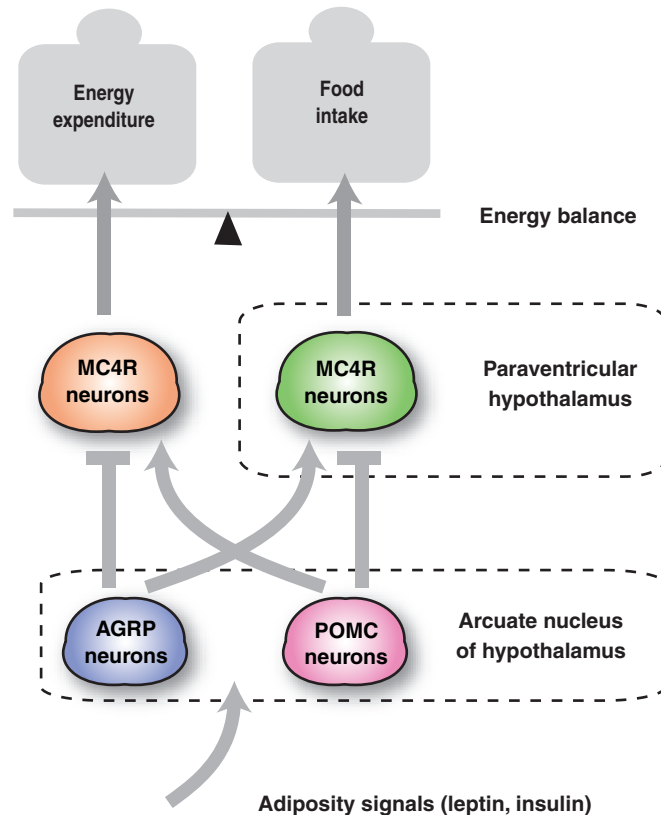


Figure 1 Energy balance is achieved when food intake precisely matches energy expenditure. Energy balance requires first-order neurons to sense and integrate adiposity signals such as leptin and insulin, which circulate at levels proportional to the body's fat mass. POMC and AGRP neurons are important first-order neurons, and they project to multiple areas of the brain to control the activities of MC4R neurons. It is now known, at least in rodents, that food intake and energy expenditure are separately regulated by anatomically distinct MC4R neurons: MC4R neurons in the PVN of the hypothalamus control food intake, whereas MC4R neurons elsewhere control energy expenditure.

turns out that MC4R reactivation in the paraventricular hypothalamus (PVH) normalizes food intake, but not energy expenditure, suggesting that MC4R-expressing neurons in the PVH control food intake whereas energy expenditure is controlled by other MC4R neurons. The discovery of this region-specific role for MC4R neurons will facilitate future identification of neuropeptides or neurotransmitters produced by these anatomically distinct MC4R neurons that are involved in opposite sides of energy balance regulation.

In the hierarchy of neuroendocrine networks, MC4R-expressing neurons are con-

sidered second-order: MC4R signaling is modulated by the first-order neuropeptides alpha-melanocyte stimulating hormone (α -MSH) and agouti-related protein (AGRP), whose own expression and neuronal release are modulated by circulating adiposity signals, such as leptin and insulin⁴. MC4R is activated by α -MSH (a post-translational derivative of pro-opiomelanocortin (POMC)) and inhibited by AGRP. Consequently, POMC neurons promote decreased food intake and increased energy expenditure, whereas AGRP neurons promote increased food intake and decreased energy expenditure (Fig. 1). Thus, regulation

of energy balance at the level of first-order neurons seems to be compartmentalized in terms of afferent signals (too much versus too little body fat storage), but the work by Balthasar *et al.* demonstrates that energy balance at the level of second-order neurons is compartmentalized in terms of efferent signals (food intake versus energy expenditure).

Although the work by Balthasar *et al.* increases our understanding of energy homeostasis, several mysteries remain, including the location of neurons that specifically control energy expenditure. (MC4R neurons in the intermediolateral nucleus of the spinal cord are probable candidates.) Several lines of evidence also suggest that the simple view of first-order neurons described above is more complicated—POMC neurons do not always act in perfect opposition to AGRP neurons, particularly in meal initiation and satiety. For example, AGRP neurons but not POMC neurons receive input from ghrelin, an important meal initiation signal⁵. Conversely, POMC neurons but not AGRP neurons are found in the solitary tract nucleus of the hindbrain, an important site for receiving satiety signals. Also, AGRP neurons seem to inhibit POMC neurons but not vice versa^{6,7}, whereas POMC neurons are important for compensatory refeeding in a manner that AGRP neurons are not⁸. Finally, there is an unappreciated heterogeneity to POMC and AGRP neurons within the hypothalamus: some respond directly to leptin, some respond directly to insulin and some respond to neither or both^{9,10}. Moreover, POMC neurons themselves are anatomically compartmentalized within the hypothalamus: POMC neurons in the rostral hypothalamus are significantly more responsive to leptin

than POMC neurons in the caudal hypothalamus¹⁰. Therefore, MC4R neurons in various brain regions may not receive identical inputs from the first-order neurons, adding another layer of complexity to the divergence of the second-order neurons.

An important challenge for most areas of experimental biology relevant to human disease is translation to clinical practice, and it is unclear if the results of Balthasar *et al.* will translate to humans. For example, the administration of leptin in rodents causes decreased food intake together with increased energy expenditure¹¹ and the same is true for melanotan II (MTII), a synthetic peptide that activates MC4R and MC3R receptors^{12,13}. However, a similar phenomenon does not occur in human patients with congenital leptin deficiency, in whom leptin replacement therapy causes a marked decrease in food intake with no change in metabolic rate¹⁴. Likewise, whereas *Mc4r*-knockout mice show increased food intake and decreased energy expenditure^{1,15}, MC4R-deficient humans overeat as well but have normal energy expenditure². The two sides of the energy balance equation are clearly still linked in humans, as acute perturbations of food intake cause compensatory changes in energy expenditure. Thus, it seems unlikely that different mechanisms have evolved in rodents and humans to confer independent control of food intake and energy expenditure; instead, rodents may simply rely on changes in energy expenditure more heavily, so to speak, than do humans. This clearly applies in the case of thermogenesis via mitochondrial uncoupling, where the ability of brown fat stores to create heat (resulting in energy expenditure) are tremen-

dously important for animals or human neonates with a large surface area to mass ratio, but less important for large, non-hibernating animals and adult humans.

From a therapeutic perspective, one of the most interesting questions posed by the work of Balthasar *et al.* is whether dual control of food intake and energy expenditure by discrete neuronal subgroups will be associated with discrete pharmacologic targets. An important lesson learned by attempts to treat obesity with changes in diet or physical activity is that one rarely works without the other. Treatment strategies that target first-order neurons are appealing because this is the point of system integration; however, this is also a point of considerable redundancy. Molecules or pathways in MC4R-expressing neurons that specifically control food intake may offer a new way of thinking about combination therapy.

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Reaching out to their neighbors

Axon and dendrite initiation is studied mostly in dissociated cultures, where neurons start out as round balls shorn of their processes. Immature neurons *in vivo* are not balls—they are already polarized cells, undergoing directional migration in cortex, or being part of a neuroepithelial layer in the retina. Morgan *et al.* (page 85 of this issue) imaged early bipolar cells (labeled in the picture with GFP in green and the marker CaBP5 in pink) as they connected themselves into the retinal network. These cells began as typical neuroepithelial cells, spanning the entire retina with an apical and a basal process. Over a few days, they grew dendritic branches directly from their apical process, and axonal branches from their basal process. Whereas new apical branches appeared close to the final dendritic layer, basal axonal branches grew more randomly, and seemed to be selectively stabilized in the correct layer through unknown mechanisms. Eventually the distal ends of the two neuroepithelial processes retracted, resulting in mature bipolar morphology. This 'economical' type of axon and dendrite growth serves to connect a nascent bipolar cell to its nearby pre- and postsynaptic partners—which are already in place—with minimal fuss.

Annette Markus

