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REVIEW

The structural and functional complexity of the integrative hypothalamus

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The hypothalamus ("hypo" meaning below, and "thalamus" meaning bed) consists of regulatory circuits that support basic life functions that ensure survival. Sitting at the interface between peripheral, environmental, and neural inputs, the hypothalamus integrates these sensory inputs to influence a range of physiologies and behaviors. Unlike the neocortex, in which a stereotyped cytoarchitecture mediates complex functions across a comparatively small number of neuronal fates, the hypothalamus comprises upwards of thousands of distinct cell types that form redundant yet functionally discrete circuits. With single-cell RNA sequencing studies revealing further cellular heterogeneity and modern photonic tools enabling high-resolution dissection of complex circuitry, a new era of hypothalamic mapping has begun. Here, we provide a general overview of mammalian hypothalamic organization, development, and connectivity to help welcome newcomers into this exciting field.

The hypothalamus makes up just 2% of brain volume but is essential for fundamental life processes and survival. The term hypothalamus was first introduced by Wilhelm His in 1893 and was further annotated by Santiago Ramón y Cajal in the 1890s, Albrecht von Haller in the 1900s, and Ernst and Berta Scharrer in the 1920s. For centuries, the hypothalamus has fascinated scientists for its nodal position and dense nuclear clustering of neurons, and this allure only increased as early lesioning studies revealed the outsized role of the hypothalamus in a range of physiologies and behaviors. Today, the hypothalamus is known to receive, process, and integrate sensory inputs to drive bidirectional communication with a range of behavioral, autonomic, and endocrine pathways. The development and interaction of the circuits by which the hypothalamus maintains homeostatic set points, overcomes stressors, and drives behaviors are continually being defined.

The intricate organization of the hypothalamus

The hypothalamus resides in the ventral-most region of the forebrain and drives diverse processes through a complex cytoarchitecture localized around the third ventricle (*1*, *2*) (Fig. 1A). Along the rostral-caudal axis, the hypothalamus can be divided into the preoptic, anterior, tuberal, and mammillary regions (Fig. 1, B to E), each of which is further partitioned into lateral, medial, and periventricular zones (*3*). These hypothalamic regions are populated by a patchwork of neurons that form discrete three-dimensional (3D) clusters, or nuclei, which contrasts with the relatively flat laminar structure of the cerebral cortex. Most rostrally, the preoptic area (POA) includes the median (MnPO), medial (MPO), ventrolateral (VLPO), anteroventral periventricular (AVPV), and periventricular (PVpo) preoptic nuclei. The anterior region is composed of the anterior hypothalamus (AH) and supraoptic (SON), suprachiasmatic (SCN), and paraventricular (PVN) nuclei. The median eminence (ME) and pituitary stalk lie ventral to the tuberal hypothalamus, which contains the loosely defined lateral hypothalamic area (LH) as well as the dorsomedial (DMH), ventromedial (VMH), arcuate (Arc), and tuberal (TuN) hypothalamic nuclei. Finally, the premammillary (PM), supramammillary (SuM), tuberomammillary (TM), and posterior hypothalamic (PH) nuclei make up the mammillary hypothalamus.

The mapping of functional roles and accompanying pathways onto individual nuclei has revealed extensive overlap and high coordination (*1*, *2*) (Fig. 1F). For example, various aspects of feeding, energy balance, and thermoregulation are strongly regulated by neurons residing in the Arc, VMH, DMH, LH, and TuN within the tuberal hypothalamus, but they also loop in circuits within the MnPO, VLPO, PVN, AH, and PM. Similarly, corticotropin-releasing hormone (CRH) neurosecretory cells in the PVN are central to stress responses and integrate inputs from the MPO, DMH, LH, and PH (*4*). Further, sleep-wake is orchestrated by the SCN—the principal circadian pacemaker—in conjunction with the VLPO, MnPO, PVN, DMH, VMH, LH, TM, and SuM (*5*). Although the interconnectedness of spatially segregated nuclei is a core feature of hypothalamic function, more recently this concept has evolved to appreciate the concerted contributions of extrahypothalamic circuits as well. For example, the hypothalamus facilitates circuitry for reward, salivation, smell, and hunger alongside the physical act of foraging for food, which provides a framework for studying complex behaviors mediated by the hypothalamus, such as feeding.

Some hypothalamic nuclei are known to be sex dimorphic. The MPO is one of the best-known sex-biased brain regions, with male sexual behaviors in rats influenced by a greater volume and neuronal number compared with that in females (*6*). Notably, this anatomical size difference is also observed in the third interstitial nucleus of the anterior hypothalamus (INAH-3), the presumptive homologous cell group in humans (*7*). In mice, distinct subpopulations of tyrosine hydroxylase– and progesterone receptor–expressing neurons in the AVPV and the VMH, respectively, are sexually dimorphic and mediate a range of sexual, parental, and aggressive behaviors (*8*, *9*), whereas female-biased differences in pro-opiomelanocortin (POMC) neuronal numbers and activity in the Arc have been reported to influence energy balance (*10*). High-resolution mapping of hypothalamic circuitry mediating sex-specific behaviors is an emerging area of research (*11*).

Extraordinary diversity of hypothalamic cell types

To better understand the cellular basis of hypothalamic functions, attention has largely focused on the resident neurons that are staggering in diversity. Elegant biochemical studies in the 1950s identified various neuropeptidergic neurons across the hypothalamus (*12*), with the true extent of hypothalamic neuron heterogeneity now being realized with the emergence of single-cell omics, particularly single-cell RNA sequencing (scRNA-seq). Although hypothalamic neurons historically have been classified on the basis of neuropeptide phenotype or fast neurotransmitter identity, scRNA-seq–defined transcriptional signatures highlight the complexity of hypothalamic neuronal populations. For example, some neuropeptide transcripts are preferentially expressed in glutamatergic neurons (e.g., *Oxt*, *Grp*, and *Ucn3*), whereas others are found in γ-aminobutyric acid–expressing (GABAergic) neurons (e.g., *Npy* and *Agrp*) or even both (e.g., *Sst* and *Crh*) (*13*). Concomitantly, the broad categorization of excitatory glutamatergic or inhibitory GABAergic populations can be further stratified into subpopulations that express a combination of neuropeptides and transcription factors (*14*–*16*), such as *Hcrt* and *Pnoc* distinguishing clusters of *Lhx9*+ putative glutamatergic neurons within the LH (*15*). Seventeen hypothalamic scRNA-seq datasets have been integrated into a consolidated and comprehensive mouse hypothalamic cell atlas, serving as a valuable resource for studying hypothalamic function with unprecedented cell subtype–specific precision (*17*).

The challenge now is to translate this transcriptional profiling into higher-resolution insights of the cell type-function relationships. For instance, a subset of *Gal*+ and *Avpr1a*+ neurons in the POA are associated with enriched cFos expression after pup exposure, which suggests a role in parenting behavior (*18*); subtypes of orexigenic agouti-related peptide (AgRP) neurons in the Arc exhibit differential transcriptional responses to fasting, potentially representing

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discrete, energy state–responsive populations (*19*); and molecularly distinct clusters of neurons in the mammillary bodies are not only spatially segregated but also project to different regions of the anterior thalamus as perhaps parts of parallel memory subcircuits (*20*). To build on these largely correlative links and establish function, fine dissection of the component parts of diverse hypothalamic circuits will require combining newly identified subtype-specific markers with targeted genetic tools. For example, chemogenetic activation of LH *Sst*+ neurons increases locomotor activity (*21*), whereas activating VMH *Esr1*+ neurons elicits both locomotion and heat production (*22*), which highlights that circuits mediating movement behaviors are dependent on context. In addition, chemogenetic and optogenetic manipulations of broad VMH *Esr1*+ neurons reveal this population to be necessary and sufficient for female aggression (*23*), whereas stereotaxic injection of small interfering RNAs (siRNAs) against *Rprm*, which is expressed in a subset of VMH *Esr1*+ neurons, increases body temperature in female mice, consistent with a sex-specific thermoregulatory role for this neuronal subpopulation (*22*). These studies underscore the arduous task of fine mapping the hypothalamic cell types and circuits that control complex behaviors.

Despite a neuron-centric view of hypothalamic cell type diversity, the hypothalamus also contains heterogeneous glial populations, defined originally in classical morphological studies and more recently by multi-omics approaches. These glial cells, including astrocytes, oligodendrocyte precursor cells, oligodendrocytes, microglia, ependymal cells, and tanycytes, are not simply structural support but are active participants in modulating hypothalamic circuits (*24*). Astrocytes in the SCN, for instance, show rhythmic expression of clock genes and influence circadian locomotor behavior (*25*), and microglial activity in the developing POA is required for mating behaviors (*26*). Glial contributions to the hypothalamic control of energy balance are also extensive, ranging from leptin response modulation by astrocytes and oligodendrocyte precursor cells; to melanin-concentrating hormone (MCH)–responsive cilia beating by ependymal cells, which may in turn facilitate volume transmission of MCH through the cerebrospinal fluid; to nutrient sensing, metabolite transport, and neurogenesis by multifunctional tanycytes (*24*, *27*). Altogether, mounting evidence indicates that different classes of glia play essential roles across neuroendocrine networks, with further studies needed.

Developmental programs of the hypothalamus

Given the diversity of cellular phenotypes found in the hypothalamus, an understanding of when and how these cell identities are acquired has lagged. According to the columnar model of early hypothalamic organization, and with the exception of the telencephalon-derived POA, the hypothalamus begins to develop from the ventral diencephalon at approximately embryonic day (E) 8.5 in mice or gestational week (GW) 5 in humans, with both extrinsic and intrinsic factors directing regional patterning (*13*). Sonic hedgehog (Shh) drives the expression of the classic hypothalamic marker *Nkx2.1* and is required to induce a hypothalamic fate in diencephalic progenitors, whereas Bmp and Wnt signaling play important roles in establishing the dorsal-ventral and rostral-caudal axes, respectively (*28*). After early patterning, the developing hypothalamus is populated by neurons, then glia, following the typical pattern for other neural tube–derived brain regions: Radial glia divide at the ventricular zone, giving rise to fate-committed neuronal and glial precursors that undergo radial migration into the parenchyma (*2*). In the developing mouse and human hypothalamus, hypothalamic radial glia lining the third ventricle can divide asymmetrically to generate mantle radial glial cells, which serve as intermediate progenitor cells (IPCs) located in the mantle zone (*29*) (Fig. 2A). Notably, live-cell imaging of mantle radial glia reveals that in addition to undergoing asymmetric neurogenic divisions, they can also produce cells expressing the progenitor marker SOX2 (*29*). Some of these daughter cells have basal processes and likely function to maintain the mantle radial glia pool, whereas others that lack processes are speculated to increase the diversity of hypothalamic progenitors and/or to contribute to gliogenesis (*29*).

Early hypothalamic precursors are heterogeneous (*30*), supportive of a cascade diversification model for hypothalamic neurogenesis. Unlike

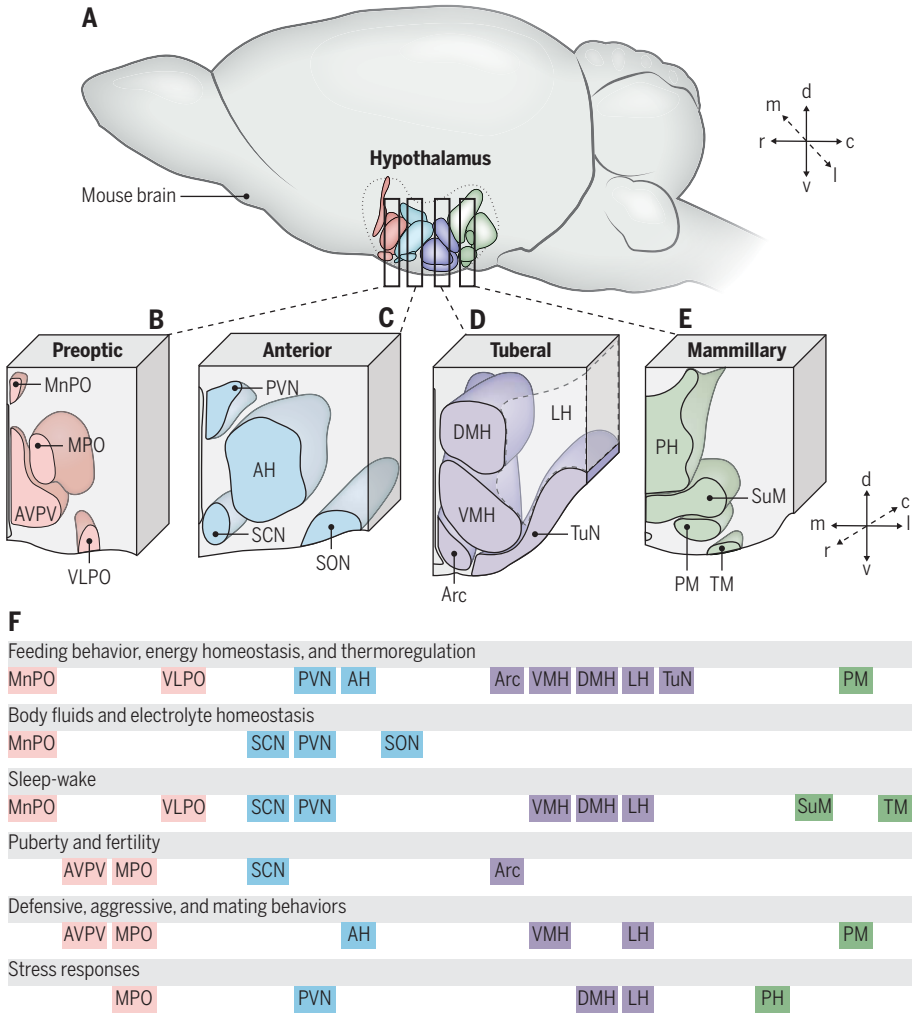

Fig. 1. A schematic representation of hypothalamic subdivisions in the mouse brain, highlighting individual nuclei and associated physiologies. (**A**) The hypothalamus resides at the base of the brain, with its nuclei forming a complex 3D patchwork around the third ventricle across four rostral-caudal regions. (**B** to **E**) Representative coronal sections of the preoptic (B), anterior (C), tuberal (D), and mammillary (E) regions demonstrate the diversity and intercalation of hypothalamic nuclei. (**F**) The hypothalamic control of diverse physiologies and behaviors critical to survival involves coordinated activity across multiple nuclei.

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the fate-predetermined and stochastic models of the cortex and the retina, respectively, the cascade diversification model posits that the myriad neuronal subtypes required for hypothalamic function are generated in a stepwise amplification of radial glial cells, to intermediate progenitors, to neurons (*31*). In brief, hypothalamic radial glia give rise to *Ascl1*+ IPCs, competent to generate both glutamatergic and GABAergic neurons, and *Neurog2*+ IPCs, which primarily produce glutamatergic neurons (*31*) (Fig. 2A). The postmitotic neuronal progeny of these complementary pools of intermediate progenitors ultimately populate diverse nuclei. In their nascent state, the coexpression of multiple peptides in a given neuronal subtype, along with pseudotime analyses, suggest that several neuronal phenotypes can emerge from a common, initially ambiguous transcriptional state (*31*), which points to hypothalamic complexity from its origin. Notably, in the Arc, fate-mapping experiments reveal that both somatostatin (SST) and AgRP neurons are derived from SST-expressing nascent neurons (*31*), and distinct subpopulations of neurons defined by combinatorial *Tbx3*, *Otp*, and *Dlx1/2/5/6* expression are generated from a common multipotent progenitor domain in a mosaic and synchronous fashion (*32*), providing evidence for this fate diversification.

As in other areas of the brain, neuronal and glial cells in the hypothalamus emerge during specific temporal windows (Fig. 2B). Hypothalamic neurogenesis occurs at approximately E10.5 to E16.5 in mice (*33*) and GW8 to GW10 in humans (*34*), with neuronal precursors specified by the combinatorial expression of transcription factors and their corresponding regulons (*15*, *16*, *31*). In mice, neuronal fate commitment is marked by the *Dlx1/2/5/6* and *Sox11/12* regulons (*16*), the former of which also characterizes neurogenic progenitors in the developing human hypothalamus (*34*). Further region- and nucleus-specific identity is conferred by additional gene regulatory networks, such as *Lhx5* for the mammillary hypothalamus and *Foxa1* for the SuM (*15*), and neuronal generation proceeds in an outside-in, rostral-caudal pattern (*32*, *34*). Comparatively less is known about gliogenesis, but the process initiates upon the decline of neurogenesis (*16*, *34*) under the control of the *Hes5*, *Sox9*, and *Nfia* regulons (*16*). Tanycytes are among the first-born glial cells, with most generated between E13 and E15 (*31*), followed by the onset of oligodendrocyte lineage cells (E13.5 or GW10), astrocytes (E15.5 or GW15) (*34*, *35*), and finally ependymal cells (E17.5 or GW18) (*16*, *34*) (Fig. 2B). Notably, oligodendrocyte development occurs relatively early in humans compared with mice, with in silico cell-cell communication prediction suggesting a potential human-specific role for early oligodendrocyte lineage cells in facilitating neuronal survival and maturation through Wnt and integrin signaling (*34*). The role of glia in neuronal maturation and circuitry establishment continues to emerge as the genetic programs governing hypothalamic development are revealed.

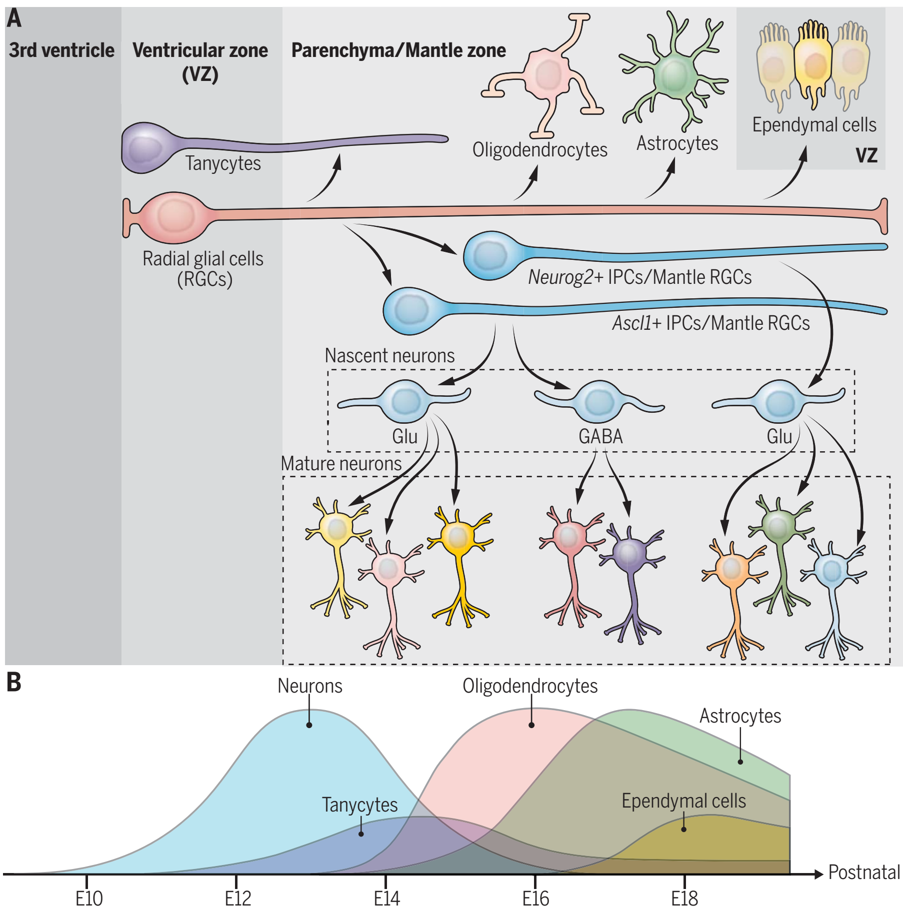

Fig. 2. Developmental programs of the mouse hypothalamus. (**A**) Radial glial cells (RGCs) divide at the ventricular zone (VZ) and give rise to both glia (tanycytes, oligodendrocytes, and ventricular zone–residing ependymal cells) and neurons. Hypothalamic neurogenesis is proposed to occur through a cascade diversification model in which ventricle-adjacent radial glia generate *Neurog2*+ and *Ascl1*+ intermediate progenitors located in the mantle zone. These intermediate progenitors then generate nascent glutamatergic or GABAergic neurons that ultimately differentiate into the range of neuronal subtypes found in the mature hypothalamus. (**B**) Schematic representation of the timing of neurogenesis and gliogenesis in the developing hypothalamus. Glu, glutamatergic; GABA, GABAergic.

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Environmental influences on hypothalamic development

Curiously, the developing hypothalamus is influenced by the external environment, changing programs in response to the cues perceived to define the environment in which it will live (Fig. 3A). This concept of fetal programming or developmental origins of heath and disease (DOHaD) is not new, but it does seem especially pronounced in the murine fetal hypothalamus, perhaps reflecting the eventual central role of the hypothalamus as a sensing brain region responsible for adjusting a range of homeostatic and behavioral systems to match the current environment.

Accumulating evidence illustrates the permanent effects of environmental inputs during development on proper hypothalamic function later in life. In both human and animal models, a maternal high-fat diet can induce metabolic disorders in the offspring by altering Arc neuropeptides, signaling, neuroinflammation, and/or epigenetics (*36*). In mice, maternal cold stress causes hypothalamic fetal microglia to sex-specifically disrupt oxytocin neuronal numbers (*37*), and maternal fever (through exogenous immune activation) accelerates the onset of puberty with accompanying changes in hypothalamic *Kiss1*, *Tac2*, and *Kiss1r* gene expression (*38*). Furthermore, anthropological chemicals, especially endocrine-disrupting chemicals that mimic hormones, such as the xenoestrogen bisphenol A (BPA), can perturb developmental hypothalamic programs. Maternal exposure to environmentally relevant levels of BPA can cause precocious neurogenesis in the SCN of embryonic mice, leading to a concomitant disruption of circadian rhythms (*39*) as well as changes in the development of

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kisspeptin and gonadotropin-releasing hormone (GnRH) neurons, resulting in fertility issues in females (*40*). Beyond bisphenols, many studies have shown that exposure to air pollution particle matter, diesel exhaust, polychlorinated biphenyls, and nicotine can interfere with hypothalamic neuro- and gliogenesis (*41*). The true impact of environmental inputs on human hypothalamic development is less defined, with large cohort studies needed to further delineate their influence.

Hypothalamic connectivity that drives physiology and behaviors

Given that hypothalamic development is a protracted series of steps whereby a neuron born at a specific time migrates to the correct location and forms appropriate synaptic connections, it is perhaps not surprising that complex developmental programs progress into intricate circuit formation. As a neural integration center, the hypothalamus connects to the endocrine system via the pituitary and the circumventricular organs (CVOs) as well as to other neural centers through intra- and extrahypothalamic circuits that control behaviors (Fig. 3). The sophistication of these connections is central to the myriad physiologies and behaviors that the hypothalamus controls.

Neuroendocrine connectivity

The hypothalamus connects to the anterior and posterior lobes of the pituitary via the infundibulum (also known as the pituitary stalk). Briefly, magnocellular neurons that originate in the PVN and SON send axons that travel through the infundibulum and terminate in the posterior pituitary, where they secrete arginine-vasopressin and oxytocin to be released into peripheral circulation. The connection to the anterior pituitary is less direct, with neurons in the POA and other regions secreting hypothalamic-releasing hormones (e.g., GnRH and CRH) into the ME, where the hypophyseal-portal vasculature transports them to the anterior pituitary to stimulate the synthesis and secretion of pituitary hormones (e.g., luteinizing hormone, follicle-stimulating hormone, and adrenocorticotropic hormone) that then act on peripheral endocrine organs (*42*). Notably, the release of these neuropeptides or hormones is either contributed by multiple nuclei (i.e., arginine-vasopressin and oxytocin by the PVN and SON) or is the result of interactions among multiple nuclear clusters (e.g., the release of follicle-stimulating hormone by the interactions among AVPV, POA, and Arc), which emphasizes the extensive cooperation between hypothalamic nuclei (Fig. 1F).

The neuroendocrine hypothalamus also relies on highly permeable brain regions that reside outside the blood-brain barrier to sense peripheral cues, referred to as the CVOs: the organum vasculosus of the lamina terminalis (OVLT), the subfornical organ (SFO), and the ME (Fig. 3A). The OVLT resides in the anterior wall of the third ventricle and detects circulating chemokines and cytokines to initiate body temperature changes as well as monitor solute levels to drive regulation of water balance and thirst (*43*). The SFO is positioned in the dorsal surface of the third ventricle and is the primary sensor of circulating angiotensin II to communicate with brain centers that regulate blood pressure, sympathetic outflow, and neuroendocrine factors (*43*). The ventral-residing ME's primary function is to provide a portal for the secretion of hypothalamic-releasing hormones into the anterior pituitary, but its capillary bed is also in direct contact with hypothalamic ventricular cells and Arc neurons, which suggests a role in sensing peripheral signals (*43*). Recently, a SCN-OVLT vascular portal was identified that retrogradely transports arginine-vasopressin from the hypothalamus to the OVLT for secretion into broader circulation (*44*), thereby creating a second portal system outside the hypophyseal vasculature, which leads to intriguing questions as to whether hypothalamic neurons can secrete neuropeptides for peripheral circulation independent of the pituitary.

Hypothalamic neural connectivity

Individual hypothalamic nuclei are highly interconnected to facilitate the coordinated regulation

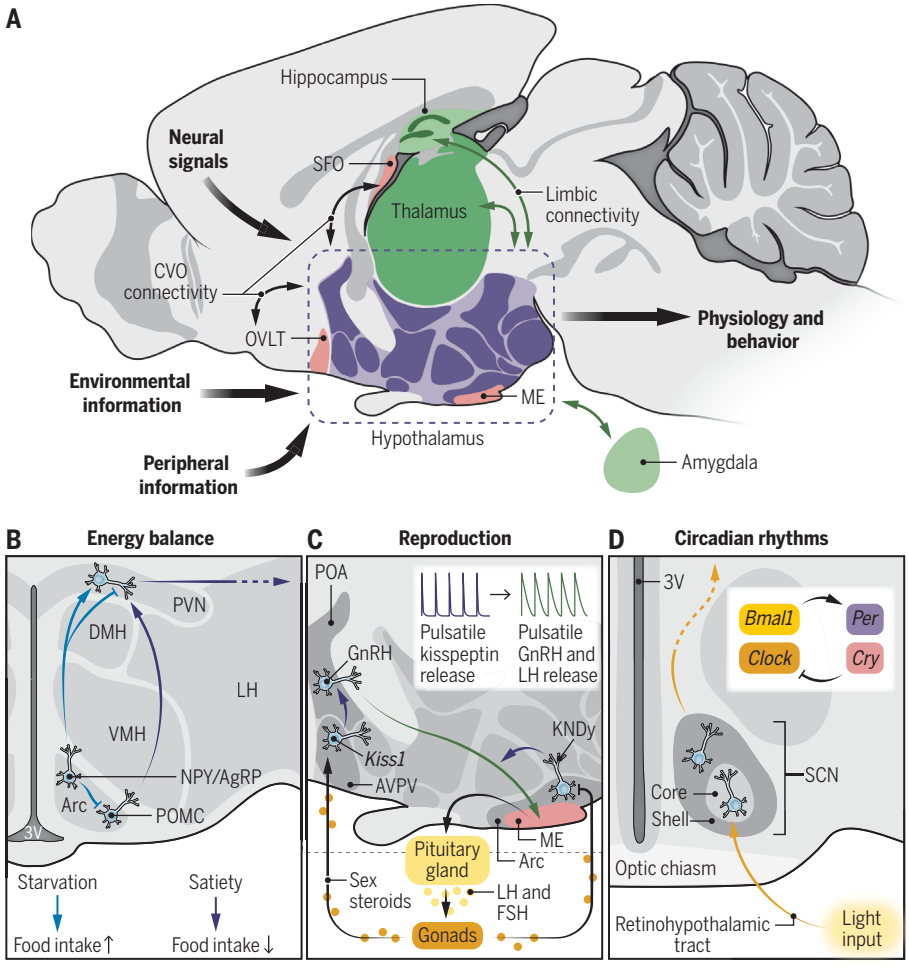

Fig. 3. The hypothalamus as an integration center driving key physiologies. (**A**) The hypothalamus receives and integrates environmental information, peripheral information, and neural signals to regulate physiologies and behaviors via connections to the endocrine system and intra- and extrahypothalamic neural centers. (**B**) After food intake, POMC neurons in the Arc that project to PVN neurons increase satiety, thus decreasing food intake (purple). During fasting, NPY/AgRP-expressing neurons in the Arc that project to the PVN increase food intake while also decreasing satiety by inhibiting (i) POMC neurons directly and (ii) the anorexigenic POMC inputs on PVN neurons (blue). (**C**) *Kiss1* neurons in the AVPV together with KNDy neurons in the Arc act on GnRH neurons in the POA to control the pulsatile release of GnRH, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) to regulate female fertility. (**D**) Responding to light input from the retinal-hypothalamic tract, the SCN maintains circadian rhythms through the autoregulatory transcriptional-translational feedback loops of clock genes, such as *Bmal1*, *Clock*, *Period1,2,3*, and *Cry1,2*. 3V, third ventricle.

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of energy balance, reproduction, sleep-wake, stress responses, thermoregulation, osmoregulation, and blood pressure. Moreover, the hypothalamus's central positioning within the brain facilitates broader neural connectiveness, such as through the dorsal longitudinal fasciculus to the brainstem, across the medial forebrain bundle to the neocortex, via the stria terminalis to the amygdala, and within the mammillothalamic tract to the thalamus, among others. Each of these pathways contributes to a range of neural processes, making the hypothalamus an important contributor to a multitude of behaviors (*45*). Herein, we provide a high-level overview of the hypothalamus's role in the limbic system plus three well-established circuits to illustrate the interconnectiveness of hypothalamic nuclei: energy balance, reproduction, and circadian rhythms.

Alongside the amygdala, thalamus, and hippocampus, the hypothalamus is a key component of the limbic system. The limbic circuitry serves as a crucial interface between cognitive functions, emotions, and adaptational responses and allows for the formation of memories, learning from experiences, and behavioral adaptations in response to the environment. The major hypothalamic-limbic connections include the hippocampal-centered circuits that connect via the fornix to regulate the anterograde episodic memory and the amygdala-centered circuits interconnected with the stria terminalis and thalamus that are considered fundamental for the processing of mood, affect, and emotions (*42*, *45*) (Fig. 3A).

Centrally, the hypothalamus relies on a complex interplay of hypothalamic circuitry to regulate energy balance. Simplistically, the drive to forage is mediated by neurons coexpressing orexigenic neuropeptide Y (NPY) and AgRP (NPY/AgRP) in the Arc that activate melanocortin hormone– and orexin-expressing neurons in the LH that increase food intake, whereas satiety is primarily controlled by POMC neurons in the Arc that project to PVN neurons to release α-melanocyte–stimulating hormone and decrease food intake. Second-order neurons in the PVN, VMH, DMH, and LH also send additional intra- and extrahypothalamic projections that help regulate feeding (*46*). Notably, when energy levels are low, NPY/AgRP-expressing neurons can directly inhibit POMC neurons to effectively block satiation (*47*) (Fig. 3B).

Alongside energy balance, the hypothalamus plays a fundamental role in regulating reproduction. Prenatally, GnRH neurons migrate from the olfactory placode to the POA, where they remain quiescent until acted upon at puberty by pulsatile kisspeptin release from Arc neurons that coexpress kisspeptin, neurokinin B, and dynorphin (KNDy) and drives synchronized GnRH secretion (*48*) (Fig. 3C). KNDy neurons together with the kisspeptin (*Kiss1*) neurons in AVPV control the feedback loop that regulates this pulsatile gonadotropin release and induces sex steroid synthesis and secretion from the gonads as well as gametogenesis (*49*). KNDy neurons are involved in the negative feedback of sex steroids, whereas *Kiss1* neurons in the female AVPV are involved in the positive feedback of hormones leading to the preovulatory luteinizing hormone surges (Fig. 3C). Given that reproduction is an energy-demanding event, a tight relationship between metabolic status and reproduction exists, with melanocortin neurons and kisspeptin neurons in the Arc influencing each other (*50*), although the full mechanisms are still emerging.

The hypothalamus is the central circadian pacemaker in mammals, with the SCN receiving light input from the retinohypothalamic tract to entrain the environmental light-dark cycle (Fig. 3D). Mechanistically, neurons of the SCN generate autonomous cellular circadian oscillations through the autoregulatory transcriptional-translational feedback loops of clock genes, such as *Bmal1*, *Clock*, *Period1,2,3*, and *Cry1,2*, which then regulate large gene networks to control synchrony of cellular activities (*51*). These SCN neurons regulate circadian rhythms by interacting with other wakefulness- and sleep-promoting circuits in the POA, DMH, PVN, and LH, releasing transforming growth factor–α (TGF-α) and prokineticin 2 and sensing changes in body temperature (*5*), for example. The LH is especially crucial in controlling sleep-wake cycles, with orexin neurons promoting wakefulness and maintained arousal during the day (*5*).

Emerging circuits of the hypothalamus

Advancements in cellular techniques, such as cell type–specific viral transfections, antero- and retrograde tracing, and chemogenetic and optogenetic manipulations, facilitate a higher-resolution probing of hypothalamic connectivity, leading to the identification of emerging circuits. Recently, the PVN and the LH were revealed to help regulate pain, with parvocellular oxytocin neurons in the PVN simultaneously projecting to magnocellular oxytocin neurons in the SON and deep-layer neurons in the spinal cord to suppress nociception and promote analgesia (*52*). Moreover, activation of PVN neurons that project to the neocortex reduces pain sensitization and anxiety-like behaviors (*53*), whereas LH orexin neurons that project to the dorsal horn of the spinal cord mediate antinociceptive effects (*54*). These findings implicate the hypothalamus as an underappreciated region involved in the regulation of autonomic and motivational responses to pain.

Moreover, the hypothalamus is emerging as a neural integration center for gut-derived hormones and microbiota metabolites. Serotonin, cholecystokinin, and peptide YY hormones secreted by the gut can inhibit the activity of hunger-promoting hypothalamic AgRP neurons to induce satiety (*55*), whereas secretin released from colon endocrine cells indirectly activates neurons in the nucleus of the solitary tract that project to the PVN to modulate sodium homeostasis (*56*). In addition, muropeptides derived from gut bacteria can be transferred to the hypothalamus, where they act on GABAergic neurons to regulate food intake and body temperature (*57*). Less understood is the influence of the maternal microbiome on developmental programs of the hypothalamus, although maternal treatment with the so-called good bacterium, *Lactobacillus*, corrects a gut microbial imbalance from a maternal high-fat diet, restoring oxytocin levels in the developing PVN and reversing social deficits in offspring (*58*). Mapping the hypothalamic circuitry responsive to the gut microenvironment will require a multidisciplinary approach that combines microbiome profiling and multi-omics integration with rodent gnotobiotic and behavioral studies.

The hypothalamus as a debated adult neurogenic center

A less-recognized output of the hypothalamus is its influence on adult neurogenesis in the two key neurogenic centers: the neocortex and the hippocampus (Fig. 4A). In response to metabolic cues, POMC neurons in the Arc send projections to the ventricular-subventricular zone of the neocortex and regulate neural stem cell proliferation that drives adult neurogenesis of deep granule neurons (*59*). Similarly, optogenetic stimulation of hypothalamic SuM neurons robustly promotes adult neuronal birth in the hippocampal dentate gyrus, leading to increased production of adult-born neurons and improved memory performance (*60*). These studies raise the compelling possibility that the hypothalamus serves as a conduit, translating sensory inputs into outcomes in adult neurogenic niches.

At the same time, an ongoing debate continues as to whether the hypothalamus itself is a bona fide adult neurogenic center. The ventricular zone of the adult hypothalamus has neurogenic potential, with low levels of adult-born hypothalamic neurons found in the POA and the tuberal hypothalamus. Tanycytes, specialized glia that reside in this niche, have a radial glia–like morphology and transcriptomic signature (*61*), which suggests a shared role in cell genesis (Fig. 4B). Short-term exposure to a high-fat diet induces hypothalamic ventricular cells to proliferate and give rise to neurons (*62*), with genetic fate mapping linking adult neurogenesis to tanycytes in the ME (*63*). Evidence is not conclusive as to whether these newly born neurons are functionally integrated into hypothalamic circuits, although these newly born tanycyte-derived neurons display some electrophysiological properties of functional neurons (*64*), and transplanted immature hypothalamic neurons reconstitute leptin responsiveness in obese *db/db* mice (*65*), demonstrating the feasibility of adult integration. Given the low numbers of hypothalamic adult-born neurons relative

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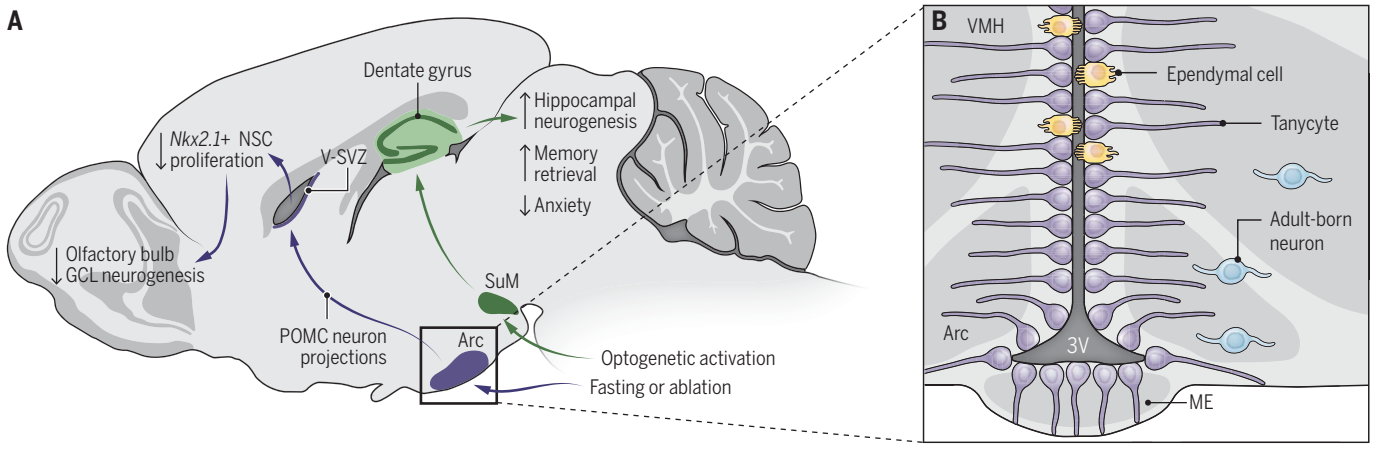

Fig. 4. Adult neurogenesis mediated by the hypothalamus. (**A**) In response to environmental cues, projections from POMC Arc neurons to the ventricular-subventricular zone (V-SVZ) regulate neural stem cell (NSC) proliferation, which then modulates adult neurogenesis in the deep granule cell layer (GCL) of the olfactory bulb. Optogenetic activation of SuM neurons increases adult neurogenesis in the hippocampal dentate gyrus, which improves memory performance after putative circuit integration. (**B**) Radial glia–like tanycytes are thought to contribute to adult neurogenesis in the Arc and VMH, among other hypothalamic regions.

to those produced by the neocortical and hippocampal niches (*62*), questions continue regarding the functional relevance of these adult hypothalamic neurons.

Future directions

The hypothalamus is an ancient brain region that predates the evolution of the vertebrate brain, with hypothalamic neurosecretory cells found in insects and annelids (*66*). Comparative studies show species-specific hypothalamic programs that are either gained or lost during evolution, which raises compelling questions about the developmental and functional relationships between the hypothalamus and specialized areas of the mammalian brain, especially the neocortex. For example, the hypothalamus is more complex in cytoarchitecture and cell diversity compared with the neocortex, where the columnar units are relatively consistent across the cortical plate despite mediating an extensive range of cognitive functions. This observation perhaps reflects an evolutionary simplification of the potentially surplus networks used by the hypothalamus or, alternatively, reveals the necessary redundancy in circuitry needed to ensure survival. Relatedly, the hypothalamus and neocortex use different strategies to yield neuronal diversification, which may further reflect an adoption of simplified programs in the neocortex. Indeed, some hypothalamic cell types common across vertebrates, such as ciliated ependymal serotonergic cells, are absent in the mammalian hypothalamus (*67*), but related homologs are found in the lateral ventricles, which raises questions as to whether some primitive hypothalamic functions have been assumed by other brain regions. Molecular and genetic tools, such as spatial transcriptomics and cell type–specific Cre lines, can provide insights into the relationship between hypothalamic cell types and the functional roles of related cells in other brain regions, especially the neocortex.

Historically, hypothalamic neurons have received the lion's share of research attention, but today, neuron-to-glia, glia-to-neuron, and glia-to-glia communication is more appreciated. Glial cells in the ventricular zone not only give rise to all cell types in the hypothalamus but also sense peripheral cues via the CVOs and transfer these signals to nearby neurons. Moreover, parenchymal glia, consisting of microglia, astrocytes, and cells of oligodendrocyte lineages, interact with neurons to control synaptic transmission, among other roles. Chronic high-fat diet in mice, for instance, can cause astrogliosis and microglia activation, leading to neuroinflammatory effects on nearby hypothalamic neurons. Although most hypothalamic axons are not myelinated, many input projections are, which makes defects in myelination potentially relevant to hypothalamic diseases, which remain poorly characterized. As the mapping of hypothalamic connectivity continues, the influence of neural glia on these circuits must also be examined.

Modern molecular tools have generated a wealth of data that enable the cataloging of hypothalamic cell types, which now must be interrogated for their roles in development and function. To do so, specialized Cre lines that target increasingly discrete subpopulations are needed. For instance, *Sst*+ neurons in the LH were recently stratified into four molecularly distinct populations (*21*), potentially each requiring their own Cre line to enable functional characterization. Furthermore, Cre drivers can be used in combination with chemogenetic and optogenetic lines to activate or inhibit neuronal populations, such as with diptheria toxin to ablate specific cell types, stereotaxic injections of siRNAs and/or short hairpin RNAs (shRNAs) to knock down target genes, or floxed reporters to study lineages. Furthermore, dead CRISPR-Cas9 enzymes (dCas9s) are increasingly used to activate or inhibit targeted transcription factors to study the combinatorial code driving developmental programs (*68*). Finally, the field must continue to pursue human model systems, including hypothalamic primary and organoid cultures enriched with various combinations of hypothalamic nuclei, to confirm developmental and network findings (where feasible) observed in rodents. In conclusion, deciphering the fundamental mechanisms of hypothalamic development and circuitry establishment is critical to our understanding of physiological homeostasis and behaviors and should facilitate the discovery of the underlying etiology of many disease states.

REFERENCES AND NOTES

1. C. B. Saper, B. B. Lowell, *Curr. Biol.* **24**, R1111–R1116 (2014).
2. Y. Xie, R. I. Dorsky, *Development* **144**, 1588–1599 (2017).
3. E. A. Markakis, *Front. Neuroendocrinol.* **23**, 257–291 (2002).
4. J. P. Herman *et al.*, *Compr. Physiol.* **6**, 603–621 (2016).
5. T. E. Scammell, E. Arrigoni, J. O. Lipton, *Neuron* **93**, 747–765 (2017).
6. R. A. Gorski, R. E. Harlan, C. D. Jacobson, J. E. Shryne, A. M. Southam, *J. Comp. Neurol.* **193**, 529–539 (1980).
7. L. S. Allen, M. Hines, J. E. Shryne, R. A. Gorski, *J. Neurosci.* **9**, 497–506 (1989).
8. N. Scott, M. Prigge, O. Yizhar, T. Kimchi, *Nature* **525**, 519–522 (2015).
9. C. F. Yang *et al.*, *Cell* **153**, 896–909 (2013).
10. C. Wang *et al.*, *Nat. Commun.* **9**, 1544 (2018).
11. D. W. Bayless *et al.*, *Cell* **186**, 3862–3881.e28 (2023).
12. R. Guillemin, *Science* **202**, 390–402 (1978).
13. M. Benevento, T. Hökfelt, T. Harkany, *Nat. Rev. Neurosci.* **23**, 611–627 (2022).
14. R. Chen, X. Wu, L. Jiang, Y. Zhang, *Cell Rep.* **18**, 3227–3241 (2017).
15. D. W. Kim *et al.*, *Nat. Commun.* **11**, 4360 (2020).
16. R. A. Romanov *et al.*, *Nature* **582**, 246–252 (2020).
17. L. Steuernagel *et al.*, *Nat. Metab.* **4**, 1402–1419 (2022).
18. J. R. Moffitt *et al.*, *Science* **362**, eaau5324 (2018).
19. J. N. Campbell *et al.*, *Nat. Neurosci.* **20**, 484–496 (2017).
20. L. E. Mickelsen *et al.*, *eLife* **9**, e58901 (2020).
21. L. E. Mickelsen *et al.*, *Nat. Neurosci.* **22**, 642–656 (2019).
22. J. E. van Veen *et al.*, *Nat. Metab.* **2**, 351–363 (2020).
23. K. Hashikawa *et al.*, *Nat. Neurosci.* **20**, 1580–1590 (2017).
24. J. Clasadonte, V. Prevot, *Nat. Rev. Endocrinol.* **14**, 25–44 (2018).
25. O. Barca-Mayo *et al.*, *Nat. Commun.* **8**, 14336 (2017).

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26. K. M. Lenz, B. M. Nugent, R. Haliyur, M. M. McCarthy, *J. Neurosci.* **33**, 2761–2772 (2013).
27. S. Nampoothiri, R. Nogueiras, M. Schwaninger, V. Prevot, *Nat. Metab.* **4**, 813–825 (2022).
28. S. Burbridge, I. Stewart, M. Placzek, *Compr. Physiol.* **6**, 623–643 (2016).
29. X. Zhou *et al*., *Nat. Commun.* **11**, 4063 (2020).
30. D. W. Kim *et al*., *Cell Rep.* **38**, 110251 (2022).
31. Y. H. Zhang *et al*., *Cell Stem Cell* **28**, 1483–1499.e8 (2021).
32. T. Ma, S. Z. H. Wong, B. Lee, G. L. Ming, H. Song, *Neuron* **109**, 1150–1167.e6 (2021).
33. J. Altman, S. A. Bayer, *Adv. Anat. Embryol. Cell Biol.* **100**, 1–178 (1986).
34. X. Zhou *et al*., *Cell Stem Cell* **29**, 328–343.e5 (2022).
35. C. M. Marsters *et al*., *Neural Dev.* **11**, 20 (2016).
36. B. Harmancıoğlu, S. Kabaran, *Front. Genet.* **14**, 1158089 (2023).
37. J. M. Rosin, S. Sinha, J. Biernaskie, D. M. Kurrasch, *Dev. Cell* **56**, 1326–1345.e6 (2021).
38. X. Zhao, M. Erickson, R. Mohammed, A. C. Kentner, *Dev. Psychobiol.* **64**, e22278 (2022).
39. D. Nesan, K. M. Feighan, M. C. Antle, D. M. Kurrasch, *Sci. Adv.* **7**, eabd1159 (2021).
40. C. Pivonello *et al*., *Reprod. Biol. Endocrinol.* **18**, 22 (2020).
41. L. Koshko, S. Scofield, G. Mor, M. Sadagurski, *Front. Endocrinol.* **13**, 938094 (2022).
42. H. L. Müller *et al*., *Nat. Rev. Dis. Primers* **8**, 24 (2022).
43. G. I. Uwaifo, *The Human Hypothalamus: Anatomy, Dysfunction and Disease Management* (Springer, 2020).
44. R. Silver, Y. Yao, R. K. Roy, J. E. Stern, *J. Neuroendocrinol.* **35**, e13245 (2023).
45. P. Y. Risold, R. H. Thompson, L. W. Swanson, *Brain Res. Rev.* **24**, 197–254 (1997).
46. S. G. Bouret, in *Appetite and Food Intake: Central Control*, R. Harris, Ed. (CRC Press, ed. 2, 2017), pp. 135–154.
47. K. Timper, J. C. Brüning, *Dis. Model. Mech.* **10**, 679–689 (2017).
48. Y. Uenoyama, N. Inoue, S. Nakamura, H. Tsukamura, *Front. Endocrinol.* **10**, 312 (2019).
49. V. M. Navarro, M. Tena-Sempere, *Nat. Rev. Endocrinol.* **8**, 40–53 (2011).
50. V. M. Navarro, *Nat. Rev. Endocrinol.* **16**, 407–420 (2020).
51. J. S. Takahashi, *Nat. Rev. Genet.* **18**, 164–179 (2017).
52. M. Eliava *et al*., *Neuron* **89**, 1291–1304 (2016).
53. X. H. Li *et al*., *Cell Rep.* **36**, 109411 (2021).
54. Y. Jeong, J. E. Holden, *Neuroscience* **159**, 1414–1421 (2009).
55. L. R. Beutler *et al*., *Neuron* **96**, 461–475.e5 (2017).
56. Y. Liu *et al*., *Sci. Adv.* **9**, eadd5330 (2023).
57. I. Gabanyi *et al*., *Science* **376**, eabj3986 (2022).
58. S. A. Buffington *et al*., *Cell* **165**, 1762–1775 (2016).
59. A. Paul, Z. Chaker, F. Doetsch, *Science* **356**, 1383–1386 (2017).
60. Y. D. Li *et al*., *Nat. Neurosci.* **25**, 630–645 (2022).
61. T. Goodman, M. K. Hajihosseini, *Front. Neurosci.* **9**, 387 (2015).
62. S. Yoo, S. Blackshaw, *Prog. Neurobiol.* **170**, 53–66 (2018).
63. D. A. Lee *et al*., *Nat. Neurosci.* **15**, 700–702 (2012).
64. S. Yoo *et al*., *Sci. Adv.* **7**, eabg3777 (2021).
65. A. Czupryn *et al*., *Science* **334**, 1133–1137 (2011).
66. L. A. Lemaire, C. Cao, P. H. Yoon, J. Long, M. Levine, *Sci. Adv.* **7**, eabf7452 (2021).
67. T. K. Sato *et al*., *Neuron* **43**, 527–537 (2004).
68. C. T. Breunig *et al*., *PLOS ONE* **13**, e0196015 (2018).

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