REVIEW

Molecular and functional mapping of the neuroendocrine hypothalamus: a new era begins

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Abstract

Background Recent advances in neuroscience tools for single-cell molecular profiling of brain neurons have revealed an enormous spectrum of neuronal subpopulations within the neuroendocrine hypothalamus, highlighting the remarkable molecular and cellular heterogeneity of this brain area.

Rationale Neuronal diversity in the hypothalamus reflects the high functional plasticity of this brain area, where multiple neuronal populations flexibly integrate a variety of physiological outputs, including energy balance, stress and fertility, through crosstalk mechanisms with peripheral hormones. Intrinsic functional heterogeneity is also observed within classically 'defined' subpopulations of neuroendocrine neurons, including subtypes with distinct neurochemical signatures, spatial organisation and responsiveness to hormonal cues.

Aim The aim of this review is to critically evaluate past and current research on the functional diversity of hypothalamic neuroendocrine neurons and their plasticity. It focuses on how this neuronal plasticity in this brain area relates to metabolic control, feeding regulation and interactions with stress and fertility-related neural circuits.

Conclusion Our analysis provides an original framework for improving our understanding of the hypothalamic regulation of hormone function and the development of neuroendocrine diseases.

Keywords Hypothalamus · Energy balance · Fertility · Stress · Neuroendocrinology · Neuronal heterogeneity

Abbreviations

3V	Third ventricle
ACTH	Adrenocorticotropic hormone
AgRP	Agouti-related protein
ARC	Arcuate nucleus
AVP	Arginine vasopressin
AVPV	Anteroventral periventricular nucleus
CRH	Corticotropin-releasing hormone
Crhr	Corticotropin-releasing hormone receptor
DMH	Dorsal medial hypothalamus
Drd1	Dopamine receptor D1
DYN	Dynorphin
ECM	Extracellular matrix
ECS	Extracellular space
ERα	Oestrogen receptor alpha
FSH	Follicle-stimulating hormone
GABA	Gamma-aminobutyric acid
GLP1	Glucagon-like peptide-1
Glp1r	Glucagon-like peptide-1 receptor
GnRH	Gonadotropin hormone-releasing hormone
HFD	High-fat diet
HPA	Hypothalamic-pituitary-adrenal axis
HPG	Hypothalamic-pituitary–gonadal axis
Kiss1	Kisspeptin
Lepr	Leptin receptor
LH	Luteinising hormone
MC4R	Melanocortin 4 receptor
ME	Median eminence
mENK	Methionine enkephalin
MePO	Median preoptic nucleus
NKB	Neurokinin B
NPY	Neuropeptide Y
OV	Organum vasculosum of the lamina terminalis
OXT	Oxytocin
Pdyn	Prodynorphin
POMC	Proopiomelanocortin
PVN	Paraventricular nucleus
RCA	Retrochiasmatic area
Rprm	Reprimo

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SST   Somatostatin
TH   Tyrosine hydroxylase
VMH   Ventral medial hypothalamus
VMHvl   Ventrolateral division of VMH
αMSH   Alpha-melanocyte-stimulating hormone

Introduction

The hypothalamus, with its intricate bidirectional communication between neurons and hormones, holds a central position in regulating a multitude of physiological outputs and behaviours essential for survival. These encompass vital functions such as food and water consumption [1, 2], energy balance and thermal regulation [3, 4], osmotic balance [5], circadian rhythm [6], reproduction [7, 8], stress response [9, 10], and goal-oriented behaviours [11, 12]. The orchestration of multiple outputs requires considerable functional adaptability, which is reflected in the organization of endocrine neurons into multiple subpopulations. Traditionally, subpopulations of hypothalamic neuroendocrine neurons have been classified into functionally homogeneous clusters based on their molecular, topological and electrophysiological properties. However, this conventional view is now being challenged. The advancement of the single-cell molecular profiling technique has revealed the presence of sub-clusters in each neuronal subpopulation that may be involved in distinct physiological roles and modes of action, unravelling a more intricate and subtle working model [13–18]. The identification of new molecular markers within 'defined' neuroendocrine subpopulation and the development of genetically engineered mouse models that allow precise manipulation of these subpopulations using chemogenetic and optogenetic techniques have led to the emergence of new perspectives on the functional heterogeneity of the hypothalamus.

Amidst this progress, several critical questions emerge: Does heterogeneity among endocrine neuronal populations remain static throughout an organism's lifespan? Can physiological and environmental stimuli dynamically influence hormonal responses by modifying the heterogeneity of endocrine neurons? How does neuronal heterogeneity influence the onset and progression of neuroendocrine diseases? In this review, we address these central questions by discussing emerging models of hypothalamic neuroendocrine neuron heterogeneity. We will review potential molecular and cellular mechanisms underlying the functional heterogeneity and adaptability of hypothalamic neurons involved in hormonal regulation. We will also investigate the impact of environmental and internal cues on shaping neuronal diversity and adaptability in the hypothalamus across the lifespan, and explore the implications for understanding the aetiology of neuroendocrine diseases such as obesity and infertility disorders.

Current and evolving models of neuronal heterogeneity in the neuroendocrine hypothalamus

Neurochemical heterogeneity

Current knowledge suggests that endocrine neurons in the hypothalamus can be divided into functionally distinct populations based on the expression of specific neuropeptidergic markers and peptide hormones that regulate specific endocrine functions. For example, in the arcuate nucleus (ARC) of the hypothalamus, proopiomelanocortin (POMC)-expressing neurons are characterised by the expression of the neuropeptidergic precursor POMC, which undergoes post-translational cleavage to produce various biologically active neuropeptides, such as α-melanocyte stimulating hormone (α-MSH), which suppress appetite in response to peripheral hormonal actions [19]. When POMC neurons are activated by an interoceptive stimulus, such as in response to changes in peripheral hormones, the resulting release of α-MSH from the axon terminals activates melanocortin-4 receptors (MC4R) on target secondary order neurons to suppress food intake and increase energy expenditure [20]. This central regulatory mechanism involves MC4Rs expressed in the paraventricular nucleus (PVN) or the dorsomedial hypothalamus (DMH) of the hypothalamus, thereby forming a crucial circuit that dynamically regulates feeding behaviour and metabolism in response to changes in the body energy status, commonly known as the central melanocortin circuit [19, 21].

The melanocortin circuit is co-regulated by a second key neuronal population defined by the co-expression of the neuropeptide agouti-related protein (AgRP) and neuropeptide Y (NPY). Unlike POMC neurons, which are typically considered excitatory, AgRP neurons are inhibitory in nature. They release AgRP, which is an endogenous antagonist of MC4R [22]. AgRP counteracts the effects of α-MSH by competing for MC4R binding; the inhibition of MC4R subsequently increases appetite and reduces energy expenditure. AgRP/NPY neurons exert their influence not only by antagonising POMC neurons at their target sites, where MC4Rs are located, but also by directly inhibiting POMC perikaryal [23]. This direct inhibition involves a 36 amino-acid neuropeptide, NPY [23–25], which suppresses POMC neuronal activity whenever AgRP/NPY neurons are active [23].

POMC neurons are typically inhibited under conditions of low energy availability, whereas they are activated by hormonal and nutrient-related signals indicating a surplus of body energy reserve. Activation of POMC neurons in response to energy supply, such as after a meal, is

necessary to maintain a stable body weight by reducing food intake and increasing energy expenditure [26, 27]. Conversely, when energy reserves in the body are low during fasting, AgRP/NPY neurons are activated to stimulate hunger and reduce energy expenditure, thereby promoting energy repletion via both POMC neuron-dependent and independent mechanisms.

Thus, the melanocortin circuit is adept at responding to continuous changes in peripheral endocrine signals that inform the brain of changes in the whole-body energy status. Among these peripheral signals, the orexigenic hormone ghrelin is known to induce hunger by activating AgRP/NPY neurons, whereas anorexigenic endocrine signals such as insulin and leptin act in an opposite manner by inhibiting AgRP/NPY neurons while activating POMC neurons [26–28] respectively. The neuroendocrine significance of this feedback loop is evidenced when it fails to respond properly to anorexigenic metabolic hormones, leading to detrimental metabolic consequences. Insulin and leptin receptors are expressed in POMC and AgRP/NPY neurons [28], and disruptions in these hormonal signals pave the way to metabolic disorders. Nevertheless, despite acting on the same receptor, the mechanisms by which the same hormone, such as leptin, can exert contrasting neurobiological effects on POMC and AgRP neuronal populations remain a subject of intense research and never-ending debate, as extensively discussed in previous work [29–32]. Regardless, the heterogeneous response of different neurochemically defined neuronal populations to a specific hormonal signal is likely to be a general critical feature for normal hypothalamic function, allowing rapid and accurate monitoring of metabolic status and subsequent energetic adjustments.

A similar neuropeptidergic model can be used to explain how the hypothalamus orchestrates other neuroendocrine axes, such as stress and fertility, by 'defined' populations of neurons that act via the release of 'defined' sets of neuropeptide hormones. To regulate reproductive cycles, kisspeptin (Kiss1)-expressing neurons in the anteroventral periventricular nucleus (AVPV) influence the release of pituitary and gonadal hormones to modulate the hypothalamic-pituitary–gonadal (HPG) axis [33, 34]. During pubertal development in female mice, kisspeptin-immunoreactive fibres from the AVPV innervate gonadotropin-releasing hormone (GnRH) neurons in the organum vasculosum laminae terminalis (OV) and the median preoptic nucleus (MePO) around postnatal day 25 (P25) [35]. These AVPV fibres project directly to GnRH neurons in the OV and MePO [36], which express the kisspeptin receptor [37]. The preovulatory surge of oestradiol in the ovary triggers AVPV Kiss1 neurons to stimulate GnRH release [38], subsequently leading to a rise in luteinising hormone (LH) and follicle-stimulating hormone (FSH). This hormonal cascade plays a crucial role in guiding the onset of puberty during sexual maturation [39].

Following the same model, when eliciting fight-or-flight behaviour in response to stressors, a population of neurons in the PVN plays a central role in modulating behavioural and metabolic adaptation to cope with the cue through the release of corticotropin-releasing hormone (CRH) [40]. In response to environmental stressors. PVN CRH neurons are activated and rapidly release CRH into the pituitary portal circulation, followed by activation of the hypothalamic–pituitary–adrenal (HPA) axis. At the same time, stress-induced glucocorticoid secretion from the adrenal glands provides negative feedback to override the HPA axis and prevent system overactivation. The regulation of CRH release from PVN neurons is complex and involves multiple neurotransmitters, hormones and feedback mechanisms. Interoceptive signals such as glucocorticoids (including cortisol), vasopressin and neurotransmitters, such as serotonin and norepinephrine can modulate CRH release. In addition, feedback loops involving cortisol and other components of the HPA axis help to regulate CRH secretion to maintain neuroendocrine homeostasis [41].

These examples provide just a glimpse of the intricate mechanisms by which defined populations control specific neuroendocrine outputs. However, the complexity within neuronal populations extends far beyond neuropeptidergic control. In addition to neuropeptides, different neuronal populations exert their influence through a variety of downstream secreted factors, including fast acting neurotransmitters. These multiple interactions play a crucial role in shaping the plasticity during neurotransmission, highlighting the rich complexity of neural activity regulation within the neuroendocrine hypothalamus. As recently demonstrated by single-cell RNA sequencing data, neurochemically defined populations in the hypothalamus can express a repertoire of different neurotransmitters, including glutamate, gamma-aminobutyric acid (GABA) or both [42]. Glutamatergic signalling in the brain is recognised for its capacity to elicit excitatory responses, whereas GABAergic neurons exert inhibitory effects. Thus, despite expressing the same neuropeptidergic markers, different neurons in each 'defined' population may act dichotomously to modulate the excitatory-inhibitory balance of postsynaptic neurons, potentially displaying intrinsic heterogeneity. Accordingly, neuropeptides and neurotransmitters do not operate in isolation, as neuropeptides are involved in fine-tuning neurochemical transmission to elicit rapid responses while adjusting signal strength. POMC neurons, for example, are classically thought to induce satiety once activated by promoting fast-acting glutamatergic transmission to satiety-supportive neurons in the PVN [43]. However, accumulating evidence suggests that POMC neurons can be divided into GABA and glutamatergic subtypes [44–49], and that the two distinct subpopulations may play different roles in food intake regulation. Mice with a *Pomc* gene loss-of-function mutation

typically develop obesity. When the *Pomc* gene is selectively re-expressed during postnatal life specifically in GABAergic POMC neurons, food intake is reduced, effectively reversing the obese phenotype [50]. This suggests that GABAergic POMC neurons may act through yet-to-be-defined mechanisms to promote food intake and weight gain, thus challenging the classical paradigm in which POMC neurons consistently act as satiety signals. Recent findings align with this notion, demonstrating that pharmacological inhibition of the mechanistic target of the rapamycin (mTOR) pathway in mice activates GABAergic POMC neurons and induces hyperphagia by mimicking low cellular energy levels of POMC neurons [44].

The presence of highly plastic neurobiological pathways connecting neuropeptides and fast-acting neurotransmitters extends beyond ARC neuronal populations. Orexin neurons (hypocretin neurons) are located primarily in the lateral hypothalamus and adjacent areas [51]. They are named after the neuropeptides they produce, orexin-A (hypocretin-1) and orexin-B (hypocretin-2), which play crucial roles in regulating various physiological processes, including sleep–wake cycles, energy homeostasis, reward processing, and arousal [52]. Synaptic transmission in orexin neurons involves a complex interplay of neurotransmitters, receptors and neural circuits. Glutamate is the primary excitatory neurotransmitter in synaptic transmission in orexin neurons [53, 54]. Glutamatergic inputs from other brain regions, such as the locus coeruleus and the basal forebrain, can excite orexin neurons and promote their firing activity [55]. This excitatory input is crucial for regulating arousal and wakefulness, as orexin neurons are most active during wakefulness and play a key role in promoting arousal and maintaining wakefulness. Notably, the activity of orexin neurons is also modulated by several neuromodulators, including dopamine [56], serotonin [57], and acetylcholine [58], which affect the excitability and synaptic transmission of orexin neurons, further regulating their activity and function. Thus, overall synaptic transmission in orexin neurons is finely tuned by a balance of excitatory and inhibitory inputs from different brain regions and neurotransmitter systems, which ultimately influence neuropeptide release and downstream actions.

In summary, the coexistence of glutamatergic and GABAergic subtypes within populations traditionally considered homogeneous functional units in the neuroendocrine hypothalamus, alongside various extracellular signals modulating neurotransmission, underscores the necessity for developing more comprehensive models of functional heterogeneity of the neuroendocrine hypothalamus. Novel conceptual frameworks are essential to elucidate how the neurochemical diversity of hypothalamic neuronal populations translates into specific functional properties, going beyond the conventional 'one population, one neuropeptide, one physiological function' paradigm.

Spatial heterogeneity of the neuroendocrine circuits in the hypothalamus

Early electrophysiological and histological studies have provided preliminary evidence that the anatomical position of a hypothalamic neuron population expressing the same peptidergic marker may determine their responses to circulating hormones [59, 60]. For example, leptin and glucagon-like peptide-1 (GLP1), which are involved in regulating glucose metabolism and stimulating satiety via POMC neurons expressing the leptin receptor (*Lepr*) and glucagon-like peptide-1 receptor (*Glp1r*), may regulate different subpopulations of POMC neurons localised in specific spatial locations Most *Lepr*-expressing POMC neurons are located in the anterior division of ARC, while *Glp1r*-expressing neurons are, to a larger extent, located in the posterior ARC [61]. Leptin is regarded as a regulator that coordinates long-term energy balance [62], whereas GLP1 exerts an acute effect on promoting satiety and suppressing food intake [63, 64]. During the progression of obesity, obese patients develop resistance towards these hormones, which exert opposing effects on POMC neurons. Leptin activates POMC neurons to promote glucose metabolism [65], while insulin can suppress hepatic gluconeogenesis by inhibiting POMC neuron activity [66]. In response to acute insulin or leptin application, leptin-excited POMC neurons are mainly located in the lateral division of the retrochiasmatic area (RCA) and medial division of the ARC. In contrast, insulin-inhibited POMC neurons are primarily localised to the medial RCA and anterior ARC, which agrees with the observed insulin receptor distribution [59]. POMC neurons in the anterior ARC project primarily to autonomic areas [67, 68], and those in the medial posterior ARC project mainly to hypothalamic nuclei [69, 70]. Thus, certain subpopulations of POMC neurons may be more or less sensitive to certain hormones, depending on their spatial organisation, thereby fine-tuning different types of downstream brain circuits.

The physiological purpose underlying the spatial arrangement of various neuroendocrine subpopulations in the hypothalamus remains enigmatic. Specific neuronal subtypes may need strategic positioning to receive and transmit specific signals from the peripheral bloodstream. Neurons in the ARC are located in close proximity to the third ventricle (3V) and the median eminence (ME), which is a region more exposed to nutrients and signalling molecules release due to the presence of fenestrated blood vessels [71] and the tanycytic barrier [72, 73]. In addition, the organisation of the extracellular matrix (ECM) in the ARC-ME region may affect the diffusion of hormones and nutrients [74, 75]. The molecular composition, pore size, and electrical charge properties of ECM influence the molecular diffusivity [76, 77]. ARC GABAergic neurons in the vicinity of ME are enveloped by a condensed form of ECM composed mainly

of hyaluronan and chondroitin sulphate proteoglycans, which are known as the perineuronal net (PNN) [78]. The PNN wraps the somata of ARC GABAergic neurons but leaves space for the access of specific afferent synaptic boutons. While the functions of PNNs are still being debated in the scientific community, they are thought to play a crucial role in regulating synaptic transmission and plasticity in the brain and the proper development of neural circuits [79–82]. This includes the maturation of ARC neurons, possibly via mechanisms controlled by the metabolic hormone leptin [78]. Of note, PNNs may also serve as physical tunnels or scaffolds that facilitate the transport of hormones and nutrients.

PNNs show a heterogeneous organisation within the ARC. Approximately 80% of NPY/AGRP neurons are surrounded by PNNs, whereas only about 50% of POMC neurons are surrounded by this specific extracellular matrix [78]. This suggests that functional differences between and within populations may be influenced by the properties of the extracellular matrix, thereby affecting sensitivity to extracellular homeostatic signals. The heterogeneous distribution of PNNs around ARC neurons, located in a brain region with a permeable blood–brain barrier, supports the hypothesis that specialised mechanisms that regulate the flow of signalling molecules from the bloodstream to specific spatial locations within the brain are crucial for synaptic plasticity and neuroendocrine responses.

The spatial distribution of endocrine neurons may not only be related to the purpose of communicating with blood-borne hormonal signals. Oxytocin (OXT) neurons in PVN are located where there is a lack of contact with fenestrated blood vessels. These neurons are classified into two distinct subpopulations, including magnocellular neurons, characterised by larger somata, and parvocellular neurons, with smaller somata, each displaying unique dendritic structures and electrophysiological properties [83]. While magnocellular and parvocellular OXT neurons regulate social and emotional behaviours and physiological responses such as uterine contractions and milk ejection [84], the two populations are localised in different spatial positions in PVN [83]. Magnocellular neurons primarily reside in the anterior PVN, facilitating an efficient release of signals into the bloodstream to regulate peripheral physiological functions. Conversely, parvocellular neurons in the posterior PVN are strategically positioned to modulate central neural circuits involved in social and emotional behaviours [85]. A subset of approximately 30 parvocellular OXT neurons has been identified that have collateral projections to magnocellular OXT neurons and deep spinal cord neurons [86]. This subset has been implicated in regulating nociception and analgesia in an animal model of inflammatory pain, further demonstrating how intrinsic differences in the spatial organisation of molecularly 'defined' populations can lead to different physiological outcomes [86]. Thus, the inherent

spatial diversity within neuropeptidergic populations is indispensable for orchestrating changes in peripheral hormonal responses alongside the modulation of secondary-order brain neurons responsible for processing behavioural and metabolic responses, among other functions.

Sex dimorphism of the neuroendocrine circuits in the hypothalamus

Sex hormones, such as testosterone and oestrogen, play a pivotal role in shaping sex-specific neural circuits in the hypothalamus during development [87], influencing sexual differentiation [88].

In adult rats, Kiss1 neurons in the AVPV are sexually dimorphic, with females having more Kiss1-expressing cells than males [89]. This sexual dimorphism of the AVPV Kiss1 circuit is influenced by the hormonal environment, independent of neurogenesis [90], during the postnatal period. The AVPV neuronal population is susceptible to testosterone or its aromatised metabolite oestradiol during perinatal development [89, 91, 92]. During P7-10 days, male neonates produce testosterone, while females produce negligible levels of sex steroids [93]. The testosterone surge during this critical period potentially triggers cell death of AVPV Kiss1 neurons in male neonates [94], and the apoptotic event can be prevented by gonadectomy [92]. A similar phenomenon can be recapitulated in female neonates upon exogenous testosterone administration [89, 92, 95]. Without perinatal testosterone surge in female mice and rats, *Kiss1* mRNA transcript and protein expression become detectable between P10 and P15 [96, 97]. From P15 to adulthood, AVPV *Kiss1* expression increases steadily [96], while AVPV Kiss1 fibres innervate GnRH neurons in female mice at P25 [35]. Ovariectomy at P15 significantly reduces AVPV *Kiss1* levels in adult females [96], indicating the essential role of ovarian sex hormone secretion in the maturation of the AVPV Kiss1 circuit, which can be restored by oestrogen replacement [96]. Other studies demonstrate the involvement of oestrogen receptor alpha (ERα). Furthermore, blunting oestrogen signalling by *ERα* deletion in Kiss1 neurons diminishes AVPV *Kiss1* expression in adult females [98], confirming the involvement of oestrogen signalling in the postnatal development of the AVPV Kiss1 circuit. It is currently uncertain why males require fewer AVPV Kiss1 neurons than females, probably due to the absence of ovulation in males [8].

Other than the AVPV kisspeptin circuits, gonadal hormones regulate the sexual dimorphism of reprimo (*Rprm*), tachykinin 1 (*Tac1*), and prodynorphin (*Pdyn*) neuronal subpopulations in the ventral medial hypothalamus (VMH) via oestrogen signalling [99]. These differences contribute to the modulation of thermogenesis [99], physical activity [100], and potentially reproductive behaviours in a sex-specific manner. Female VMH is enriched by *Rprm* and

Tac1-positive neurons, whereas male VMH is enriched by *Pdyn*-positive neurons. *Rprm* and *Tac1* clusters are eliminated in males, and the *Pdyn* cluster is eliminated in females and is regulated by gonadal hormones but not sex-linked chromosomes [99]. In females, *Rprm* gene silencing in VMH during adulthood increases core temperature [99], and genetic deletion of *Tac1* in VMH mildly reduces locomotor activities [101]. In males, testosterone can be aromatised to oestradiol to defeminise the VMH by suppressing *Tac1* and *Rprm* expressions through ERα signalling and to reinforce the male-specific identity of VMH by maintaining *Pdyn* expression via testosterone in adulthood [99]. *Pdyn*-expressing cells in the VMH are the cold-sensitive cells in males, which are activated by cold shock and suppressed by heat, as shown by in vivo calcium recording [102]. Thus, sexually dimorphic circuits and nuclei shape different metabolic outcomes and reproductive behaviours in response to sex hormones, which are vital in directing the functional heterogeneity of the neuroendocrine circuits that control fertility and reproductive behaviours.

Sex dimorphism is also observed in the neural circuits that affect body energy balance. Females are more protected by hypercaloric diet-induced metabolic disorder [103, 104], but the protection is abolished when female sex hormones are depleted in menopause [105, 106] or prematurely by ovariectomy in rodent models [107, 108]. From a neuroanatomical standpoint, female mice have more ARC POMC neurons, which display higher firing rates than males [109]. At the molecular level, male and female POMC neurons may recruit different molecular machinery to regulate energy balance and diet-induced obesity susceptibility [110, 111]. Obesity or diet-induced obesity is exclusively observed in a sex specific manner when specific genes, for example, 5-hydroxytryptamine 2c receptor (5-HT$_{2C}$R), in males or oestrogen receptors in females are deleted in POMC neurons [109, 112–115]. These collective findings demonstrate that sex hormone regulation can shape sexual dimorphism in molecularly defined neuronal populations in the hypothalamus to orchestrate physiological outcomes associated with

both reproductive outcomes and energy balance regulation (Table 1).

One population, multiple functions: integrative models of the neuroendocrine circuits in the hypothalamus

According to the central dogma of neuroscience, neurons in the mammalian brain establish their specific function, including their neurochemical identity, before birth through the action of regulatory proteins known as 'terminal selectors'. Once this developmental process is complete, each neuron is thought to maintain its function within its designated group throughout its lifetime. However, this view does not take into account that hypothalamic neurons often monitor and flexibly adapt their neuronal and hormonal regulation, as well as their neuropeptidergic machinery, to both internal physiological needs and environmental cues. Thus, while the notion of 'one population, one physiological function' still prevails in the field, there is increasing evidence that the functional roles of specific neuronal populations in the neuroendocrine hypothalamus are much more complex (Fig. 1), as discussed below.

Energy balance vs reproduction

In the ARC, POMC and AgRP neurons are widely recognised as important regulators of energy balance, whereas Kiss1 neurons have a more specialised role in fertility control. However, a closer examination reveals that these different neuronal populations can often control the same physiological outcome. Sexual maturation and fertility are critical for mammalian survival, and these neuroendocrine outputs are integrated with the regulation of whole-body energy homeostasis. Nutritional status, on the other hand, plays a critical role in reproductive health and outcomes, possibly through intimate cross-talk mechanisms between melanocortin neurons, Kiss1 neurons and the HPG axis.

Table 1 Sexually dimorphic neurons in the neuroendocrine hypothalamus

Hypothalamic nucleus	Neuronal population	Sex-specific responses to sex hormones		
		Anatomical features	Hormone/neurotransmitter triggering physiological responses	Sex-specific physiological outcomes
AVPV	Kiss1 [98]	Fewer in males	Oestrogen (♀)	Regulating pre-ovulatory LH surge (♀)
VMH	Rprm & Tac1 [99] [101]	Female-enriched	Oestrogen (♀)	Basal thermogenesis & physical activity (♀)
VMH	Pdyn [102]	Male-enriched	Testosterone (♂)	Cold-induced thermogenesis (♂)
ARC	POMC	More in females with higher firing rates	Oestrogen (♀)	Protective effect on diet-induced obesity (♀) [110]
			Serotonin (♂)	Physical activity (♂) [111]

Fig. 1 Challenging the 'one population, one physiological function' paradigm, rodent studies have provided new insights into how populations of neuroendocrine neurons in different hypothalamic nuclei can regulate multiple physiological functions. For example, the AgRP neuronal population and many others are involved in the regulation of reproductive functions and the stress response, in addition to the previously known function of regulating food intake and energy balance. These neuronal populations integrate various external stimuli, including energy needs, reproductive needs and environmental stress, to ensure real-time coordination of multiple physiological needs
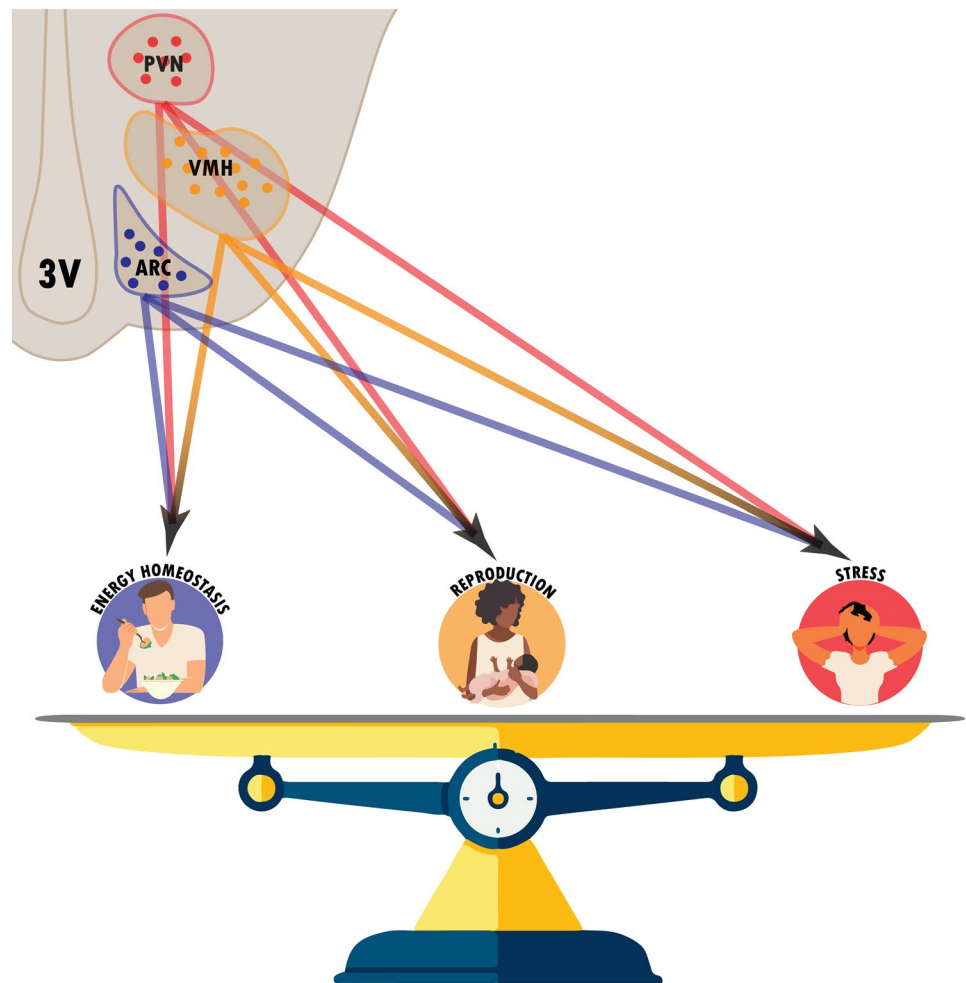
After a period of extended fasting, leading to diminished energy reserves, suppression of the HPG axis is observed, particularly in females, which is orchestrated by heightened activity in AgRP neurons [116, 117]. Conversely, following a meal, the surge in postprandial leptin levels suppresses the activity of AgRP/NPY neurons in the ARC, indicating an increase in energy availability and prompting meal cessation. The effect of leptin on feeding behaviour may involve a specific subset of AgRP neurons expressing *Lepr* [117]. In a transgenic mouse model, the absence of *Lepr* in AgRP neurons leads to increased adiposity. Female mutants display a discernible delay in the onset of puberty compared to controls, highlighting how distinct molecularly defined clusters of 'hunger neurons' can synergistically regulate reproductive parameters. Supporting this view, the genetic rescue of *Lepr* expression solely in AgRP neurons restores puberty onset, oestrous cyclicity, and fecundity in female *Lepr*-null mice, denoting the alternative role of AgRP neurons in regulating female fertility [118].

Following continuous chemogenetic activation of AgRP neurons for 14 days, which simulates chronic food deprivation, disrupted ovarian cycles and fertility are observed in adult female rodents [119], indicating that changes in the AgRP neuronal activity in response to long-lasting alterations in whole-body energy demands can override reproductive cycles. Similar observations have been made in a model of undernourished neonatal mice raised in large litters. Neonatal malnutrition in this model causes delayed onset of puberty, and chemogenetic inhibition of AgRP neuronal activity from postnatal day 26–30 reverses the delay in preputial separation and vaginal opening in male and female pups, respectively [120]. However, chemogenetic activation or inhibition during prepubertal development does not affect puberty onset when juveniles are nutritionally nourished during postnatal development [120], indicating how nutritional deprivation during early development can upset the energy balance, thereby dampening sexual maturation through the modulation of AgRP neuron. This multifaceted function may be controlled by AgRP neurons projecting to kisspeptin neurons in both the ARC (Kiss1^{ARC}) and the anteroventral periventricular nucleus (Kiss1^{AVPV}) [119], which are implicated in regulating the female ovarian cycle and initiating puberty onset, respectively [121]. Of note, kisspeptin can directly act on the melanocortin circuit

by activating POMC neurons and inhibiting NPY neurons [122], indicating that AgRP neurons are part of a neuronal circuit co-regulated by Kiss1 neurons, which is sensitive to changes in central oestrogen signalling [123]. Therefore, changes in overall energy availability during early development or adulthood could disrupt fertility by affecting neurons traditionally linked to food intake or energy balance regulation. This challenges the conventional model of 'One Population, One Physiological Function' that has been previously established.

While energy balance impacts reproductive needs and feeding regulation, the opposite relationship has also been demonstrated. The female ovarian cycle substantially influences appetite [124, 125], mainly when food is limited [126]. Cyclic fluctuations in female sex hormones affect food intake and body weight through changes in AgRP neuron activity [127], which are likely ERα dependent [128]. Xu et al. have established a transgenic mouse line in which AgRP neurons are ablated due to AgRP neuron-specific deletion of the mitochondrial transcription factor A gene [129]. In this model, the disrupted AgRP neural activity does not affect oestrous cyclicity but abolishes the cycle-dependent, oestrogen-suppressed food intake [127]. Also, female mutants disply blunted responses to E2-inhibited food intake [127]. Peripheral oestradiol fluctuation can also influence female receptivity to reinforce reproductive success. AgRP/NPY neurons achieve this output through a multi-order circuit targeting the ventromedial hypothalamus (VMH) to facilitate lordosis behaviour and thus reproductive outcomes in female mice in response to E2 [130–136]. Hence, hunger neurons may have diverse roles in synchronising fluctuations in overall energy levels with hormonal and behavioural responses that regulate reproductive function via central E2 signalling.

Energy balance is defined as the state achieved when energy intake equals energy expenditure, and the second part of the equation can also be integrated with reproductive needs. A molecularly defined hypothalamic population in the ventrolateral division of VMH and expressing ERα (VMH^{ERα}) seems vital in this context. E2 acts on the VMH to regulate energy expenditure by increasing physical activity and adaptive thermogenesis [99, 100, 137]. Food deprivation inhibits the neuronal firing of VMHvl^{ERα} neurons [138], while reduced VMHvl^{ERα} neuronal firing is linked to reduced female receptive behaviours during mating [136]. Blunting oestrogen signalling by genetic deletion of *ERα* in VMH^{SF1} neurons results in weight gain, reduced metabolic rate during the inactive phase, reduced thermogenesis in brown adipose tissue, and increased susceptibility to HFD-induced obesity in a female-specific manner [113]. Oestrogen deficiency in ovariectomised females blunts the responses to cold exposure or food deprivation, indicating a need for endogenous ovarian hormones to coordinate thermoregulatory responses and nutritional states. Conversely,

chemogenetic activation of VMH^{ERα} neurons during the inactive phase significantly increases spontaneous activity and promotes BAT thermogenesis in females [99]. It is noted that VMHvl^{ERα} neuronal firing is increased during the receptive period [80], indicating that VMH^{ERα} neurons promote energy expenditure during the reproductive receptive phase, while impaired oestrogen signalling in these neurons can blunt energy dissipation and abolish receptive behaviours. Collectively, the evidence emphasises the bidirectional communication of oestrogen signalling and energy balance through VMH^{ERα} neurons and suggests that activating these neurons might potentially restore metabolic homeostasis, even when oestrogen signalling is impaired.

Energy balance vs stress

Stress disrupts the delicate equilibrium between energy homeostasis and reproductive demands, a balance overseen by a central population of CRH-expressing neurons in the PVN, which are among the postsynaptic targets of AgRP neurons [139]. Food deprivation can disinhibit PVN^{CRH} neurons by triggering AgRP neuron firing [139], suggesting that negative energy balance may activate the HPA axis with elevated circulating glucocorticoid levels. Consequently, the glucocorticoid surge allows the body to mobilise glucose to fuel energy demands [125], like food-seeking activity, and promote energy storage [140]. Notably, liver-expressed antimicrobial peptide 2 is secreted from the gut during food deprivation, whereas ghrelin is secreted after a meal. These gastrointestinal hormones can suppress and activate AgRP neurons via the growth hormone secretagogue receptor [141], suggesting how feeding status may counter-modulate the stress response via the periphery-to-brain axis.

Similar to food deprivation, when exposed to environmental or physical stressors such as foot shock, air puff, tail suspension, immobilisation or cooling, PVN^{CRH} neurons become activated. However, neuronal activity can be rapidly and significantly reduced when exposed to appetitive or palatable stimuli [142, 143]. Thus, the reward system may prevent excessive HPA axis activation by modulating a common circuit that controls energy homeostasis. Accordingly, glucocorticoid receptors present in PVN^{CRH} neurons facilitate the negative feedback of glucocorticoids [144] by inhibiting presynaptic norepinephrine [145] or glutamatergic [146] activation, thereby reducing CRH secretion and suppressing the HPA axis. By activating the reward system, the act of eating may help alleviate the effects of physical and environmental stressors [147]. This concept is particularly relevant to our understanding of how stress influences the consumption of 'comfort food' in eating disorders such as bulimia nervosa [148, 149]. Therefore, these studies suggest that the classically known 'stress neurons' can detect and adapt to both energy and environmental demands, whereas

satiety and reward-associated feeding behaviours can reduce PVN[CRH] neuronal overactivation, highlighting that specific neuronal populations typically regulate more than one bodily function in an integrated manner over time.

Stress vs energy balance vs reproduction

Encountering adverse early-life experiences, such as being raised in an impoverished environment with unpredictable maternal care during a critical developmental period, is associated with altered brain maturation [150] and emotional vulnerabilities [151]. Rodent studies have shown that unpredictable maternal care during neonatal development can lead to emotional deficits, including anhedonia [152–154], meaning that offspring have diminished pleasure towards previously perceived rewarding stimuli [155]. In the animal kingdom, disruptions in maternal care, such as restricted access to nesting and bedding materials after birth, can result in lasting traumatic experiences for offspring, possibly via increased glutamatergic transmission to the PVN[CRH] circuit during neonatal development [156, 157]. Such functional identity reprogramming may be due to defective microglial action in shaping the circuit [157] and changes in the intrinsic gene expression profiles of PVN[CRH] neurons [158]. Conversely, optimal rearing conditions with maternal care have been shown to reduce excitatory synapses to PVN[CRH] neurons [159, 160] and the expression of the stress-related hormone CRH [159, 161, 162]. Neonates exposed to maternal separation exhibit changes in serum corticosterone and leptin levels. By postnatal day 4 (P4), traumatised neonates tend to have lower leptin and higher corticosterone levels compared to those receiving proper maternal care [163]. Although traumatised juveniles may initially exhibit reduced body weight before puberty [163], these changes in hormone levels during neonatal development may predispose individuals to obesity [164] and hyperactivity of the HPA axis [165] later in life.

PVN[CRH] neuron intrinsic excitability is regulated across the oestrous cycle with a peak in the preovulatory phase and a nadir in the oestrus phase [166], implicating the potential involvement of ovarian hormones in regulating stress response through manipulating the CRH neuron firing. Conversely, CRH neurons may also play a role in restraining reproductive receptivity during periods of stress. Pharmacological antagonism of CRH receptors before stress exposure can prevent stress-induced changes in LH pulses [167, 168]. Specifically, circulating oestradiol can potentiate the effects of stress induced by insulin-induced hypoglycaemia [169–171]. Exposure to acute psychosocial stress before the preovulatory phase disrupts the LH surge [172]. This finding is artificially recapitulated by exposure of female mice to acute stress upon E2 administration. CRH elicits dose-dependent effects on GnRH neuron firing activity in stress-free conditions, stimulating at lower concentrations and inhibiting at higher concentrations, depending on the CRH receptor subtypes involved [173]. Thus, CRH neurons can influence GnRH neuron activity, with oestrogen signalling playing a crucial role in determining whether CRH has stimulatory or inhibitory effects on these neurons.

The intensity and duration of stress experiences can affect food intake and reproductive cycles by involving a specific population of neuroendocrine neurons defined by CRH production. Thus, the evidence presented here supports the emerging model that the same population of neuroendocrine neurons can play a central role in orchestrating and integrating multiple physiological demands, responding adeptly to the ever-changing environmental cues encountered during both early postnatal development and adult life.

Intrinsic heterogeneity of neuroendocrine neurons: multiple subsets and multiple purposes?

Over a decade ago, pioneering ex vivo studies investigating the electrochemical properties of hypothalamic pro-opiomelanocortin (POMC) neurons revealed the remarkable intrinsic diversity of this population. This work has identified a wide range of electrophysiological properties and demonstrated a distinct responsiveness to different neurotransmitters and hormones [59, 174]. Recent advances in single-cell transcriptomic approaches have further elucidated the complexity among POMC neurons, unravelling several molecularly distinct clusters [13, 175, 176]. Thanks to these efforts, we are now uncovering that hypothalamic POMC neurons, previously thought to be a homogeneous functional pool, comprise distinct subpopulations organised based on spatial, molecular and electrophysiological features, which may play different roles in physiological conditions or the pathogenesis of hypothalamic diseases [49, 175–177]. This emerging framework, which has been extensively reviewed [177] and is beyond the scope of this paper, holds great promise for elucidating how different subsets of neurons in well-defined populations can affect food intake and body weight regulation. However, this inherent diversity raises new fundamental questions: Is heterogeneity an exclusive property of POMC neurons? Or can it also be found in other important hypothalamic neuroendocrine populations?

Intrinsic heterogeneity of melanocortin neurons

AgRP/NPY neurons have classically been regarded as a molecularly homogeneous cluster of neurons in the ARC, predominantly propagating GABAergic transmission [178]. However, recent studies have shown that refeeding after a period of fasting can lead to a significant decrease in spiking
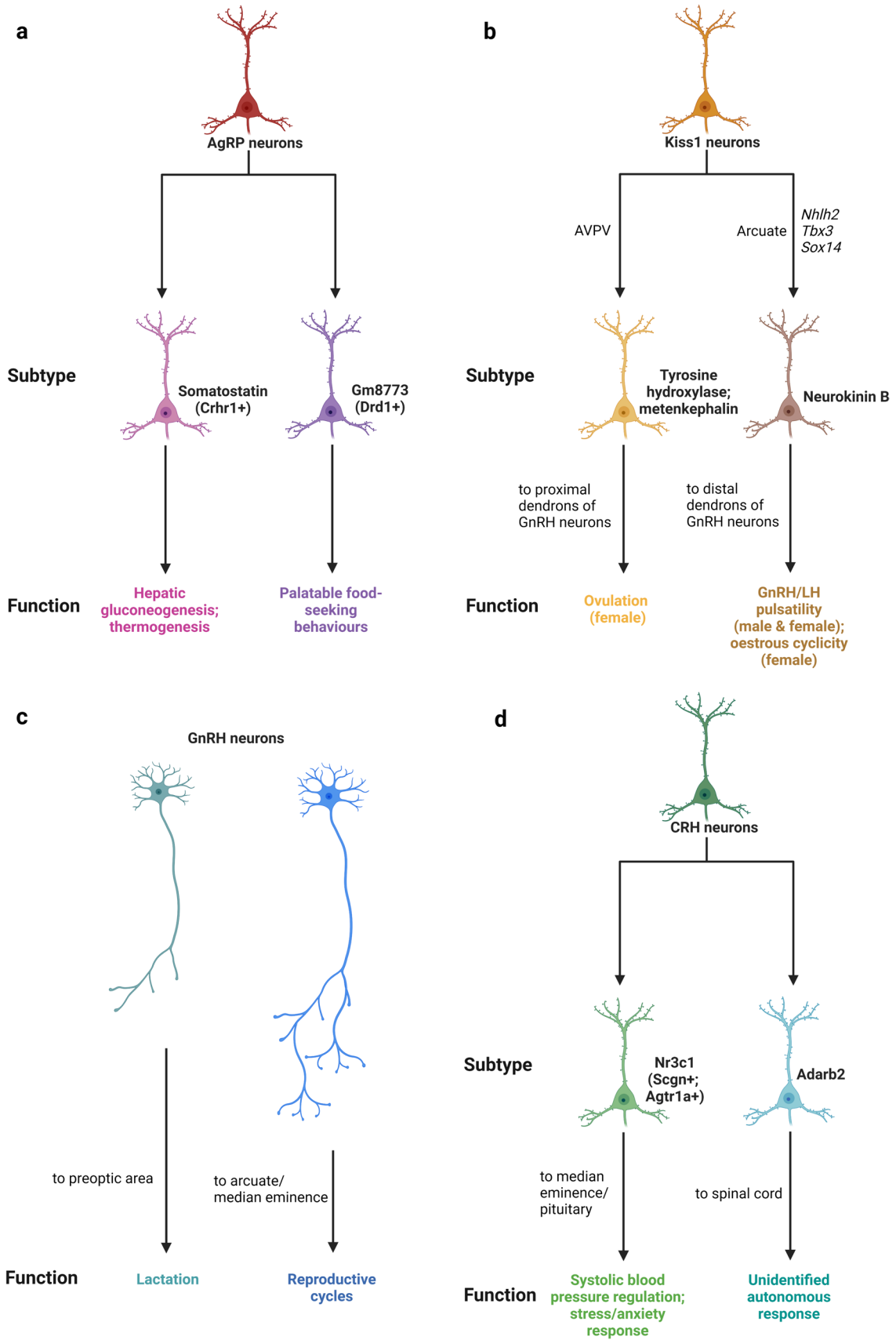
◄ **Fig. 2** Possible models for intrinsic heterogeneity within different neurondocrine populations in the hypothalamus. (a) AgRP neurons may be functionally subdivided into somatostatin (SST) and newly identified Gm8773 subtypes. (b) Kiss1 neurons may be subclassified on the basis of their spatial distribution in the AVPV and ARC. Their identities are further marked by differential expression of peptidergic markers. (c) GnRH neurons may exert diverse functions by projecting to different hypothalamic subnuclei. (d) CRH neurons have recently been subdivided into the Nr3c1 subtype, which expresses the glucocorticoid receptor, and the Adarb2 subtype. The identification of neuronal subpopulations demonstrates their possible involvement in specific endocrine responses

activity in approximately two-thirds of AgRP neurons while causing an increase in firing rates in less than one-third of AgRP neurons [179], suggesting intrinsic functional heterogeneity.

Single-cell transcriptomic analyses have identified two distinct subtypes of AgRP neurons: somatostatin-expressing (AgRP^{SST}) and Gm8773-expressing (AgRP^{Gm8773}) neurons [176, 180] (Fig. 3a). However, the specific functional roles of these neuronal subpopulations remain elusive. Interestingly, Drop-seq analysis, which reads the gene expression profile of thousands of individual neurons and determines their molecular identity, has revealed that *Gap43*, a gene encoding a protein involved in axonal regeneration [181], is significantly upregulated in the AgRP^{Gm8773} subpopulation but downregulated in the AgRP^{SST} population following fasting [176], suggesting differential structural plasticity to food deprivation in these subpopulations.

Corticotropin-releasing hormone receptor type 1 (*Crhr1*) is predominantly expressed by the AgRP^{SST} subpopulation [176]. These neurons receive innervation from PVN^{CRH} neurons and project back to the PVN [182], potentially relaying feedback signals to the PVN [183]. Corticotropin-releasing hormone has been shown to suppress the neuronal firing of AgRP^{Crhr1} [182]. Additionally, genetic deletion of *Crhr1* from AgRP neurons has been found to affect hepatic gluconeogenesis and thermogenesis during the active dark phase following overnight food deprivation without significant alterations in feeding behaviour or body weight [182]. Hence, the AgRP^{Crhr1} subpopulation may regulate glucose levels and body temperature in response to stress compared to other classical AgRP hunger neurons responding to the change in body energy status.

Dopamine signalling plays a crucial role in regulating feeding-associated behaviours, particularly in favour of palatable food consumption [184]. Dopamine deficiency may lead to failure to ingest food and subsequent death from starvation [185], while the effect of hyperactivating dopamine signalling on overconsumption remains uncertain. Among the dopamine receptor D1 (*Drd1*)-expressing subpopulations in the ARC, the AgRP^{Gm8773} subpopulation constitutes the primary neuronal cluster expressing *Drd1*. To explore the role of *Drd1* in AgRP/NPY neurons in food intake, an adenovirus expressing chemogenetic activation receptor has been injected into the ARC of *Drd1::Cre; Npy::Flp* mice, thereby allowing to precisely modulate by chemogenetic activation the subpopulation of AgRP/NPY^{Drd1} neurons [186]. Chemogenetic activation of this specific population induces acute voracious feeding behaviour. In contrast, genetic deletion of *Drd1* in AgRP/NPY neurons reduces daily consumption of a high-fat diet, diminishes high-fat diet consumption following acute fasting, and attenuates foraging behaviour to access chow pellets [186], suggesting that the AgRP^{Gm8773} populations may drive feeding behaviours through an increased dopamine level in response to energy-rich food stimulus [187–189].

In summary, these findings raise the possibility that different molecularly defined clusters of AgRP neurons may control divergent physiological outcomes, with some more specialised in homeostatic energy balance and others more likely to control hedonic feeding behaviour (Fig. 2a).

Kisspeptin neurons

In the mouse hypothalamus, *Kiss1* expression is restricted to the ARC and the AVPV regions, where each harbour functionally distinct Kiss1 subpopulations [190]. AVPV kisspeptin neurons are considered to be the impulse generators [191] responsible for the onset of puberty. In contrast, ARC *Kiss1* neurons are regarded as the pulse generator [192], responsible for the cyclicity of the female ovarian cycle. These two topographically distant populations express different peptidergic markers. Indeed, tyrosine hydroxylase (TH) and metenkephalin (mENK) are found colocalised with AVPV *Kiss1* neurons [193, 194], whereas ARC Kiss1 neurons are frequently co-localised with neurokinin B (NKB) and dynorphin (DYN) [195]. Recent studies on single-cell transcriptomic profiling suggest that (pro)dynorphin may not necessarily be a specific marker for Kiss1^{ARC} neurons [176, 196].

Whether or not ARC or AVPV kisspeptin neurons display intrinsic heterogeneity is poorly understood. Approximately half of the AVPV Kiss1 neurones co-express *TH* with Kiss1TH innervating GnRH neurons [197]. The functional role of this kisspeptin subpopulation has yet to be discovered. However, since the specific deletion of *TH* in kisspeptin neurons has no effect on the onset of puberty and fertility in both males and females [198], it could be speculated that this subtype does not follow the classical functional behaviour of Kiss1 neurons.

NKB can promote kisspeptin release through NKB receptors on Kiss1 neurons via autocrine control. NKB signalling stimulates GnRH secretion [199], which is essential for puberty onset in both sexes. Impaired NKB signalling results in hypogonadotropic pubertal delay in humans [200]. Both mENK and DYN modulate opioidergic signalling of
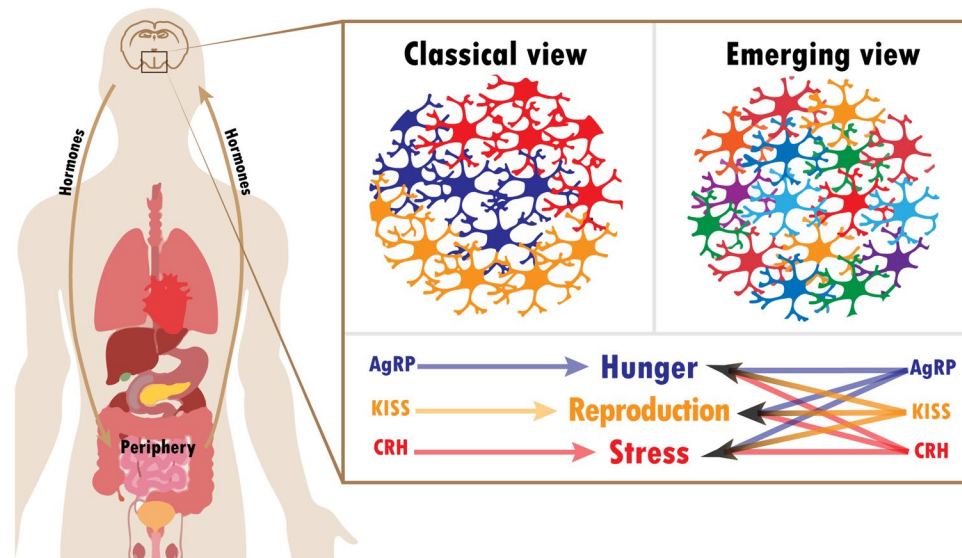
Fig. 3 The classical neurobiological view (left panel) depicts hypothalamic endocrine neurons as discrete populations, each characterised by a specific neuropeptidergic profile, programmed by development to elicit singular physiological functions throughout life. However, emerging evidence challenges this 'One Population, One Physiological Function' paradigm. The integrated view (right panel) presents a more in-depth understanding, illustrating that hypothalamic endocrine neurons exhibit additional layers of plasticity and functional heterogeneity. Contrary to the classical notion, these neural circuits are highly heterogeneous and interconnected, participating in a network where functions related to reproduction, stress, and hunger are intricately intertwined. This model highlights the dynamic nature of hypothalamic endocrine neurons, emphasising the need for a holistic experimental approach to dissect the neuroendocrine system and the underlying neural correlates

kisspeptin neurons [201]; in particular, they inhibit the release of kisspeptin through κ-opioid receptors and δ-opioid receptors, respectively, via autocrine fashion. While the role of mENK in AVPV Kiss1 neurons is uncertain, ablation of *Kiss1* in ARC *Pdyn* neurons does not affect the onset of puberty. Still, it leads to a disrupted ovarian cycle, hypogonadism and female infertility [202], further suggesting the existence of multiple sub-populations of Kiss1 neurons that operate via divergent modes of action.

In adult mice, the AVPV and the ARC Kiss1 populations have different sensitivity to oestrogen, possibly because ERα binds to a different regulatory site on the *Kiss1* locus in AVPV compared to ARC Kiss1 neurons [203]. The differential transcriptional control of kisspeptin through oestrogen signalling could thereby lead to ARC Kiss1 neurons control GnRH/LH pulses via mediating the oestrogen-negative feedback action and that the AVPV Kiss1 neurons control GnRH/LH surge via mediating the oestrogen-positive feedback action [204]. In female rodents, the ARC expresses a high level of *Kiss1* when the circulating oestradiol level is low at diestrus, which can be suppressed by oestrogen treatment [205]. Conversely, the AVPV *Kiss1* expression level is high during the preovulatory period and can be further augmented by oestrogen treatment in rodents [205]. Indeed, analyses from bulk RNA sequencing from laser-capture microdissection fluorescently-labelled Kiss1 neurons show that ARC Kiss1 neurons display an entirely different transcription profile than AVPV Kiss1 neurons in response to E2 treatment [206].

Interestingly, the expression of the transcription factors *Nhlh2, Tbx3*, and *Sox14* is specific to Kiss1[ARC] neurons [194], suggesting that different developmental origins may account for the regulation of different downstream circuits and possibly the functional dichotomy of different subpopulations of Kiss neurons in response to oestrogen action [194] (Fig. 2b).

GnRH neurons in the hypothalamus

The presence of diverse sub-populations of gonadotropin-releasing hormone (GnRH) neurons may be central to orchestrating the patterns of gonadotropin secretion essential for puberty onset and fertility. GnRH neurons are dispersed across the anteroposterior axis of the brain, including the medial septum, rostral preoptic area (POA), anterior hypothalamus, and medial basal hypothalamus [207]. Yet, they originate from distinct pools of progenitor cells derived from *Islet-1/2* or *Wnt1* origins [208], located respectively in the placodal ectoderm and neural crest rather than the hypothalamus. During development, GnRH neurons embark on a long-distant migration from the nasal septum, facilitated by olfactory ensheathing cells [209], astrocytes [210], and hormonal cues [211, 212], to the forebrain, which ultimately projects dendrites towards the preoptic area, hypothalamus,

and median eminence [213]. While the molecular profiles of GnRH neurons in different brain regions require further investigation at the single-cell level, it is plausible that GnRH neurons located in different anteroposterior positions express different subsets of chemotactic and hormone-sensing machinery, facilitating their migration and localisation within specific hypothalamic nuclei during development.

The majority of GnRH neurons project to the median eminence, where they release GnRH peptide into the portal blood in a pulsatile manner to control gonadotropin secretion from the pituitary gland, a mechanism crucial for the initiation of puberty and the preservation of fertility [214]. Despite challenges in the detection of GnRH neurons due to their limited cell numbers by single-cell transcriptomic analysis, the use of the more sensitive multiplexed error-robust fluorescence in situ hybridisation (MERFISH) technique has identified that GnRH-expressing clusters in the POA that highly express kisspeptin receptors [215]. Sexual dimorphism emerges in the kisspeptin fibre innervation patterns onto the dendrites of GnRH neurons. Female mice receive denser kisspeptin fibre innervation in rostral preoptic GnRH neurons than males [35], which probably originates from the AVPV Kiss1 neurons.

In addition to ME, GnRH neurons project onto kisspeptin and tyrosine hydroxylase-expressing neurons in the POA and ARC [216], denoting their structural heterogeneity (Fig. 2c). The GnRH neurons projecting to POA may have implications on regulating lactation [216]. Moreover, a subset of GnRH neurons targets tuberoinfundibular dopaminergic neurons in the ARC, showing hormonal regulation of their innervation patterns across the oestrous cycle [217]. Under physiological conditions, TH-expressing neurons in ARC received GnRH fibre innervation at proestrus and estrus but no fibre innervation at diestrus, suggesting an ovarian hormone-dependent regulation of GnRH fibre innervation.

Corticotrophin-releasing factor neurons in PVN

While the role of CRH neurons in regulating stress response is well established, surprisingly, the heterogeneity of this neuronal population has been barely investigated. In a single-cell transcriptomic study, however, Romanov et al. have revealed that CRH neurons residing in the PVN are glutamatergic, contrary to those GABAergic subtypes residing in DMH and the preoptic area [218]. By single-cell RNA-sequencing, Berkhout et al. have identified two main populations of CRH neurons, including the pituitary-projecting population expressing $Nr3c1$ glucocorticoid receptor and the spinal cord-projecting $Adarb2$ population [219] (Fig. 3d). The latter has yet to be studied, but the CRH^{Nr3c1} population expresses glucocorticoid receptors to facilitate feedback mechanisms to suppress HPA axis overactivation [220]. This population also expresses Avp, which strongly potentiates the release of CRH-driven adrenocorticotropic hormone (ACTH) from the anterior pituitary. Arginine vasopressin (AVP) is present in both magnocellular and parvocellular divisions of the PVN [221], and the latter population of AVP is colocalised with CRH [222]. A pioneering study has demonstrated the co-release of CRH and AVP upon stressful stimuli, whereas their syntheses can be suppressed by glucocorticoid [223]. By exposing neonates to maternal separation from postnatal day 2 to 9, Short and Tai et al. showed that Avp is strongly expressed among the glutamatergic CRH neurons [158]. At P10, mice expressing tdTomato under the CRF^{Avp} resided primarily in the dorsal region of PVN [158]. CRH^{Nr3c1} also expresses $Scgn$ encoding secretagogin. Secretagogin appears to be a calcium ion sensor that regulates intracellular vesicle transport of CRH and facilitates its extracellular release [224]. Gene silencing of secretagogin induces CRH accumulation in the PVN, suggesting an impairment in the intracellular translocation of CRH. Besides, ACTH and corticosterone production are abolished after secretagogin knockdown in animals exposed to acute formalin stress. Lastly, $Agtr1a$, encoding angiotensin type-1a receptor, also expresses the CRH^{Nr3c1} population. Photo-stimulation of CRH^{Agtr1a} neurons activates the HPA axis and increases systolic blood pressure, whereas inhibiting CRH^{Agtr1a} neurons suppresses the activity of the HPA axis and reduces anxiety-like behaviour [225]. Notably, secretagogin [224] and angiotensin type-1a receptor [225] co-express with CRH axons in the ME.

In conclusion, although the PVN CRH neurons share a common peptidergic marker in the same hypothalamic nucleus, differential expression of additional markers, Nr3c1 and Adarb2, may define subpopulations that project to and control different circuits, thereby conferring intrinsic functional heterogeneity (Fig. 2d).

Conclusion and open questions

The current neurobiological model used to explain how hypothalamic neuroendocrine neurons operate implies that a specific neuronal population, defined by a set of neuropeptides and other secreted molecules, is programmed by development always to elicit one physiological function throughout life. However, this traditional 'One-Population-One-Function' model does not take into account that additional layers of plasticity and heterogeneity are observed with each molecularly defined cluster of neuroendocrine neurons (Fig. 3). For example, 'hunger neurons' promote food intake when activated by integrating this behavioural output with reproductive needs and environmental cues that affect stress-related circuits. No single circuit in the brain operates as an isolated functional entity, and each population modulates different downstream circuits in response to presynaptic and postsynaptic cross-talk between neuropeptides,

neurotransmitters, and other released factors such as cytokines that fine-tune neurotransmission. Since neuroendocrine neurons are in constant dialogue with hypothalamic non-neuronal cells, microglia, oligodendrocytes, astrocytes, tanycytes, to maintain body homeostasis [226–228], non-neuronal cells may play a key contributing role in this context, although this remains a key question to be answered.

Advancements in single-cell analysis reveal that even neuronal populations identified by their shared neurochemical or neuropeptidergic identities harbour distinct subpopulations, likely participating in distinct neurobiological mechanisms. Hence, it is plausible that neuronal populations classically known to control one unique output, such as hunger, stress, or fertility, may contain special subtypes that are capable of integrating all these different outputs.

In this evolving framework, additional important questions arise. Is functional heterogeneity a static property throughout mammalian life, or can this property dynamically change in response to environmental or internal cues? Exposure to an unbalanced diet during embryonic development induces persistent gene-specific DNA methylation changes [173–176], leading to permanent metabolic reprogramming and the development of neuroendocrine diseases such as obesity in adulthood. Following exposure to a maternal hypercaloric diet, offspring mice show increased circulating leptin levels at weaning [177] and hypermethylation of the *Pomc* gene, highlighting the long-term imprinting effect of maternal diet on offspring gene expression and whole-body energy homeostasis. Maternal overnutrition during lactation in mice reduces embryonic (E12.5) neurogenesis and impairs postnatal neurotrophic development in the hypothalamus. When dams consume a high-fat diet during pregnancy and early lactation, their male neonates develop more 'hunger neurons' and fewer 'satiety neurons' in the hypothalamus. This alteration in NPY-to-POMC neuron ratio sets the stage for them to become obese later in life [229], suggesting that metabolic and dietary challenges may alter the functional heterogeneity of hypothalamic circuits and influence how these circuits respond to changes in hormonal signalling across the lifespan.

The prevailing lifestyle in modern societies, characterised by a lack of physical activity, excessive calorie intake, and repetitive behaviours, is widely recognised as one of the main causes of the global burden of obesity, leading to maladaptive regulation of energy balance, eating behaviour and body weight control. When laboratory mice are subjected to environmental enrichment, which involves augmenting their rearing environment with increased physical activity and exposure to sensory, cognitive, and social stimulations, significant changes in synaptic plasticity are observed in the neuroendocrine hypothalamus. Specifically, an increase in the excitation-inhibition ratio in α-MSH neurons and a decrease in the excitation-inhibition ratio in AgRP neurons

are observed, which is accompanied by an improved hypothalamic leptin sensitivity, highlighting how environmental cues can reprogram hypothalamic neuroplasticity [230].

Interestingly, hypothalamic neuronal functions can be maximally enhanced by environmental stimuli that are applied during developmental stages [230]. During post-weaning, when hypothalamic neuronal projections in animals undergo plastic changes, voluntary wheel running selectively reduces obesity in rats bred to develop diet-induced obesity compared to those bred to be diet-resistant. Remarkably, three weeks of exercise in juvenile rats (4 week old) has a sustained effect in preventing obesity development later in adult life, even when the animals stop exercising and continue to consume a diet relatively high in fat and calories [231]. The protective effect of exercise during juvenile life is associated with substantial differences in the hypothalamic expression of various neuropeptides, such as NPY, AgRP, or POMC [231] and increased central leptin signalling, suggesting functional reprogramming. Consistent with this, it has been recently shown using whole-cell patch-clamp electrophysiology that exercise training promotes a rapid reorganisation of synaptic inputs and biophysical properties in POMC and NPY/AgRP neurons [232].

These data collectively emphasise the intriguing possibility that environmental stimuli can reprogram the functional heterogeneity of hypothalamic neural circuits not only during juvenile development but also, albeit to a lesser extent, in adulthood. Such findings hold substantial promise for therapeutic applications, particularly in light of the growing interest in developing pharmacological anti-obesity treatments to emulate the beneficial effects of environmental stimuli on hypothalamic neuronal plasticity. Some of the effects of environmental enrichment or exercise on hypothalamic neurobiology and central leptin sensitivity may be mediated by the increased expression of brain-derived neurotrophic factor (BDNF) and its influence on neuronal plasticity [233]. The selective serotonin reuptake inhibitor fluoxetine is an antidepressant drug with similar effects to environmental stimulation [233]. Fluoxetine-treated obese mice experience reduced weight gain, increased energy expenditure and improved central leptin sensitivity [234]. Patch-clamp recordings from POMC neurons further indicate that fluoxetine increases the firing rate of this neuronal population and excitatory AMPA-mediated transmission while concurrently reducing presynaptic inhibitory GABAergic currents, potentially through heightened activity of the mTOR pathway [235]. The case of BDNF and fluoxetine therefore illustrates how further research into the mechanisms of hypothalamic functional plasticity may pave the way for innovative therapeutic solutions.

Elucidating the neurobiological mechanisms underlying the functional heterogeneity of neuroendocrine neurons in the hypothalamus is a complex and dynamic area of research

with several important outstanding questions (see Box 1). Addressing these questions will significantly improve our understanding of the hypothalamus-mediated control of hormonal action under physiological conditions and during neuroendocrine disease.

Box 1 Outstanding questions

Investigating the functional heterogeneity of neurons in the hypothalamus is a complex and dynamic area of research. Here are some open questions about the functional heterogeneity of hypothalamic neurons that remain in our understanding of how different neuronal subtypes contribute to regulating different physiological functions via hormonal cross-talk.

Plasticity and adaptation: How do neuropeptides interact with the fast-acting excitatory-inhibitory neurotransmission to elicit rapid and precise responses to regulate the neuroendocrine systems?

Spatial organisation: What is the functional relevance of spatial patterning of different neuronal subpopulations in the neuroendocrine hypothalamus?

Extracellular space: What is the role of the ECM and extra-cellular space (ECS) homeostasis in shaping the functional heterogeneity of neuroendocrine neurons? And what about the role of non-neuronal hypothalamic cells?

Physiological outcome integration: How do the heterogeneous neural circuits in the hypothalamus orchestrate and prioritise our physiological needs? Does maintaining energy homeostasis always take precedence over reproductive needs or stressful cues?

Temporal organisation: Can neuroendocrine neurons change their neurochemical identity throughout development, ageing, circadian and oestrous cycles?

Sexual dimorphism: How do sex hormones participate in shaping heterogeneous hypothalamic circuits to protect the female from diet-induced metabolic syndrome?

Pathophysiology: Since hypothalamic neurons are functionally and molecularly heterogeneous, how do other sub-populations restore physiological functions if one is compromised? What are the roles of specific neuron subtypes in the onset and progression of neuroendocrine disorders?

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and the ideas. All authors have read and agreed to the published version of the manuscript.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval This study does not contain any studies with animals performed by any of the authors.

Informed consent For this type of study formal consent is not required.

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