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POMC neuronal heterogeneity in energy balance and beyond: an integrated view

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Author contributions

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Abstract

Hypothalamic AgRP and POMC neurons are conventionally viewed as the yin and yang of the body's energy status, since they act in an opposite manner to modulate appetite and systemic energy metabolism. However, although AgRP neurons' functions are comparatively well understood, a unifying theory of how POMC neuronal cells operate has remained elusive, probably due to their high level of heterogeneity, which suggests that their physiological roles might be more complex than initially thought. In this Perspective, we propose a conceptual framework that integrates POMC neuronal heterogeneity with appetite regulation, whole-body metabolic physiology and the development of obesity. We highlight emerging evidence indicating that POMC neurons respond to distinct combinations of interoceptive signals and food-related cues to fine-tune divergent metabolic pathways and behaviours necessary for survival. The new framework we propose reflects the high degree of developmental plasticity of this neuronal population and may enable progress towards understanding of both the aetiology and treatment of metabolic disorders.

Since the discovery of the hormone leptin and its powerful metabolic effects[1], the field of neuroendocrinology has made enormous progress in understanding how the brain orchestrates appetite and peripheral metabolism, and a population of neurons expressing the peptidergic precursor pro-opiomelanocortin (POMC) has emerged as a key piece of this puzzle.

POMC neuronal cells are mostly located in the hypothalamus and release bioactive molecules (melanocortins) as a result of the post-translational cleavage of POMC, which signals via specialized brain metabolic receptors (melanocortin receptor type 4 (MC4R)) to modulate food intake and systemic energy metabolism[2].

POMC neurons are switched off under conditions of low energy availability while being stimulated by hormonal and nutrient-related signals of positive energy. Their activation in response to energy supply, such as after a meal, promotes maintenance of a stable body weight by reducing food intake and increasing energy dissipation[3,4].

A second neuronal population co-expressing neuropeptide Y (NPY) and the agouti-related protein (AgRP) is instead activated by negative energy balance. Following activation, NPY/AgRP neurons inhibit POMC neurons[5,6] and antagonize central MC4R signalling via the release of AgRP[7], ultimately stimulating food intake and reducing energy expenditure.

This simple neurobiological model has offered a foundation for understanding how the brain regulates whole-body energy handling, and a mechanistic rationale explaining why loss-of-function mutations affecting the *POMC* or *MC4R* gene cause severe forms of obesity[8–11]. In fact, synthetic MC4R agonists are now being scrutinized for the treatment of obesity[12–14].

The canonical view whereby AgRP and POMC neurons behave as yin–yang partners, however, may still be incomplete, and perhaps too simplistic. On the basis of recent chemogenetic (ligand-based) and optogenetic (light-based) approaches, which provide real-time information on how neuronal activities translate into behavioural or metabolic outputs, POMC neuronal activation under specific conditions can produce similar, rather than opposite, behavioural effects relative to NPY/AgRP neuronal stimulation, including the promotion of feeding[15]. Moreover, rapid in vivo activation of NPY/AgRP neurons promotes hunger independently of POMC neuronal activity[16–18], suggesting that these supposedly antagonistic populations do not always influence appetite in an interdependent manner.

One possible explanation for these differences lies in the heterogeneous nature of POMC neurons. More than 10 years ago, ex vivo studies addressing the electrochemical properties of hypothalamic POMC neurons surprisingly revealed that POMC neurons are electrophysiologically diverse, as they respond differently to neurotransmitters and hormones[19,20]. Now that molecular profiling of hypothalamic neurons at a single-cell resolution is increasingly used[21–23], the field can no longer ignore the fact that POMC neurons form several, molecularly distinct clusters[21–23]. This molecular complexity probably translates into functionally divergent effects and might explain why an overarching theory on the mode(s) of action of these neurons has not yet been achieved.

In this Perspective, we propose that the physiological purpose of POMC neurons is broader and more complex than was originally predicted, owing to mechanisms of intracellular and intercellular plasticity that have yet to be fully explored. Thus, we present new models that interrogate how the heterogeneous nature of POMC neurons can be linked with appetite regulation, metabolic control and obesity pathophysiology.

One population, multiple effects: changing POMC neurons' dogma

According to the prevailing view, POMC neuronal activity is modulated by the body's energy status. Indeed, *Pomc* messenger RNA (mRNA) expression, POMC peptide release and POMC neuronal activation (as assessed by the marker c-Fos) decrease when systemic energy levels drop (fasting) and increase when the body's energy levels recover (feeding)[24–27].

An often-underappreciated aspect, however, is that only 20–50% of all POMC neurons show activity changes in response to nutritional variations[26–29], which may be the result of cellular heterogeneity (see below) but may also be due to one intrinsic technical caveat of these observations: c-Fos, the neuronal activation marker used in the majority of these studies, does not provide temporal information. Neurons with rapid/transitory activation in response to the stimulus (food) may thus not show c-Fos immunoreactivity by the time the marker is assessed, which is typically within 1–2 h after the stimulus.

The use of fibre photometry and optetrode electrophysiology, which record real-time neuronal activity in awake mice, has provided unexpected insights into POMC neuronal regulation. Rapid activation is detected after the presentation of regular chow or palatable food, before the food is actually consumed, and in association with food-related sensory

cues[25,30,31]. When food is presented to a mouse, the animal starts eating and continues to eat avidly, sometimes well after the initial and sustained POMC neuronal activation[32], which appears to contradict the established model that POMC neurons encode a satiety signal[32].

In fact, POMC neuronal activation for several hours does not influence food intake[16,33,34]; rather, chronic neuronal activation (from 24 h to multiple days) is required in order to observe the classic appetite-reducing effect[16,33–35]. Under certain conditions, POMC neurons can even promote food intake. Cannabinoid-dependent hyperphagia, for example, is amplified by chemogenetic stimulation of hypothalamic POMC neurons[15], due to the release of the less studied hunger-promoting POMC-derived opioid β-endorphin[15].

Taken together, in vivo manipulations and recordings of POMC neurons belie their proposed role as a satiety signal. Instead, these neurons appear to operate in ways that are reminiscent of the dog in Pavlov's famous experiment, which salivates in response to the ringing of a bell that predicts food availability. Accordingly, changes in molecular pathways implicated in intracellular nutrient sensing (for example, the mechanistic target of rapamycin pathway) are observed in the liver of fasted mice immediately after food presentation, and this response is primed by POMC neuronal activation[25].

Thus, in addition to affecting feeding behaviour and other metabolic outputs (see below) in response to the body's metabolic needs, POMC neurons contribute to anticipatory (or cephalic) mechanisms, which use environmental cues associated with the energy source, such as its smell or sight, to prepare the body for the imminent consumption of a meal[36].

Dismantling the bulk: multiple subsets and multiple purposes

Spatial heterogeneity.

Within the brain, POMC neurons are mainly located in the hypothalamic arcuate nucleus (ARC), a region with a leaky blood–brain barrier where blood-borne signals can rapidly enter and come into contact with the local network[37]. In the ARC, POMC neurons receive broad and dense inputs from other brain regions and target multiple forebrain centres[38].

Whereas cells positioned in the rostral ARC project mainly to autonomic areas[39], caudal $POMC^{ARC}$ neurons have mostly, but not exclusively[38], axonal connections within the hypothalamus[40]. This complex organization explains how such a relatively small population (~9,000 cells[41]) can respond to a plethora of extracellular signals, which are all implicated in whole-body metabolic control.

A small fraction of POMC neurons are also present outside the hypothalamus, in the brainstem nucleus of the solitary tract (NTS)[42], a hub within the hindbrain that is sensitive to gastrointestinal hormones that modulate food intake[43]. $POMC^{ARC}$ and $POMC^{NTS}$ subpopulations share similarities while also being different. Both clusters are equipped with specific receptors that allow responding to signals implicated in appetite and energy balance regulation, such as leptin or somatostatin[42] (see below). However, whereas acute activation of $POMC^{NTS}$ produces an immediate inhibitory feeding response[34], $POMC^{ARC}$ neurons need to be stimulated over a few hours to days to suppress food intake[16,34].

Spatially segregated POMC neuronal subpopulations may therefore operate at different time scales, possibly via divergent yet coordinated neurophysiological mechanisms, highlighting the close link between spatial and functional heterogeneity.

Heterogeneous expression of receptors.

Several extracellular messengers, including insulin, leptin and serotonin (among others) may affect systemic energy balance by acting through different POMC neuronal subpopulations. Based on single-cell transcriptomic data, leptin receptor (*Lepr*)-expressing POMC cells form a molecularly distinct cluster relative to POMC neurons expressing the serotonin receptor *Htr2c* or the insulin receptor (*Insr*)[21,22]. These subpopulations are also spatially and electrophysiologically segregated[19,20,44].

Of note, transgenic mice with deletion of the *Lepr* protein product (LepR) or the *Insr* protein product (InsR) in POMC neurons during embryonic life have broad variations in their metabolic phenotypes[45–51], which provides further evidence of functional heterogeneity. POMC-expressing progenitors, however, share developmental origins with other hypothalamic cell types[52], and developmental compensation or LepR/InsR deletion from non-POMC neurons may represent a confounding factor in these models (see also Box 1).

Mice with postnatal LepR ablation also have alterations in systemic glucose control and impaired systemic leptin production[53] without changes in body weight or food intake[53]. Conversely, postnatal ablation of the *Htr2c* protein product (5-HT_{2C}Rs) in POMC neurons promotes hyperphagia, increases the release of the hyperglycaemic hormone glucagon and favours diet-induced obesity (DIO)[54]. These observations suggest that LepR- and 5-HT_{2C}R-positive POMC neuron populations probably mediate different physiological outputs.

Distinct neuronal clusters respond to the concomitant action of multiple hormonal signals; for example, co-modulation by insulin and leptin[21]. Transgenic mice with enhanced LepR and InsR signalling in POMC neurons show better glucose handling, increased energy expenditure and adipose tissue browning (that is, the conversion of white fat cells into energy-burning brown adipocytes)[55]. Hence, neuronal subgroups responsive to multiple hormonal actions are particularly relevant for body weight control and obesity pathophysiology.

Heterogeneous expression of neuropeptides and neurotransmitters.

In adult mice, not every POMC neuron expresses high levels of the main functional marker POMC. Certain subsets (~27%) present low *Pomc* mRNA levels and high levels of the appetite-promoting neuropeptides *Agrp* and *Npy*[21]. This raises the question of whether these subsets have functional similarity to NPY/AgRP neurons.

In addition, different groups of POMC neurons with specific spatial localization can express either the inhibitory neurotransmitter GABA, the excitatory neurotransmitter glutamate or both[21,56–61]. Given the opposing neurobiological actions of GABA and glutamate, these subtypes could have distinct physiological consequences.

Interestingly, mice carrying a loss-of-function mutation for the *Pomc* gene are obese. When the *Pomc* gene is selectively re-expressed during postnatal life in POMC/GABAergic neurons of these animals, food intake is reduced, which completely reverses the obese phenotype[62]. Conversely, non-selective reactivation of *Pomc* in all POMC cells promotes only negligible anti-obesity effects[62]. Hence, POMC/GABAergic cells may act through yet-to-be-defined mechanisms that promote food intake and, possibly, body weight gain. Such a working model is supported by the recent observation that random activation of GABAergic hypothalamic ARC neurons results in obesity[63]. Accordingly, pharmacological inhibition of the mechanistic target of rapamycin pathway in mice, which leads to hyperphagia by mimicking a condition of low cellular energy levels in POMC neurons, activates POMC/GABAergic neurons[64]. Other POMC neuronal clusters (perhaps the glutamatergic ones) possibly govern opposing effects.

Additional layers of plasticity also seem to exist within each molecularly defined POMC neuron subpopulation. For instance, POMC neurons expressing the cannabinoid receptor type 1 are activated by the action of cannabinoids and promote food intake, by preferentially producing β-endorphin rather than α-melanocyte-stimulating hormone (α-MSH)[15]. Thus, following activation, certain POMC neuronal clusters can release various neuropeptides with opposing functions, possibly due to mitochondrial-related mechanims[15]. Other studies have shown that specific neuropeptide production may also be related to POMC cell ontogeny[65].

Collectively, several studies, involving both unbiased and biased methods (Fig. 1), suggest that different POMC neurons influence divergent metabolic responses as a result of their intrinsic cellular properties, including the expression of specific sets of receptors, neurotransmitters and neuropeptides implicated in metabolic control. Nevertheless, unbiased methods of classification, such as single-cell mRNA sequencing (scRNA-seq), rely on ex vivo information obtained from dissociated cells, which may not be representative of the in vivo context in which these cells normally reside. In addition, the extent to which a certain molecular profile overlaps with a specific neuroanatomical organization and functional state is not yet fully understood. Certain subsets could also operate independently of cell-intrinsic properties (for example, under the influence of heterogeneous afferent signals). A LepR-negative POMC neuron, for instance, might still respond to leptin via changes in the inhibitory inputs from leptin-sensitive NPY/AgRP neurons.

Thus, only a holistic approach that integrates the intrinsic cellular properties of POMC neurons with their spatial position in the brain and their sensitivity to afferent signals can uncover how the activity of specific POMC neuronal subsets translates into specific behavioural or metabolic effects. To effectively and rapidly establish this concept, the development of new tools is required, including methodologies that align a specific molecular profile with a defined morphological, electrophysiological and functional state (see Box 2)[66].

Scope of heterogeneity: multiple subsets, but same purpose?

Apart from regulating food intake, POMC neuronal activity has been linked to the modulation of multiple peripheral metabolic endpoints[3], although the data related to the

underlying mechanisms involved are somewhat contradictory. For instance, several studies support the idea that POMC neurons influence the release and utilization of glucose in peripheral organs[33,34,67–69], independently of changes in body weight or food intake[3], and possibly in response to the sensing of extracellular glucose fluctuations[69]. However, not all studies have found that POMC neurons are sensitive to glucose[70].

Moreover, when POMC neuronal glucose sensing is disrupted via different approaches, including alterations in ATP-sensitive potassium channel function, perturbations in specific pathways or modifications in mitochondria dynamics[69,71–74], disparate energy and glucose homeostasis phenotypes are observed[69,71–74]. In this context, chemogenetic inhibition of POMC neuronal activity in normoglycaemic mice reduces systemic blood glucose[33], which is surprising given that activation of POMC neurons in response to leptin prevents hyperglycaemia[48,49].

Cellular heterogeneity might explain these inconsistencies. On the basis of ex vivo electrophysiological investigations, certain POMC neuronal clusters increase their activity when extracellular glucose concentrations are reduced, whereas others decrease it, or exhibit a biphasic response[75]. Similarly, different POMC neuronal subsets can be inhibited or activated or can remain unaffected by the glucoregulatory hormone insulin[67]. Given that the above-mentioned genetics-based studies involve approaches that target the entire population of POMC neurons, the observed changes (or lack thereof) may reflect the modulation of specific subpopulations with divergent (that is, atypical) effects.

But what is the advantage of having different subpopulations that respond in a heterogeneous manner to the same fuel substrate? In light of the dynamic mode of action of these neurons (see previous sections), divergent mechanisms of glucose sensing may allow rapid accommodation of metabolic responses to defend euglycaemia.

During fasting, for instance, when both glucose and insulin levels drop, certain POMC neuronal subsets could either be activated or inhibited, working towards the same goal of increasing plasma blood glucose or reducing glucose uptake into peripheral organs, to maintain constant systemic glucose levels. Conversely, after a meal, these same neuronal subtypes operate in an opposite manner, but always to preserve glucose homeostasis.

Heterogeneous yet convergent mechanisms can also link POMC neuronal activity with the control of systemic lipid metabolism. For example, POMC neurons were shown to influence circulating cholesterol levels[76] and triglyceride synthesis[77], possibly in response to cell-specific mechanisms of fatty acid sensing[78,79].

Thus, heterogeneity and synergy probably represent two sides of the same coin (Fig. 2), with different POMC neuronal subsets adjusting to changes in the body’s energy status and fuel availability, not unlike the individual components of a car engine, which work in concert to regulate speed in response to movements in the brake and gas pedals.

How and when is POMC neuronal heterogeneity established?

Embryonic plasticity.

Pomc expression starts at embryonic day 10.5, when this gene is transiently expressed by the vast majority of cells in the developing ventral hypothalamus[52]. During gestation, *Pomc*-expressing immature cells can switch off POMC production and give rise to NPY/AgRP neurons or alternative cell types[52,80]. POMC and other neurons of ARC ontogeny are initiated and maintained during early embryonic development by transcriptional programmes launched by specific transcription factors, including Dlx1/2, Otp and neurogenin 3 (refs.[81,82]), among others[83]. Several microRNAs can also modulate these processes[84,85], highlighting the importance of epigenetic mechanisms.

Neonatal plasticity.

While the developmental identity of the majority of hypothalamic neurons is considered locked after early embryonic development in rodents, POMC neurons may retain plasticity during neonatal life, when the formation of axonal projections occurs, under the influence of hormonal signals[86,87]. Processes regulating these maturation steps play a critical role in programming whole-body energy balance in adulthood. Metabolic perturbations during this maturation period (for instance, those induced by maternal high-fat diet feeding) impair the POMC neuron translatome and neurocircuit development, predisposing the offspring to metabolic disorders[88–90].

Interestingly, at postnatal day 1, mice possess a significant proportion (40%) of POMC/glutamatergic neurons, whose levels progressively decline to 8% as the animals approach 8 weeks of age[91]. Conversely, a low proportion (just 8%) of POMC/GABAergic neurons are present at postnatal day 1, and their numbers progressively increase to 46% in adults[91]. The exact nature of the intracellular signals that maintain POMC neuronal identity plasticity after embryonic development is not fully known, but recent advances have identified islet-1 (ref. [92]), certain microRNAs[84] and the transcription factor T-box gene 3 (Tbx3)[26] as crucial regulators.

Plasticity in adulthood.

Loss of Tbx3 function in hypothalamic neurons alters the differentiation state and functional identity of ~50% of POMC neuronal cells during developmental maturation, ultimately leading to obesity and glucose intolerance[26]. Intriguingly, similar cellular and physiological perturbations are observed after loss of Tbx3 in fully differentiated hypothalamic neurons of adult animals[26]. Hence, specific POMC neuronal subsets may maintain intracellular programmes that possibly confer identity plasticity, even after terminal differentiation. This hypothesis is supported by the observation that some mature POMC neurons are enmeshed by perineuronal nets[93]—a condensed form of extracellular matrix implicated in neuronal developmental programming[94].

A certain fraction of POMC neuronal cells co-express high levels of *Agrp* and *Npy* mRNA in adult mice[21] (see also previous section). These subsets with such a mixed peptidergic identity and low *Pomc* expression could be embryonically derived cells where *Pomc*

transcription is never fully silenced during postnatal life, even after differentiation into NPY/AgRP neurons. Alternatively, they might represent newly committed NPY/AgRP neurons from residing neuronal stem cells, in which *Pomc* expression is yet to be fully suppressed. Indeed, hypothalamic neurogenesis can occur in adult mice[95], although its contribution to energy balance regulation is still controversial[96].

In conclusion, POMC neuronal diversity reflects the high degree of developmental plasticity of this cellular population during both embryonic and postembryonic life. It is worth noting that such plasticity may represent a conceptual obstacle when trying to decipher the postnatal functional role of these neurons using commonly available reporter mouse models (Box 1).

POMC neurons' heterogeneity beyond metabolism

The *Pomc* gene arose approximately 500 million years ago (ref.[2]), in an environment characterized by intermittent food availability, where being able to forage for supplies was imperative for survival. Our ancestors had to choose between staying immobile, safe and starving or moving and encountering potential threats or stressful/painful situations; thus, neurobiological mechanisms that orchestrate competing emotional states had to develop to overcome periods of famine[97,98]. In this evolutionary context, POMC neuronal heterogeneity may have originated as an adaptive mechanism, given that the activity of POMC neurons can affect behavioural and physiological responses associated with the evolutionary survival of our species, including stress[2,68,99,100], pain[101,102], fear[99,103] and locomotion[48,104].

Chronic restraint stress, or just an acute injection of a vehicle solution in mice, activates $POMC^{ARC}$ neurons[31,100] and translates into inhibition of dopamine neurons located in the ventral tegmental area[100]. Photo-inhibition of this $POMC^{ARC}$–ventral tegmental area circuit in chronically stressed mice increases body weight and food intake and reduces depression-like behaviours and anhedonia (that is, a deficit in the ability to experience pleasure)[100]. Certain $POMC^{ARC}$ neurons project to the nucleus accumbens[38], and α-MSH-mediated activation of MC4R in this brain area is required for chronic stress-elicited anhedonia, which can be prevented by inhibiting MC4R signalling[105]. Thus, striatal melanocortin signalling is critical for assigning negative motivational valence to harmful stimuli[106]. Other brain areas, such as the dorsal raphe nucleus, may further bridge POMC neuronal activity with the control of food intake and emotional states. Chemogenetic inhibition of dorsal raphe nucleus MC4R neurons induces depression, anxiety and reduced appetite, whereas chemogenetic activation reverses these effects[107].

Hence, one might speculate that POMC neuronal activation during stressful conditions is responsible for eliciting or amplifying negative emotional states that compete with hunger, although this contrasts with the observation that postnatal loss of function (ablation) of POMC neurons in mice leads to anxiety-like behaviours[68].

Heterogeneity, once again, may be at the core of these inconsistencies. If the ultimate physiological goal of POMC neuronal heterogeneity is to influence appetite and energy

balance, different POMC neuronal clusters may have been programmed by evolution to override potential anxiogenic feelings and painful or even fearful situations that stop locomotion and undermine food acquisition.

Effective foraging requires a proficient cardiovascular system that supports rapid and intense physical activity and subsequent oxygen delivery to internal organs during food processing. Certain POMC neuronal subsets are specialized in the modulation of cardiovascular response, due to their ability to control the sympathetic nervous system tone[108].

Accordingly, mice lacking LepR in POMC neurons do not respond to the hypertensive and sympathomimetic action of leptin[108,109]. These LepR-positive POMC cells may therefore operate via top-down pathways to increase blood pressure and heart rate in response to leptin action. Other subpopulations, instead, may have opposing cardiovascular effects, as chronic chemogenetic activation of POMC neurons reduces blood pressure[110], possibly reflecting the action of yet-to-be-identified subtypes.

Thus, multiple subsets and divergent mechanisms may allow integration of emotional, cardiovascular and behavioural outputs linked with food foraging. While reconciling conflicting data around this topic, overall, this evidence suggests that POMC neurons may have been programmed through mammalian evolution to coordinate a broader number of biological responses beyond energy balance (Fig. 3).

POMC neuronal heterogeneity in obesity

Available evidence.

The increasing worldwide prevalence of obesity is the result of genetic and epigenetic factors that interact with the environment and our lifestyle[111]. Sedentary behaviours and energy-rich/highly palatable foods promote weight gain in metabolically prone individuals. If POMC neuronal heterogeneity is the result of adaptive evolutionary mechanisms, obesity may then derive from alterations in the heterogeneous behaviour of these neuronal clusters since this disease is accompanied by POMC neuronal dysfunction[3,8–11]. This concept leads to two relevant questions: (1) are certain POMC neuronal subsets more or less susceptible to drive DIO; and (2) does POMC neuronal heterogeneity contribute to obesity and its associated cardiovascular or metabolic sequelae?

A final answer is not yet available, but several observations point to the existence of a complex link between POMC neuronal activity and obesity. Obesity is only observed after >80% of POMC neurons in the ARC are ablated[34], suggesting a large degree of redundancy[34], or that certain POMC cell clusters may somehow take over the role of dysfunctional cells during metabolic stress.

Only partial signs of POMC neuronal alterations are observed in animal models of DIO, which often involve a sub-fraction of cells. The number of inactive POMC neurons with no spontaneous action potential firing is increased in DIO mice by only ~20%[112] and long-term (8 months) exposure to hypercaloric diets leads to only 20–50% loss of POMC-expressing hypothalamic neurons[113,114]. In DIO mice, increased inflammatory and potentially

dangerous interactions between POMC neurons and the adjacent glial cells occur in a small subset (~10%) of neurons[114], resulting in synaptic derangements that are implicated in altered body weight regulation[115].

Notably, genetic approaches aimed at resolving or counteracting POMC neuronal dysfunction in DIO mice have led to divergent phenotypic outcomes. DIO activates inflammatory molecular pathways that potentially undermine neuronal activity[116], including the pathway formed by the proinflammatory protein nuclear factor κB and its upstream activator IκB kinase-β (IKK-β)[116]. POMC neuronal-specific IKK-β ablation does not prevent DIO, but it ameliorates obesity-induced hypertension[117]. Since certain POMC subsets are implicated in modulating the cardiovascular system, DIO-linked alterations in the nuclear factor κB/IKK-β pathway may involve specific subpopulations controlling cardiac outputs.

Under obesogenic conditions, perturbations in intracellular factors that are part of the InsR and LepR signalling cascade in POMC neurons (such as suppressor of cytokine signalling-3, protein tyrosine phosphatase 1B or T-cell protein tyrosine phosphatase, among others[3]) contribute to the pathogenesis of DIO. These molecular derangements promote a condition of hormonal inflexibility whereby POMC neurons are incapable of adapting their activity to extracellular hormonal signals[3]. Transgenic mice in which these common intracellular nodes have been specifically manipulated in POMC neurons to augment both insulin and leptin cellular sensitivity are protected from DIO[55], suggesting that neuronal subsets responsive to multiple hormonal signals may be particularly susceptible to DIO-induced neuronal dysfunction.

Missing evidence.

The number and exact identity of POMC neuronal subtypes affected by chronic metabolic stress may be influenced by several variables, including diet composition, length of exposure to overfeeding, genetic/epigenetic background and sex.

Understanding the potential impact of sex is particularly relevant given that obesity is growing at an alarming rate in both sexes[118]. DIO mice may have sex-specific effects on POMC neuronal dysfunction. Indeed, protein tyrosine phosphatase 1B deficiency in POMC neurons attenuates weight gain and fat mass accumulation in response to a high-fat diet in both sexes, but improves glucose tolerance and reduces hepatic lipid accumulation only in male mice[119]. Male and female mice have phenotypic differences in physical activity, energy expenditure and DIO susceptibility, and this is in part driven by subpopulations of POMC neurons expressing 5-HT_{2C}Rs[120].

Independently of how sex or other variables influence POMC neuronal dysfunction in obesity, which will require additional studies, unravelling whether and how metabolic stress alters POMC neuronal heterogeneity may significantly improve our comprehension of the neurobiology of this disease. Addressing this question may also pave the way to novel treatments that target specific POMC neuronal subtypes to safely ameliorate obesity, while bypassing potential emotional or cardiovascular side effects.

Conclusions and future directions

For those who have been working in the metabolic field for a long time, the story of how POMC neurons affect energy balance has been, and continues to be, an intriguing tale full of twists, turns and surprises.

POMC neurons were thought to decode a satiety signal in response to the internal energy state and to mainly behave as antagonistic functional partners of NPY/AgRP neurons, a model clearly too simplistic given the high degree of intercellular and intracellular plasticity discussed herein. Thus, the time is ripe for revising the conventional yin–yang action of POMC and NPY/AgRP as opposite partners in the regulation of energy balance. The molecular and functional diversity of POMC neurons now offers a foundation for understanding paradoxical effects on metabolism or non-canonical actions on emotional states and cardiovascular outputs.

Our understanding of the different POMC neuronal subsets and their various roles is still emerging, with several big questions remaining unresolved (Box 3). Addressing these questions is a priority as it may enable progress towards our understanding of both the aetiology and treatment of metabolic disorders.

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Box 1 |

Methodological limitations due to POMC neuronal developmental plasticity

Two types of genetically modified mouse strain have been generated and used in a large number of studies that investigate the role of POMC neurons in energy balance. The first is a transgenic mouse model with enhanced green fluorescent protein (eGFP) reporters driven by *Pomc* promoter elements (*Pomc*-eGFP)[5], which has been extensively employed for ex vivo electrophysiological recordings. A second *Pomc*-Cre bacterial artificial chromosome transgenic strain was instead developed to restrict genetic manipulations to POMC neurons and thus evaluate their in vivo function[47]. The use of *Pomc*-eGFP mice is limited by the fact that POMC neuron visualization will happen only if the neuron expresses a sufficient amount of *Pomc* mRNA. Hence, clusters with negligible *Pomc* mRNA levels, or transient expression of this marker, are likely to be missed during the analysis.

In contrast, when using the *Pomc*-Cre transgene to manipulate the expression of a certain molecular factor in POMC neurons, off-target Cre-mediated recombination may occur in non-POMC-expressing populations[121], since POMC (and therefore Cre) expressing cellular progenitors can generate multiple cell types across development[52]. These off-target effects represent an important caveat when interpreting the phenotype observed, especially if one considers that $POMC^{ARC}$ neurons have differential effects on food intake regulation relative to ARC neurons derived from POMC-expressing progenitors[65]. Notwithstanding, a transgenic line that allows temporal (tamoxifen-based) Cre-mediated recombination in POMC neurons is also available[54] and overcomes these obstacles.

Box 2 |

New tools for studying POMC neuronal heterogeneity

Recent progress has been made in the development of spatial transcriptomics methods that exploit transcriptional cell-type classifications and map their spatial distributions[122–124]. This approach allows the investigation of specific cell types in situ, preserving the spatial context and overall brain circuit architecture.

A key recent advance in neuroscience research is the implementation of a system called INTRSECT (intronic recombinase sites enabling combinatorial targeting), which combines multiple recombinase-dependent engineered viral vectors with specific recombinase-expressing transgenic animals[125]. INTRSECT allows the targeting of particular cell types with multiple (dual/triple) defined features[126] and could therefore be used to elucidate the function of diverse POMC neuronal subsets.

The generation of new intersectional viruses might enable the application of electrophysiology, optogenetics, chemogenetics, calcium imaging or circuit mapping in specific clusters of POMC neurons. For instance, calcium imaging combined with two-photon microscopy[127] could be employed in association with INTERSECT to image single neuronal cells in immobilized animals. A microendoscopic calcium imaging approach that utilizes a head-mounted, miniaturized microscope[128], or a different and recently established technique that involves optetrode electrophysiology[30], might instead be used to assess specific subsets at single-cell resolution in freely moving mice.

Box 3 |

Outstanding questions

Heterogeneity or heterogeneities?

Different POMC neuronal subsets have been programmed by evolutionary selection to orchestrate metabolic and behavioural endpoints necessary for survival. However, can one molecularly distinct POMC subtype switch its identity and adopt multiple phenotypes? For instance, POMC neurons can release both α-MSH and β-endorphin[15,65], neuropeptides with opposing effects on food intake regulation. The release of one neuropeptide rather than the other certainly depends on specific input signals, but the cellular underpinnings have yet to be clearly elucidated. Addressing this question will also require thorough analysis of the function of specific POMC neuron subpopulations in response to changing metabolic needs or different behavioural patterns, or during DIO.

Which subtype controls which circuit?

Beyond contributing to energy balance, POMC neurons affect cardiovascular responses, pain, fear, anxiety and locomotion. Their widespread spatial projection profile[38] probably explains such functional diversity. However, the exact identity and mode(s) of action of the multiple brain circuits controlled by different POMC neuronal subtypes are substantially unknown.

Does obesity alter only specific POMC neuronal subpopulations?

DIO may selectively alter certain POMC neurons, but what defines the susceptibility of these cells to metabolic stress? A comprehensive investigation of the effects of hypercaloric diets at single-cell resolution is still missing. scRNA-seq[21–23] or other emerging spatial transcriptomic approaches (see Box 2) could be used to address this key open question. These efforts may represent a first step leading to the development of precision therapeutic tools targeting selective POMC subtypes and safely ameliorating obesity.

How does sex impact the function of POMC neurons?

Sexual dimorphism exists in energy balance and obesity pathogenesis, hampering the efficacy of anti-obesity strategies in both sexes. The underlying mechanisms are still unclear, but the female sex hormone oestrogen has long been considered a contributor[129]. Female mice have more POMC neurons in the hypothalamus than males[130] and present phenotypic differences in physical activity, energy expenditure and DIO susceptibility[120], which are partly driven by a specific POMC neuronal subpopulation[120]. Uncovering the molecular and functional identity of these POMC subtypes implicated in sex dimorphism may favour the development of novel anti-obesity treatments effective in both sexes.

Can specific POMC neuron subpopulations be targeted pharmacologically?

Obesity is a heterogeneous disease that requires combined pharmacological strategies to correct multiple metabolic pathways. Multi-agonists that concomitantly activate glucagon-like peptide-1 receptors (GLP-1Rs) and additional metabolic receptors are

under scrutiny given their exceptional preclinical efficacy[131,132]. POMC neuronal activity partially mediates the anti-obesity effects of GLP-1R agonists[133,134] and the weight-lowering drug lorcaserin[135]. It is likely that different POMC neuronal subpopulations expressing GLP-1Rs or 5-HT_{2C}Rs[21] mediate these effects. Thus, uncovering the mode(s) of action of POMC neuronal subtypes expressing GLP-1Rs, 5-HT_{2C}Rs or other druggable metabolic receptors may spur the development of potent and safe pharmacological approaches against obesity that target specific POMC neuronal clusters.

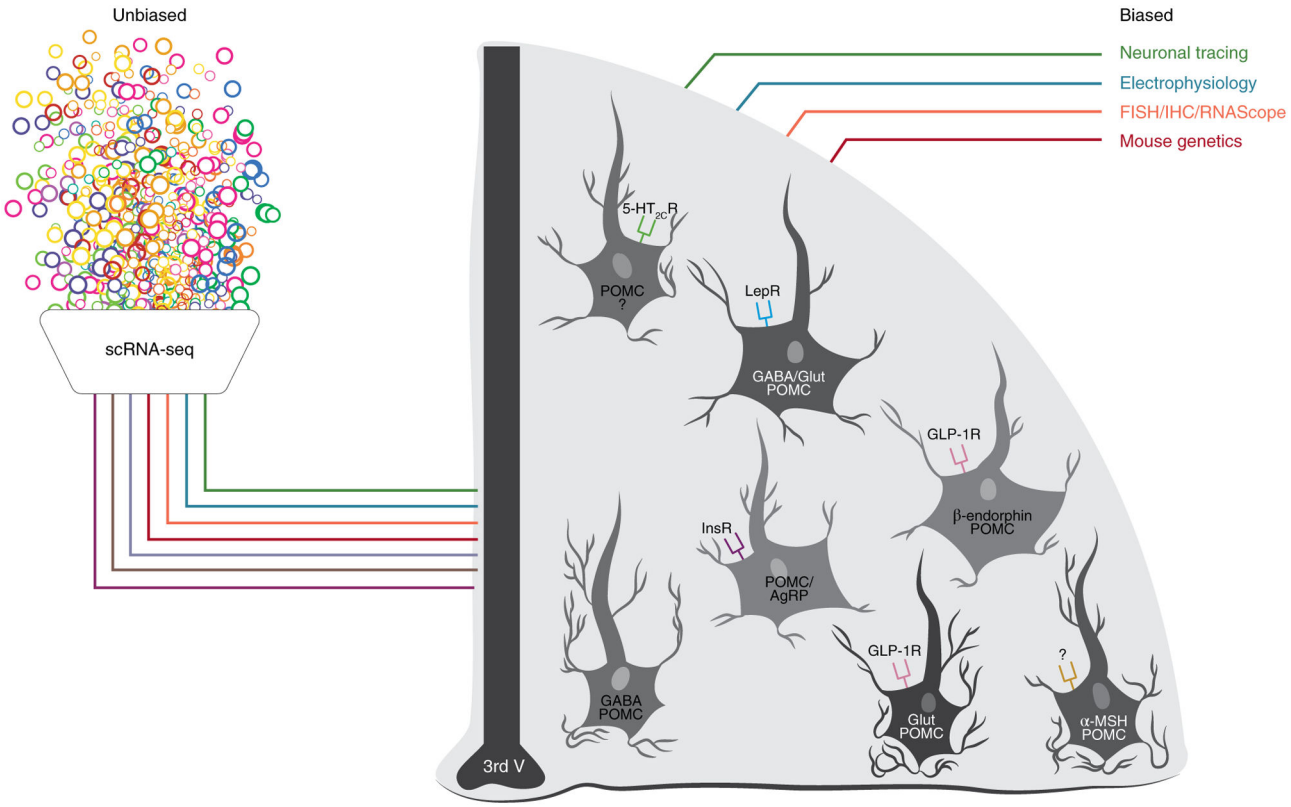

Fig. 1 |. Current classification methods used to identify POMC neuron subtypes.

Schematic of the unbiased (scRNA-seq analysis) and biased methods (neuronal tracing, electrophysiology, cell labelling (fluorescence in situ hybridization (FISH), immunohistochemistry (IHC) and RNAScope for molecular marker, neurotransmitter and neuropeptide expression) and mouse genetics) used to date for identifying and characterizing POMC neurons. Note that the expression of receptors, neuropeptides and neurotransmitters at the level of the cells is only representative. The question marks represent receptors or other molecular markers that are still to be discovered. 3rd V: third ventricle; Glut, glutamate. Figure adapted with permission from C. Padgett.

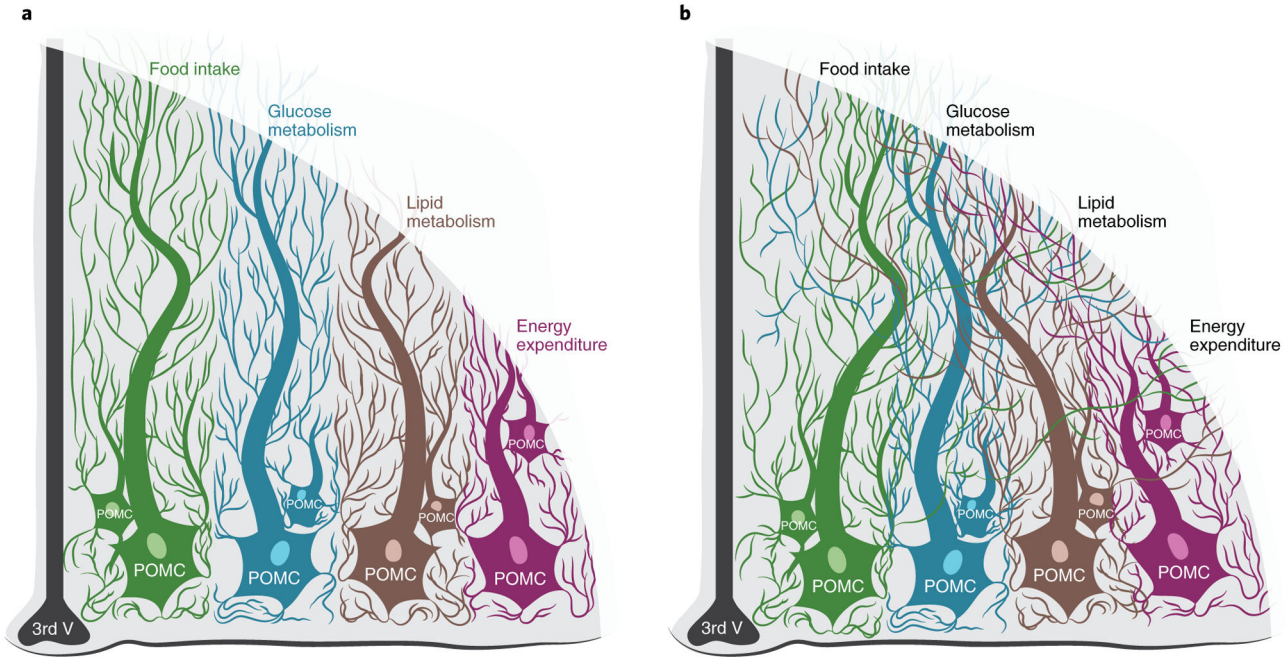

Fig. 2 |. Mode(s) of action of different subsets of POMC neurons for the regulation of energy balance.

a, Different subsets of hypothalamic POMC neurons can modulate different outputs. **b**, Different subsets of POMC neurons can also converge to modulate the same output and/or the same subsets of cells can act on multiple outputs. Figure adapted with permission from C. Padgett.

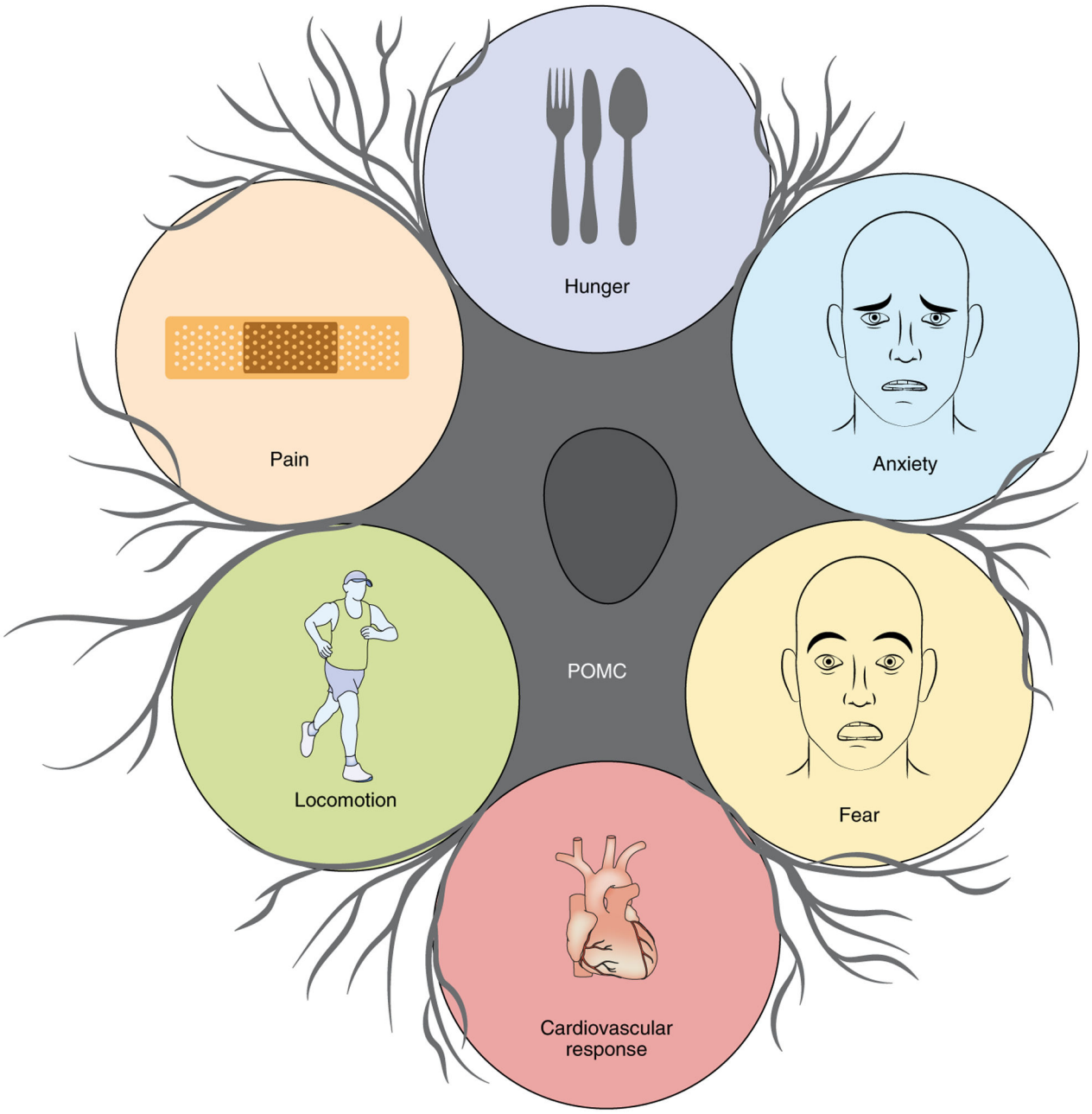

Fig. 3 |. Physiological roles of POMC neurons beyond energy balance.
Beyond a critical role in energy balance, POMC neurons orchestrate behavioural and physiological responses necessary for survival, including modulation of pain, stress/anxiety, fear, locomotion and cardiovascular responses. Figure adapted with permission from C. Padgett.