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·Review·

Dissecting the hypothalamic pathways that underlie innate behaviors

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Many complex behaviors that do not require learning are displayed and are termed innate. Although traditionally the subject matter of ethology, innate behaviors offer a unique entry point for neuroscientists to dissect the physiological mechanisms governing complex behaviors. Since the last century, converging evidence has implicated the hypothalamus as the central brain area that controls innate behaviors. Recent studies using cutting-edge tools have revealed that genetically-defined populations of neurons residing in distinct hypothalamic nuclei and their associated neural pathways regulate the initiation and maintenance of diverse behaviors including feeding, sleep, aggression, and parental care. Here, we review the newly-defined hypothalamic pathways that regulate each innate behavior. In addition, emerging general principles of the neural control of complex behaviors are discussed.

Keywords: hypothalamus; innate behaviors; neural circuit; optogenetics; neural network

Introduction

Naïve animals of many species readily display a rich repertoire of species-specific behaviors. For example, a male stickleback performs elaborate rituals to entice a female to mate and a newly-hatched herring-gull chick pecks at the red dot on the bill of its parents to stimulate feeding[1, 2]. Traditionally, ethologists refer to these behaviors as innate or instinctual. By definition, an innate behavior, which often appears in fully functional form when performed for the first time, is displayed by different individuals of the same species, and persists stably under variable conditions, even when an animal is raised in isolation. Innate behaviors allow organisms to react quickly and reliably in vital situations without needing to spend time or energy on learning. Indeed, most innate behaviors, similar to the examples of the male stickleback and the herring-gull chick, pertain to the survival of individuals and propagation of the species.

While ethologists are interested in the adaptive values of innate behaviors and the evolutionary processes that selected for their appearance, neuroscientists are intrigued by the physiological underpinnings of these behaviors. Swiss physiologist Dr. Walter Rudolf Hess, along with other great scientists of the early 1900s, pioneered the field that focuses on the neural control of behaviors. Over a span of 25 years dating back to the 1920s, Dr. Hess systematically investigated the neural substrates that regulate autonomic functions and behaviors by delivering small electrical currents that activated various brain sites in freely moving cats, a technique that he perfected[3]. This lifelong work won him the Nobel Prize in 1949 and revealed that stimulation of the hypothalamus, a ventral brain structure essential for physiological homeostasis, elicits elements of behaviors such as hunger and thirst as well as more complex behaviors such as sleep and aggression. Subsequently, other studies, which will be discussed in detail in later sections, found that lesions of the hypothalamus or

mutations of hypothalamic-enriched genes produced deficits in these behaviors. Together, these studies point to the hypothalamus as a key brain area that controls innate behaviors.

Functional Organization of the Hypothalamus

The hypothalamus lies beneath the thalamus at the ventral base of the brain. It is one of the most ancient brain structures and is highly conserved. It extends rostrally into the forebrain to the optic chiasm and caudally to include the mammillary body (Fig. 1A). Along the rostro-caudal axis, the hypothalamus is divided into four regions: the preoptic area, the anterior hypothalamus, the tuberal hypothalamus, and the mammillary body[4, 5]. Within each region, a hypothalamic nucleus is identified when there is an appearance of uniform cell groups. Such is the case for the paraventricular nucleus (PVN) and the suprachiasmatic nucleus (SCN). A hypothalamic nucleus may also be named according to its anatomical location along the dorsal/ventral and medial/lateral axes such as the dorsomedial nuclei (DMH) and the ventromedial nuclei (VMH), or according to its position relative to other landmark structures such as the premammillary nucleus (PMN), which sits rostral to the mammillary body. There are more than 20 hypothalamic nuclei, the majority of which lack clear boundaries.

During development, progenitor cells along the ventricles divide in 3 consecutive waves and migrate outwards tangentially to populate the hypothalamus in a roughly “outside-in” manner (Fig. 1B). Based on these events, the hypothalamus is divided into 3 columns that span the entire rostro-caudal length: the periventricular, the medial, and the lateral columns[4, 6]. These 3 columns appear to be functionally distinct but are extensively interconnected. Broadly speaking, the periventricular column projects to the anterior and posterior pituitary gland and regulates the hormonal milieu of the body, the medial column projects to the autonomic and motor pathways and

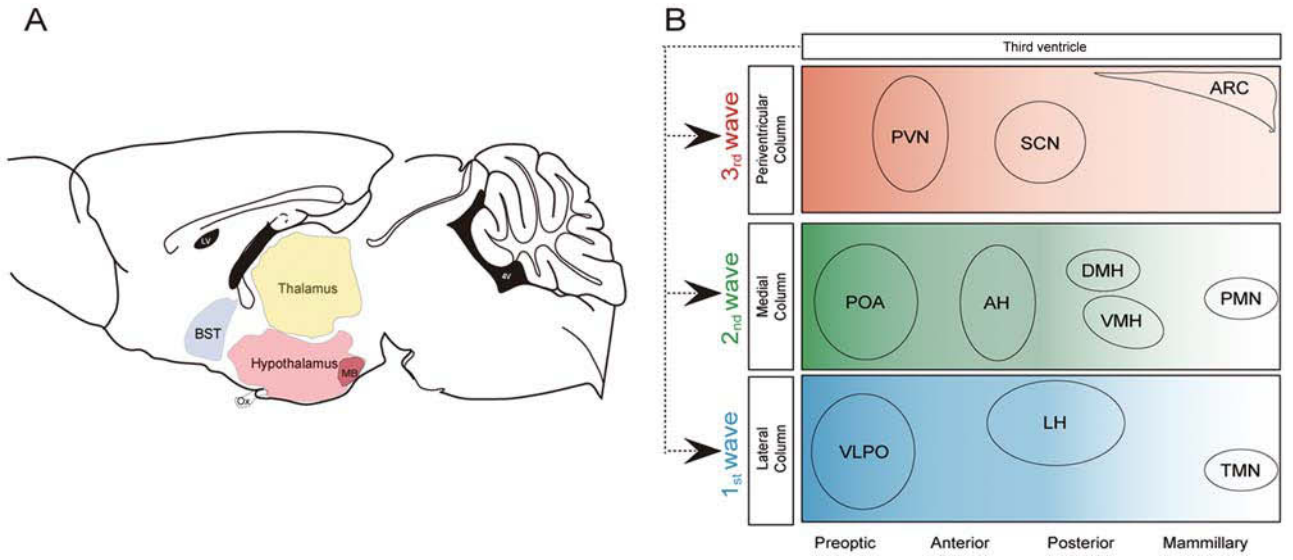


Fig. 1. Functional organization of the hypothalamus. A. Sagittal view of a mouse brain. The hypothalamus (pink) is ventral to the thalamus (yellow), caudal to the bed nucleus of the stria terminalis (BST, gray), and includes the mammillary body (MB, red) at the boundary. Along the rostral-caudal axis, the hypothalamus is divided into 4 regions: the preoptic, the anterior, the posterior, and the mammillary regions. OX, optical chiasm. B. Schematic showing a flattened view of the hypothalamus. The hypothalamus is divided into 3 columns that span the entire rostral-caudal length. During development, progenitors along the ventricles divide and migrate out in 3 consecutive waves. The first wave (blue) populates the lateral column, the second wave (green) populates the medial column, and the third wave (orange) populates the periventricular column. The relative positions of major hypothalamic nuclei are indicated. PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; ARC, arcuate nucleus; POA, preoptic area; AH, anterior hypothalamus; DMH, dorsomedial hypothalamus; VMH, ventromedial hypothalamus; PMN, premammillary nucleus; VLPO, ventrolateral preoptic area; LH, lateral hypothalamus; TMN, tuberomammillary nucleus.

controls behavioral outputs, and the lateral column contains diffuse groups of neurons traversed by the medial forebrain bundle and regulates behavioral states of the animal.

Clearly, the hypothalamus is a highly complex structure, which consists of tightly-packed nuclei and fiber tracts. The current method of grouping hypothalamic neurons according to physical proximity lacks functional insight. Moreover, it is nearly impossible to restrict surgical lesions to one hypothalamic nucleus. In addition, electrical currents or chemicals delivered to one hypothalamic nucleus can in theory spread to other sites and lead to non-specific effects. Due to these limitations, we knew very little about the specific hypothalamic pathways that govern each behavior beyond a rudimentary appreciation of the overall importance of the hypothalamus in regulating innate behaviors.

Hypothalamic Pathways Regulating Innate Behaviors

In the last decade, new tools have been developed that allow targeting and functional manipulation of genetically-defined populations of neurons without affecting neighboring cells (Table 1)[7-25]. Neuroscientists using these new methods can visualize, record, activate, inhibit, or ablate specific populations of neurons to probe the causal relationships between the activities of different populations of neurons and a specific behavior[17, 26, 27]. In addition, they can trace the synaptic inputs and outputs of these neurons and delineate the entire neural pathway that regulates a behavior[28-31]. These studies have revealed that genetically-defined populations of neurons residing in different hypothalamic nuclei and their connecting neural pathways play important yet distinct roles in feeding, sleep, aggression, and parental care. Details of these findings with regard to individual behaviors have been extensively reviewed elsewhere[7, 31-40]. In the following sections, we provide an up-to-date overview of these newly-identified hypothalamic pathways that regulate each behavior in the context of historical studies to highlight the power and necessity of cell type-specific approaches in dissecting the hypothalamic circuitry.

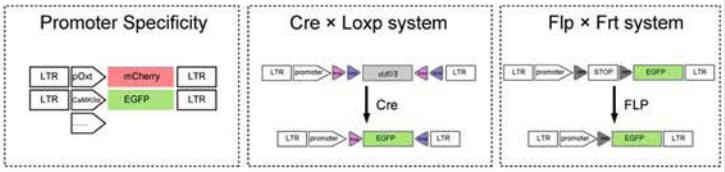
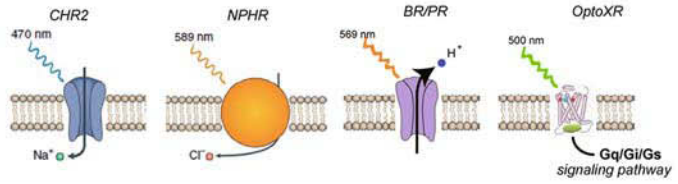
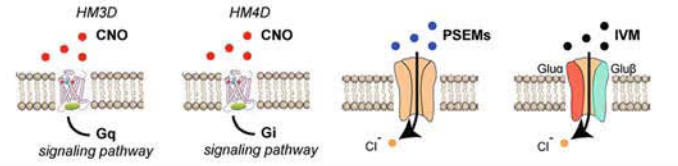
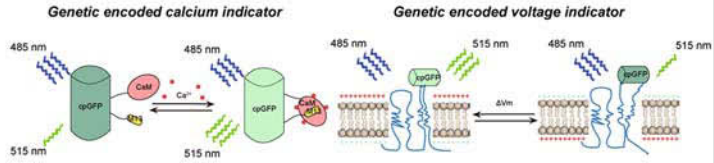
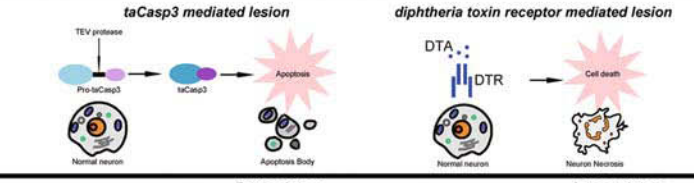
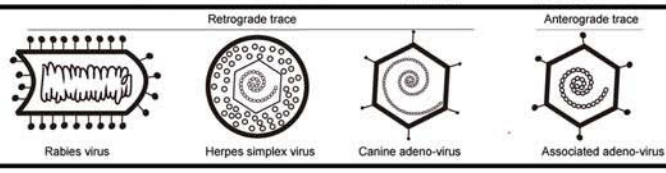
Feeding

It was first found over a hundred years ago that hypothalamic injuries led to the rapid onset of obesity in patients[41]. Follow-up studies in multiple animal species showed that destruction of the hypothalamus, especially the VMH or the PVN, invariantly caused animals to overeat and become obese, while destruction of the lateral hypothalamic nuclei (LH) led to cessation of feeding and weight loss[42-45]. These findings fueled the "dual center hypothesis", which suggested that all behaviors are governed by antagonistic "drive centers" that either promote or inhibit a given behavior[46]. Thus, the VMH was dubbed a "satiety center" and the LH a "hunger center". However, such a view has since proven to be too simplistic. For instance, a large variety of neuropeptides and neurotransmitters were found to induce or inhibit food intake when injected into the ventricles[47]. Identification of one neuropeptide, agouti-related protein (Agrp), gradually led to our current understanding of the complex hypothalamic pathways for feeding.

Agrp was first cloned based on its homology to Agouti, a skin protein that when expressed ubiquitously causes obesity in mice[48]. Similar to Agouti, Agrp antagonizes melanocortin receptor signaling. Overexpression of Agrp as well as deletion of melanocortin receptor 4 (MC4R), which is the primary melanocortin receptor in the brain, cause obesity[48, 49]. Agrp expression in the brain is restricted to a group of inhibitory neurons in the arcuate nucleus of the hypothalamus. This remarkable specificity of Agrp expression lends easy genetic access to target and functionally manipulate Agrp-expressing neurons. Indeed, using the *Agrp* locus to drive the expression of the human diphtheria toxin receptor, Dr. Richard Palmiter's group first showed that selective ablation of Agrp-expressing neurons in adults but not in neonates leads to dramatic loss of feeding behavior (aphagia) and severe starvation in mice[50]. Using similar genetic strategies to control the activity of Agrp neurons *via* optogenetic and chemogenetic methods, other groups found that inhibition of Agrp neurons in starved animals suppresses feeding while activation of these neurons elicits food foraging and voracious feeding even in satiated animals[51, 52]. Taken together, these results demonstrate that Agrp neurons occupy an obligatory node in the hypothalamic pathways that control feeding.

Agrp neurons use neuropeptide Y (NPY), γ-aminobutyric acid (GABA), and Agrp as neurotransmitters and exert an overall inhibitory effect on their targets. NPY and GABA mediate an immediate fast effect (minutes) on

Table 1. Tools to study specific populations of neurons and trace their connections

Functions	Commonly used tools	References
Cell specific targeting		Dittgen *et al.*, 2004[18] Fields *et al.*, 2012[19] Schlake and Bode, 1994[21] Sauer and Henderson, 1988[20]
Light activated receptor		Airan *et al.*, 2009[14] Boyden *et al.*, 2005[10] Gradinaru *et al.*, 2010[15]
Drug activated receptor		Armbruster *et al.*, 2007[12] Magnus *et al.*, 2011[16] Lerchner *et al.*, 2007[13]
Neuronal activity indicator		Nakai *et al.*, 2001[7] St-Pierre *et al.*, 2014[8]
Cell ablation		Yang *et al.*, 2013[17] Palmiter *et al.*, 1987[9] Buch *et al.*, 2005[11]
Circuit tracing		Callaway, 2008[22] Soudais *et al.*, 2001[23] Bak *et al.*, 1977[24] Norgren and Lehman, 1998[25]

feeding by acting on their respective receptors. In contrast, Agrp exerts a slower effect (hours) through antagonism of melanocortin receptor signaling[30, 53]. The expression levels of Agrp as well as the activities of Agrp neurons are tuned to the nutritional state of the animal; hormones that signal energy deficits upregulate Agrp expression and activate Agrp neurons, while hormones that signal energy surfeit suppress them[54]. Interestingly, food odor also rapidly modulates the firing rates of Agrp neurons[55, 56]. These regulatory mechanisms ensure that food foraging and feeding are tightly coupled to the energy needs of the animal. In addition, Agrp neurons receive excitatory synaptic inputs emanating from a small group of PVN neurons that express thyrotropin-releasing hormone (TRH) and the pituitary adenylate cyclase activating polypeptide (PACAP)[28]. Chemogenetic activation of TRH^+/$PACAP^+$ PVN neurons drives feeding in sated animals *via* activation of Agrp neurons. Conversely, inhibition of TRH^+/$PACAP^+$

PVN neurons decreases normal caloric deficiency-induced food consumption. Thus, arcuate Agrp neurons sense both hormonal and synaptic signals to regulate feeding behavior.

Agrp neurons provide an ideal starting point to trace the neural circuit for feeding. Within the arcuate nucleus, Agrp neurons project locally to inhibit a neighboring population of neurons that express proopiomelanocortin (POMC), a prohormone that is subsequently processed into α-MCH (melanocyte stimulating hormone) and other hormones[47]. In contrast to Agrp, α-MCH activates melanocortin receptors and therefore suppresses feeding. Consistent with the slower time-course of melanocortin receptor signaling, activation or inhibition of POMC neurons has little effect on acute food consumption, but instead regulates feeding over a period of 24 h[30]. Although Agrp neurons and POMC neurons are intermingled, TRH^+/$PACAP^+$ PVN neurons make contacts only on Agrp neurons. Furthermore, POMC, but not Agrp neurons, receive inputs from PACAP-expressing neurons in the VMH, a known satiety center that causes insatiable eating when lesioned[28, 57]. POMC neurons, in turn, project to many of the same targets as Agrp neurons. These observations exemplify the intricate connections among different hypothalamic nuclei and suggest a separation between the "hunger" and "satiety" pathways at the level of the arcuate nucleus. In addition to regulating acute feeding, Agrp neurons coordinate long-term appetite with POMC neurons through their balanced activities and antagonistic effects on melanocortin receptors.

Agrp neurons project extensively outside of the arcuate nucleus, most prominently to the PVN and LH, and to many other sites outside of the hypothalamus including the anterior bed nucleus of the stria terminalis (aBNST), the paraventricular nucleus of the thalamus (PVT), the central amygdala (CeA), the parabrachial nucleus (PBN), and the periaqueductal gray (PAG)[29]. Each of these target regions receives projections from a separate subset of Agrp neurons. Moreover, Agrp neurons that innervate forebrain targets are distributed more anteriorly than those that innervate the hindbrain. Independent activation of Agrp projections to the PVN, LH, aBNST, and PVT, but not to other downstream targets, elicits feeding that is comparable to the activation of Agrp neurons, which suggests a parallel and redundant configuration of Agrp neural pathways. More detailed analysis reveals that target regions of Agrp neurons are extensively interconnected. For example, GABAergic neurons in the aBNST control feeding through inhibition of LH glutamatergic neurons[58] and PVN MC4R-expressing neurons drive feeding through projections to the lateral parabrachial nucleus[59]. Thus, downstream targets of Agrp neurons likely function together as a neural network that incorporates inputs from the arcuate nucleus and other brain areas such as the cortex and the hippocampus to execute both homeostatic and hedonic control of feeding.

Interestingly, although optogenetic activation of Agrp projections to the PBN cannot evoke feeding, delivery of a GABA receptor agonist to the PBN rescues the aphagia phenotype that results from Agrp neuronal ablation[60]. PBN calcitonin-related peptide-expressing neurons normally relay information of visceral malaise associated with food poisoning or other illness and suppress feeding through projections to protein kinase C delta-expressing neurons in the CeA[61]. This "anorexia pathway" becomes chronically activated upon ablation of Agrp neurons[60-62]. Indeed, suppression of any key node in this "anorexia pathway", including neurons in the nucleus of the solitary tract (NTS) that project to the PBN or serotonergic neurons upstream of the NTS, restores feeding in animals with the Agrp neurons ablated[63].

In summary, the hypothalamic feeding circuit centers on arcuate Agrp neurons, which integrate hormonal, sensory, and synaptic inputs to coordinate neural activities in a "satiety circuit", a "hunger circuit", and an "anorexia circuit" to regulate both acute and long-term feeding (Fig. 2). Such complex control of behavior at the network level affords both redundancy and flexibility, which is perhaps to be expected given how important feeding is to the survival of an individual.

Sleep

Sleep is a rapidly reversible behavioral state of immobility with reduced sensory responsiveness. In mammals, sleep is divided into non-rapid eye movement (NREM) sleep, which is characterized by synchronized cortical activities, and rapid eye movement (REM) sleep, which is characterized by a lack of muscle tone and by the re-emergence of cortical activities that are paradoxically similar to those during the wake period. Viennese physiologist Constantin von Economo first suggested in the early 1900s that the hypothalamus harbors neural substrates that regulate sleep/arousal[64]. By studying brain

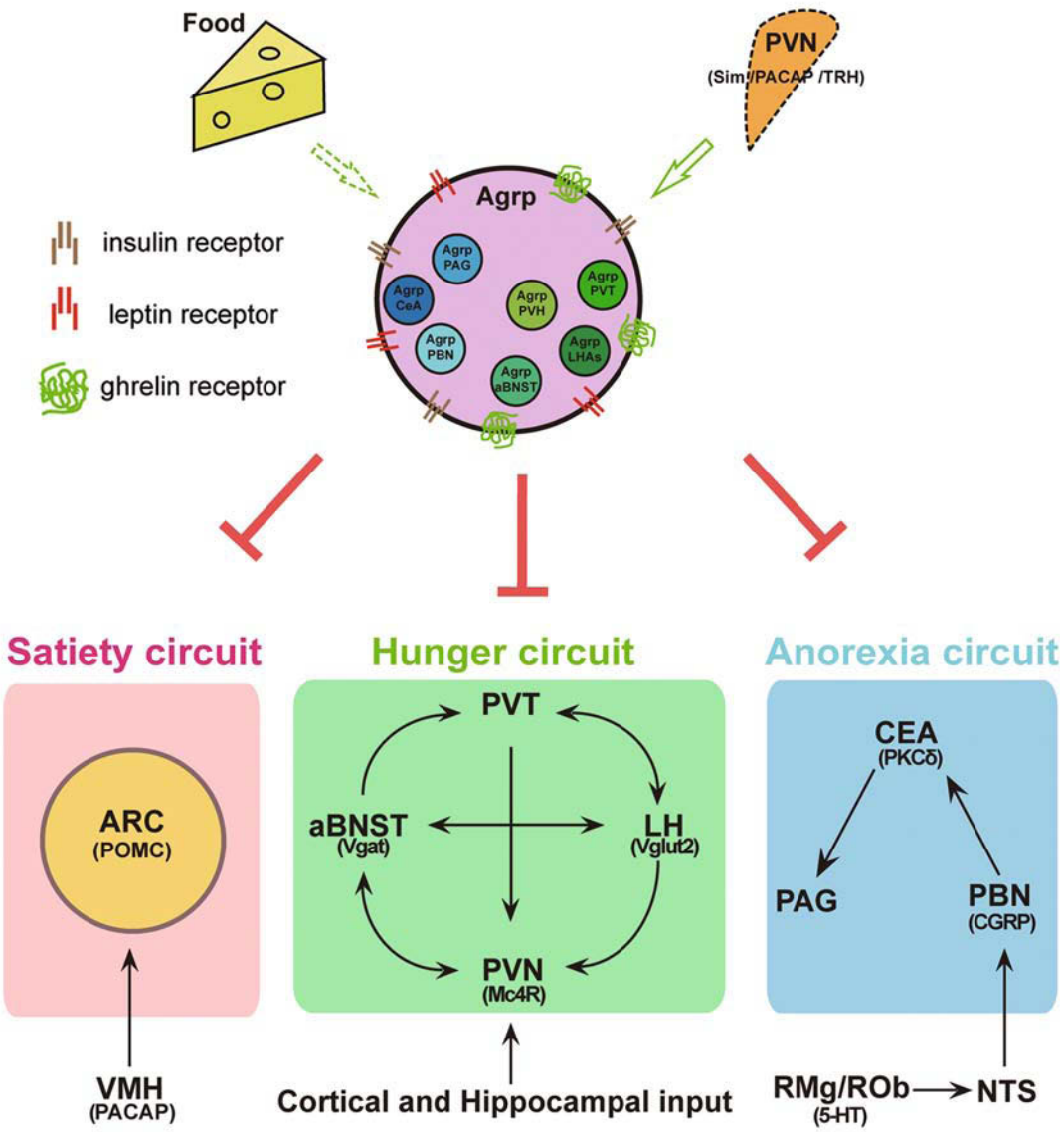


Fig. 2. The hypothalamic feeding circuit. The hypothalamic feeding circuit centers on agouti-related protein (Agrp) neurons, which incorporate hormonal, sensory, and synaptic inputs to control the activities of a "satiety circuit", a "hunger circuit", and an "anorexia circuit" to regulate feeding. Agrp neurons express receptors for insulin, ghrelin, and leptin. The activity of Agrp neurons is regulated by hormonal, sensory (food), and excitatory synaptic inputs emanating from $Sim^{+}/PACAP^{+}/TRH^{+}$ neurons in the paraventricular nucleus (PVN). Non-overlapping subpopulations of Agrp neurons (smaller circles) project to different downstream targets. These downstream targets receive synaptic inputs from other brain regions and are extensively interconnected. Within the arcuate nucleus, Agrp neurons inhibit POMC-expressing neurons, which also receive excitatory inputs from $PACAP^{+}$ neurons in the ventromedial hypothalamus (VMH). The activities of POMC neurons affect long-term feeding, thus dubbed the "satiety circuit". Outside of the arcuate nucleus, activation of Agrp projections to the anterior bed nucleus of the stria terminalis (aBNST), paraventricular nucleus of the thalamus (PVT), lateral hypothalamus (LH), and PVN (the "hunger circuit") evokes feeding, while activation of Agrp projections to the periaqueductal gray (PAG), parabrachial nucleus (PBN), and central amygdala (CeA) (the "anorexia circuit") cannot. However, suppression of the "anorexia circuit" rescues the aphagia phenotype caused by ablation of Agrp neurons (see text). Gene names in parentheses indicate the specific population of neurons that regulates feeding in the brain region above. Sim1, single minded 1; POMC, proopiomelanocortin; PACAP, pituitary adenylate cyclase activating polypeptide; TRH, thyrotropin-releasing hormone.

pathologies that accompany either insomnia or excessive sleep in patients with encephalitis lethargica, he deduced that the anterior hypothalamus promotes sleep while the posterior hypothalamus promotes arousal. These claims were later corroborated by Nauta, who found in rats that injuries to the anterior hypothalamus at the level of the preoptic nucleus led to relentless insomnia, while injuries to the posterior hypothalamus at the level of the mammillary body resulted in difficulty in staying awake[65].

In the late 1990s, Dr. Clifford Saper and colleagues stained for c-fos, a marker of neuronal activation, and showed that subsets of neurons in the ventral lateral preoptic area (VLPO) of the hypothalamus are activated during different stages of sleep in rats[66, 67]. Strikingly, destruction of the VLPO led to dramatic (50%–60%) and persistent loss of both REM and NREM sleep[68]. The majority of VLPO sleep-active neurons contain GABA and galanin, and project to the "ascending arousal pathway", which includes histaminergic neurons in the tuberomammillary nucleus (TMN) of the posterior hypothalamus, norepinephrinergic neurons in the locus coeruleus (LC), and serotonergic neurons and cholinergic neurons in the brainstem[66, 69]. VLPO sleep-active neurons are inhibited by histaminergic, noradrenergic, and serotonergic innervation[70]. Reciprocal inhibition between the VLPO, the putative sleep center, and arousal-promoting neurons is thought to mediate rapid and stable behavioral transitions during the sleep-wake cycle, similar to a "flip-flop" switch in an electrical circuit[71].

VLPO sleep-active neurons are intermingled with other neurons that do not change or decrease their firing rates during sleep[72]. The lack of genetic markers that differentiate VLPO neurons precludes a more detailed dissection of VLPO neural pathways that regulate sleep. Similarly, although c-fos immunostaining and electrophysiological recordings have identified other sleep-active neurons in the median preoptic area and the basal forebrain, whether or how these neurons regulate sleep remains to be determined[73, 74].

Cell type-specific studies in other regions where genetic markers are available have revealed nuances of regulation of the sleep-wake cycle. Of these, the best studied are the hypocretin (Hcrt)-expressing neurons in the LH. Hcrt-A and -B, which are derived from the same genomic locus, were first identified as hypothalamic specific neuropeptides that stimulate feeding[75, 76]. Hcrt neuropeptides are expressed in the LH, DMH, and the perifornical area[77]. Interestingly, a mutation in hypocretin receptor 2 (Hcrtr2) was identified in a canine model of narcolepsy, a neurological disorder of excessive daytime sleepiness and aberrant sleep-wake transitions, which suggests that Hcrt signaling is indispensable for proper regulation of the sleep-wake cycle[78]. Mice bearing a deletion of the *Hcrt* gene or ablation of Hcrt neurons exhibit a narcolepsy phenotype similar to the canine model[79]. Moreover, a deficiency in Hcrt was found in narcoleptic patients[80].

Identification of Hcrt offers a much-needed genetic entry point to dissect hypothalamic pathways that regulate sleep/arousal. Hcrt neurons are active during the wake period but are quiescent during sleep[81]. Using the *Hcrt* promoter to drive cell type-specific expression of ChR2 (channelrhodopsin-2), Dr. Luis de Lecea's group first showed that acute activation of Hcrt neurons increases the probability of wakefulness during the sleep period and decreases the latency of transition from sleep to awake[82]. Arousal-promoting effects of Hcrt neuronal activation are abolished in *Hcrt* knockout animals or when Hcrt receptors are pharmacologically blocked, which suggests that such effects depend on Hcrt signaling. Long-term activation of Hcrt neurons (~4 h) fragments sleep and increases the number of sleep-wake transitions, but it has mixed effects on total wake time depending on the particular stimulation conditions[83]. Acute silencing of Hcrt neurons induces NREM sleep[84, 85]. Chemogenetic activation and inhibition of Hcrt neurons, which affect neuronal activities on a slower time scale (hours) compared with optogenetic approaches (milliseconds), mostly confirm that Hcrt neurons bi-directionally modulate arousal[86].

In addition to diffuse projections throughout the brain, Hcrt neurons most densely innervate basal forebrain cholinergic neurons and components of the "ascending arousal pathway" that includes histaminergic TMN neurons and norepinephrinergic LC neurons, where Hcrt neurons release glutamate as well as Hcrt to excite downstream neurons[75, 87]. Although local infusion of Hcrt in each of these target regions is sufficient to promote arousal, the arousal-promoting effects of Hcrt neurons are not affected by histamine deficiency, but are blocked by concomitant inhibition of norepinephrinergic LC neurons. In contrast,

optogenetic activation of norepinephrinergic LC neurons potentiates the arousal-promoting effects of Hcrt neurons, which suggests that norepinephrinergic LC neurons are the critical downstream target of Hcrt neurons that promote arousal[88, 89]. Consistent with this idea, optogenetic activation of norepinephrinergic LC neurons causes immediate awakening in mice, an effect that is stronger and faster than those observed with activation of Hcrt neurons[90].

Surprisingly, a triple lesion study that destroyed ~93% of norepinephrinergic LC neurons, ~89% of basal forebrain cholinergic neurons, and ~75% of histaminergic TMN neurons, yielded only a mild stabilizing effects on the sleep-wake architecture, which is in stark contrast to the narcolepsy phenotype observed with similar destruction of Hcrt neurons[91, 92]. Based on these and other findings, it is proposed that Hcrt neurons regulate the vigilance state of an animal by orchestrating the activities of multiple arousal-promoting systems distributed throughout the brain[38, 93-95]. Consistent with the experimental results, such a model predicts that destruction of Hcrt neurons is more detrimental to the sleep/wake cycle than destruction of any downstream targets, which could be functionally redundant. Moreover, given the extensive afferent and efferent projections of Hcrt neurons and the proven roles of Hcrt in other physiological functions such as feeding and metabolism, Hcrt neurons likely integrate internal and external information to regulate not just vigilance states but also other bodily functions and motivated behaviors[96-98].

In the LH, Hcrt neurons are intermingled with another population of neurons that express melanin-concentrating hormone (MCH), which regulates energy homeostasis[99, 100]. MCH neurons send parallel projections to many of the same targets as Hcrt neurons, but they release GABA and are inhibitory. In contrast to Hcrt neurons, MCH neurons are active during sleep. Moreover, MCH neurons are inhibited by Hcrt neurons through local microcircuits[101]. This anatomical and physiological evidence suggests that MCH neurons play a role that is opposite to that of Hcrt neurons[102]. Indeed, using the *MCH* promoter to drive the expression of opsin or toxin, three studies found that MCH neurons promote sleep[103-105]. Specifically, they found that acute activation of MCH neurons had no effect during the wake period, but promoted REM sleep at the expense of NREM sleep. Chronic activation of MCH neurons increased both REM and NREM sleep time. Acute silencing of MCH neurons shifted the dominant EEG peak frequency toward a slower oscillation without affecting the vigilance state. Finally, ablation of MCH neurons increased wake time and decreased NREM but not REM sleep time. Together, these findings suggest that MCH neurons dynamically regulate sleep, possibly by orchestrating the activities of several downstream targets, similar to Hcrt neurons.

Other cell type-specific studies have investigated the functions of cholinergic neurons in the basal forebrain or the brainstem in sleep regulation, characterized the effects of histamine release on local and distal VLPO neural transmission, and probed the role of Hcrt inputs in the neural computation of histaminergic TMN neurons[106-108]. Together, these studies paint a picture of the sleep/wake cycle that is governed by interconnected neural networks distributed throughout the brain, whose dynamic interactions determine the vigilance state of the animal[93] (Fig 3). Although certain populations of neurons such as the galanin VLPO neurons and the Hcrt LH neurons occupy “hub” positions to promote sleep or safeguard arousal, a majority of the other neurons act as “nodes” whose functions can be compensated by other neurons. Such a highly redundant network configuration may mitigate the destabilizing effects of stochastic perturbations on the arousal state as sleep leaves an animal vulnerable to predation and therefore should be tightly controlled. In the future, it is imperative to simultaneously record and manipulate several brain areas and neuronal populations before we can fully understand the neural dynamics underlying sleep/arousal.

Aggression

Aggressive behaviors are defined from an ethological perspective as ‘‘adaptations for situations involving physical conflicts or contests between members of the same species’’ to secure food, partners, or shelters[109]. Although differing in specific motor patterns, diverse species ranging from insects to primates display innate aggression, which suggests that the genetic and neural mechanisms governing aggressive behaviors are evolutionarily conserved at some primitive levels[1]. In mammals, the display of aggressive behaviors is regulated by environmental, hormonal, and experiential factors. They are more readily observed in males, with the exception that nursing females also attack intruders to defend their

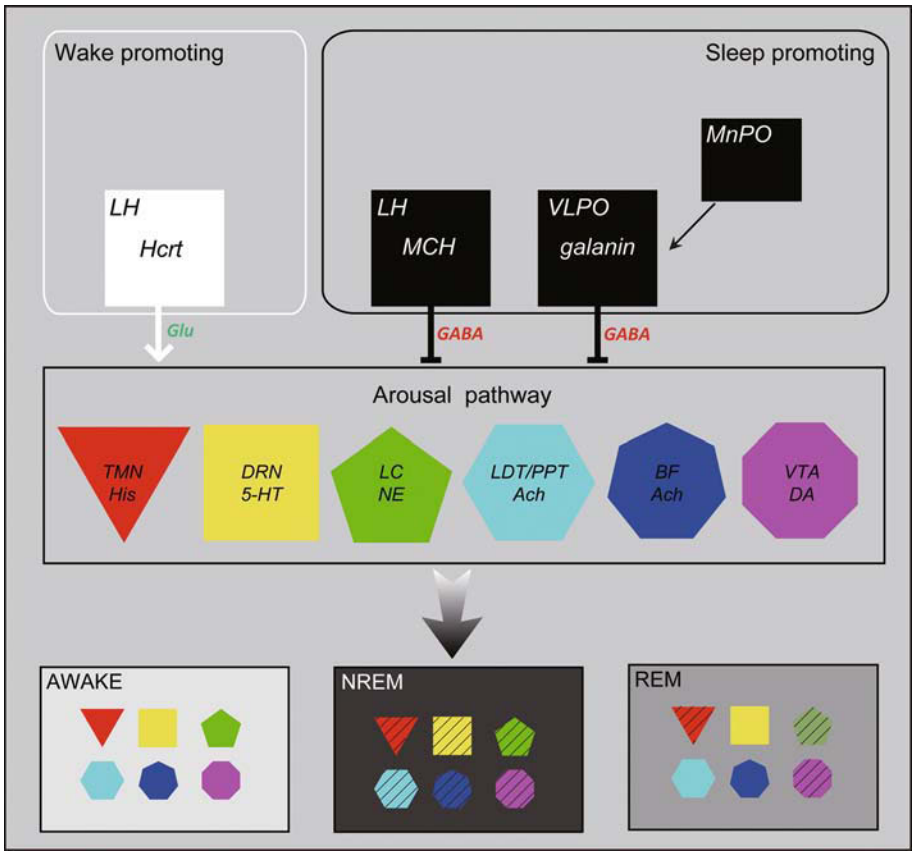


Fig. 3. The sleep/wake neural network Wakefulness-promoting neurons and sleep-promoting neurons coordinately regulate activities of the arousal neural pathway in opposite directions to produce different activation patterns and consequently different brain states. Each colored shape represents a neuronal population with the top word indicating the brain region and the bottom word indicating the neurotransmitter or genetic marker of the population. Hypocretin (Hcrt) neurons in the lateral hypothalamus (LH) use glutamate as the neurotransmitter and excite the arousal pathway. In contrast, melanin–concentrating hormone (MCH) LH neurons and galanin neurons in the ventral lateral preoptic area (VLPO) use GABA and are inhibitory. In addition, median preoptic area (MnPO) neurons project to VLPO neurons and promote sleep. Neurons within the arousal pathway are activated during the wake period, inhibited (shaded) during NREM sleep, and partially inhibited during REM sleep.

young. In laboratory settings, aggressive behaviors are typically studied by confronting singly-housed animals with unfamiliar intruders, a paradigm known as the “resident-intruder” test[110].

In the 1920s, scientists found that severing the hypothalamus from the midbrain and the brainstem blocked the display of aggressive behaviors in decorticate cats, which suggests that the hypothalamus is required for the expression of aggression[111, 112]. Subsequently, electrical stimulation of the hypothalamus was found to evoke attack behaviors in cats and many other species tested including fish, birds, rodents, monkeys, and even humans[113-117]. Plotting the placements of electrodes that elicited attacks in rats revealed a hypothalamus attack area (HAA) that does not coincide with any specific hypothalamic nucleus, but rather encompasses the ventrolateral division of the VMH (VMHvl) and a large area lateral and caudal to it[118, 119]. Moreover, the HAA partially overlaps with the hypothalamic areas that control grooming or flight behavior. Perhaps due to the limited spatial resolution of the electrical stimulation experiments that revealed the HAA, different studies found that lesions of the HAA increased, decreased, or changed the nature of aggression, which raised a question as to the exact hypothalamic sites that control aggression[119].

In the last several years, Dr. David Anderson and colleagues first achieved the difficult task of recording from hypothalamic neurons while mice were engaged in social behaviors[120, 121]. They found that a subset of VMHvl neurons reacted specifically to male-derived social cues and increased their firing rates during social interactions in a resident-intruder test. Moreover, the firing rates of these neurons were indicative of time elapsed since the last attack and predicative of the latency and intensity of future attacks. These findings suggest that a subset of VMHvl neurons transform relevant sensory information into motor commands that direct attack behaviors. Indeed, optogenetic activation of VMHvl neurons induces immediate (seconds) attacks in male mice, while chemogenetic inhibition of VMHvl neurons decreases normal aggression[121]. Interestingly, targets that are not normally perceived as threatening, such as a castrated male, a female, or even an inflated glove, precipitate attacks in animals whose VMHvl is activated, which suggests that the activation of VMHvl neurons can drive aggressive behaviors independently of sensory inputs.

To assess the identity of VMHvl neurons that regulate aggression, scientists performed double-staining of c-fos with genetic markers of the VMHvl and found that the majority (>80%) of VMHvl neurons activated during aggression also express estrogen receptor α (ERα or ESR1), a steroid hormone receptor known to regulate aggression among other social behaviors[122, 123]. Using the *ERα* genomic locus to drive cell type-specific expression of opsins, they found that activation of VMHvl ERα$^+$ but not nearby non-ERα$^+$ neurons induced attacks (seconds), while optogenetic inhibition of these neurons interrupted ongoing attacks, and decreased aggression. Consistent with the sexually dimorphic nature of aggressive behaviors, optogenetic activation of ERα$^+$ VMHvl neurons in female mice failed to evoke attacks. Thus, ERα$^+$ VMHvl neurons control aggression in a sexually dimorphic manner.

Interestingly, low-intensity activation of ERα$^+$ VMHvl neurons in males induces social investigation and mounting behavior, which escalate to attacks with increased power of stimulation, all in the same trial. Similarly, activation of ERα$^+$ VMHvl neurons in females induces social investigation and male-typical mounting behavior. These results suggest that ERα$^+$ VMHvl neurons control the outputs of several social behaviors in a scalable, dynamic, and sexually dimorphic manner. Although acute inhibition of ERα$^+$ VMHvl neurons has little effect on male mating, ablation of a closely-related population of neurons that express the progesterone receptor as well as knockdown of ERα expression in VMHvl neurons *via* RNA interference decreases male mating and female sexual receptivity along with male aggression[17]. How might a single genetically defined population of neurons be involved in the regulation of such a rich repertoire of behaviors? The VMH is interconnected with several hypothalamic nuclei also enriched in steroid hormone receptors that regulate social behaviors[124-126]. These hypothalamic nuclei receive direct synaptic inputs from the medial amygdala (MeA) and are similarly activated in aggression, mating, and other social behaviors, though the exact populations of neurons activated in each behavior may differ[121, 127, 128]. Given the neural network within which the VMH functions, it is perhaps not so surprising that manipulation of ERα$^+$ VMHvl neurons dynamically affects multiple social behaviors.

Defensive behaviors such as immobility and avoidance, which are typically elicited by a predator, are often implicitly mixed with aggressive attacks when the resident is confronted with a more combative intruder. Interestingly, studies found that the inactivation of neurons within the VMHvl decreased defensive behaviors towards a conspecific, while inactivation of neurons within the dorsomedial division of the VMH (VMHdm) decreased defensive behaviors towards a predator[129, 130]. The VMHvl and VMHdm receive topographic projections from the posterior dorsal and posterior ventral divisions of the MeA (MeApd and MeApv), respectively[128, 131, 132]. Both the VMHvl and VMHdm project to the AH and the PAG[17, 133]. Recent studies found that activation of steroidogenic factor 1-expressing neurons in the dorsomedial and central parts of the VMH (VMHdm/c) promoted immobility and avoidance behaviors, while activation of downstream AH and PAG neurons produced similar behaviors[133, 134]. Taken together, these results suggest that separate neural pathways that process conspecific (MeApd → VMHvl) or predator information (MeApv → VMHdm) converge on common downstream regions (AH and PAG) to promote defensive behaviors.

As mentioned above, VMHvl neurons receive synaptic inputs from the MeApd. Not surprisingly, optogenetic manipulation of MeApd GABAergic neurons produces

behavioral phenotypes similar to the manipulation of ERα$^+$ VMHvl neurons[135]. Interestingly, ablation of a population of MeApd neurons that express aromatase reduces both inter-male and maternal aggression, which suggests that distinct forms of aggression in both sexes may share a common MeA pathway that relays sensory information[136]. Unexpectedly, optogenetic activation of glutamatergic MeApd neurons suppresses social behaviors and promotes repetitive self-grooming, an asocial behavior. In contrast, activation of GABAergic MeApd neurons suppresses self-grooming[135]. These findings suggest that neural regulation of social *versus* non-social behaviors is separable in the MeApd along the line of inhibitory and excitatory neurons.

In summary, ERα$^+$ VMHvl neurons are both necessary and sufficient for the display of aggressive behaviors. Furthermore, the hypothalamic neural pathways that regulate aggression are embedded in a larger neural network that relays sensory information from the MeA to regulate the outputs of several innate social behaviors in a coordinated manner (Fig. 4).

Parental Care

Parental care refers to behaviors directed towards immature conspecifics that improve the survival probability of the recipient[137]. Although a variety of invertebrate and vertebrate species care for their young, parental behaviors are most developed in mammals and birds, as the offspring of these species cannot sustain themselves at birth and rely on care by others to survive. In addition to being obviously essential for survival and propagation of the species, parental behaviors have been shown to cause long-lasting and even trans-generational effects on the physiological responses and behaviors of offspring[138-140].

Our understanding of the neural mechanisms that regulate parental behaviors comes primarily from studies in rats and mice[141]. In rodents, parental behaviors consist primarily of pup-directed behaviors that include retrieval,

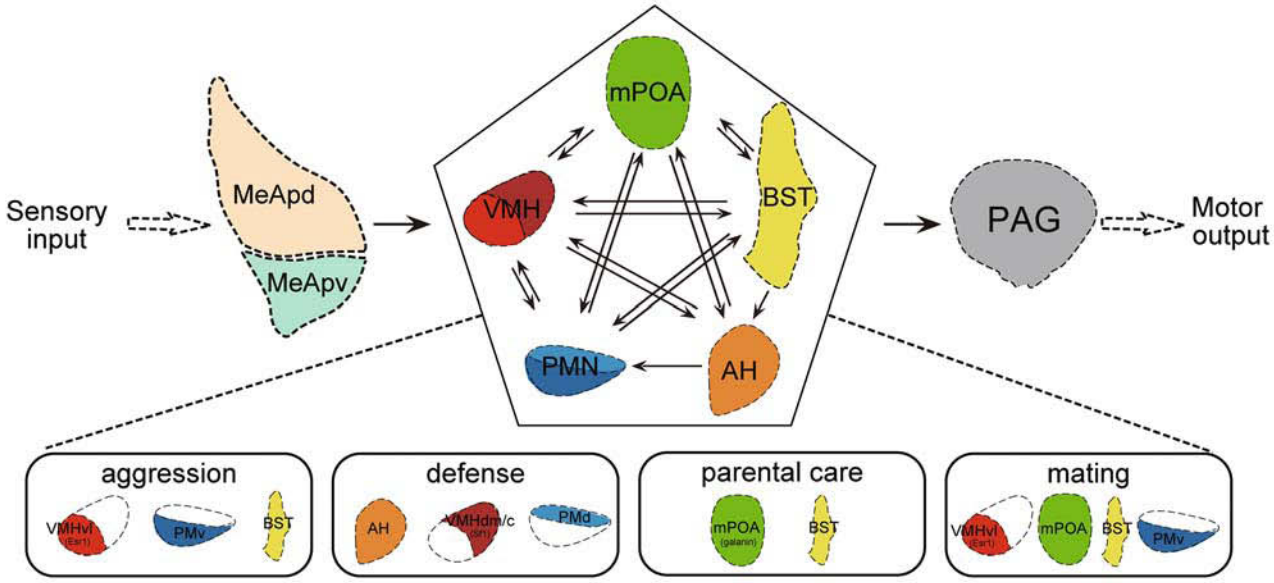


Fig. 4. The neural network governing social behaviors The hypothalamic pathways that regulate different innate behaviors are intertwined. Sensory cues emitted by pups, conspecifics, or predators are relayed *via* separate neural pathways within the posterior dorsal and posterior ventral divisions of the medial amygdala (MeApd and MeApv) to reach interconnected hypothalamic nuclei, the ventromedial hypothalamus (VMH), medial preoptic area of the hypothalamus (mPOA), premammillary nucleus (PMN), anterior hypothalamic nucleus (AH), and the forebrain structure, the bed nucleus of the stria terminalis (BST), resulting in different activation patterns of this neural network and consequently different motor outputs *via* the periaqueductal gray (PAG). Each colored shape corresponds to a nucleus of anatomically similar shape. The VMH is divided into the dorsomedial/central division (VMHdm/c) and ventromedial division (VMHvl). The PMN is divided into the dorsal nucleus (PMd) and ventral nucleus (PMv). Solid thin arrows indicate direct projections. Genetic markers in parentheses indicate specific populations of neurons within each nucleus that have been investigated with regard to regulation of the indicated social behavior.

crouching, licking, and non-pup-directed behaviors that include nest-building and maternal aggression. Similar to territorial aggression, the display of parental behaviors is also dynamically regulated by sensory, environmental, hormonal, and experiential factors. For example, virgin female rats avoid pups but can be induced to display maternal care if they are continuously exposed to pups for days or if they are treated with hormone regimens characteristic of parturient females[142, 143]. A similar behavioral switch has also been reported in males. Male rodents typically neglect or attack pups, but they show transition into parental care weeks after mating with a female, just around the birth of their own offspring[144]. These behavioral studies suggest that pup-directed cues activate antagonistic neural pathways that promote either avoidance/attack or parental care.

Classic c-fos studies revealed that the most prominent brain region activated during parental behaviors is the mPOA, a key node in the hypothalamic neural network that regulates innate social behaviors[125, 145-147]. Moreover, the extent of mPOA activation correlates with the quality of parental care[148]. Chemical or mechanical lesion of the mPOA as well as inhibition of mPOA neuronal activities abolishes parental behaviors in rats, mice, and other mammalian and avian species, which suggests that the mPOA plays an evolutionarily conserved role in regulating parental behaviors[148-152]. In addition, infusing estrogen or prolactin into the mPOA hastens the onset of maternal care in virgin female rats, which suggests that pregnancy/parturition hormones may act directly on mPOA neurons to promote maternal behaviors[153-155].

The mPOA forms reciprocal inhibitory connections with several other hypothalamic nuclei such as the AH and VMH, which regulate defensive behaviors and aggression[156-158]. Therefore, activation of the mPOA inhibits defensive/aggressive behaviors. In contrast, lesion of the AH/VMH promotes parental care[159]. Both the POA and AH/VMH receive inputs from the MeA[128, 132]. Immunostaining of c-fos suggests that the MeA, AH, and VMH are more activated in virgin males than in fathers, while the mPOA is less activated. This suggests that pup-derived cues are transmitted through the MeA in virgin males to inhibit the mPOA and activate the AH/VMH, thereby suppressing paternal behaviors and promoting avoidance/aggression behaviors[160]. Indeed, lesion of the MeA, surgical removal of the vomeronasal organ (VNO) that detects pheromone cues, or deletion of *Trpc2*, an ion channel essential for VNO signaling, decreases pup attack behaviors and promotes parental care in males[127, 159, 160].

In addition, mPOA neurons project to the ventral tegmental area (VTA), a major dopaminergic area essential for reward and reinforcement learning[161]. Pup and maternal behaviors increase dopamine release in the ventral striatum[162-165]. In addition, lesion or inhibition of the VTA as well as pharmacological blockade of dopamine receptor signaling in the ventral striatum disrupts components of maternal behaviors, which suggests that dopamine signaling is important for the display of parental care[166-169]. Indeed, severing mPOA projections to the VTA interrupts parental behaviors[167].

A recent study by Dr. Catherine Dulac's group explored for the first time the role of genetically-defined populations of mPOA neurons in behavioral switching and parental care in mice[127]. They found that ~40% of mPOA neurons activated during parental behaviors express the neuropeptide galanin. Moreover, galanin mPOA neurons are activated to a much greater extent during parental behaviors than during other social behaviors such as male mating. In correlation with the activation profile, ablation of galanin mPOA neurons causes a mild deficit in male mating but impairs all components of parental care and renders the targeted animal more prone to ignore or attack pups regardless of its sex and reproductive status. In contrast, ablation of a nearby population of mPOA neurons that express tyrosine hydroxylase has no effect on either behavior. These results suggest that galanin mPOA neurons play an essential role in parental behaviors, especially in suppressing avoidance and aggressive behaviors towards pups.

Consistent with these results, optogenetic activation of galanin mPOA neurons switches virgin male mice from pup avoidance/attack to pup grooming. Interestingly, inter-male aggression is also mildly inhibited, which raises the possibility that galanin mPOA neurons play a general role in suppressing aggressive behaviors. It remains to be determined whether the effects of galanin neurons on male mating, aggression, and pup-grooming are mediated by different subpopulations and/or *via* collateral projections to different downstream targets. Moreover, as activation of galanin mPOA neurons fails to evoke other components of

maternal behaviors such as retrieval and nest-building, it is likely that other unidentified populations of mPOA neurons play a more important role in initiating these behaviors.

In summary, galanin mPOA neurons suppress pup avoidance/attack behaviors and promote pup grooming. Similar to the VMHvl pathway for aggression, the mPOA pathway regulating parental behaviors is also embedded in a large neural network that controls many social behaviors (Fig. 4). In the future, it will be important to disentangle these neural pathways *via* more refined partitioning of the hypothalamic circuitry[170, 171].

Emerging Themes on Neural Control of Innate Behaviors

Clearly, innate behaviors are not governed by regionally defined "drive centers" as once thought. Rather, these behaviors are controlled by highly complex and interconnected neural networks distributed within and outside of the hypothalamus[46]. For example, the VMH, once thought of as the "satiety center", has been shown to regulate mating, aggression, and defensive behaviors. Similarly, the LH, the "hunger center", regulates sleep and other motivated behaviors in addition to feeding. A single hypothalamic nucleus likely regulates diverse behaviors by harboring different populations of neurons that project to distinct downstream regions and/or by dynamic neural interactions with other nuclei[122, 133]. Much research still needs to be done before we can truly understand these processes. Nevertheless, current studies have revealed several common features shared by neural networks that govern different innate behaviors. In the last section, we discuss these features in the hope that it will enlighten us on the general neural mechanisms underlying complex behaviors.

Hierarchy

Current findings suggest that various neural networks that govern different innate behaviors, similar to many other complex biological networks studied, are hierarchically organized. One key feature of this hierarchical organization is the existence of "hub" neurons that are extensively connected. Arcuate Agrp neurons form one such hub in the neural network controlling feeding. Agrp neurons integrate sensory, hormonal, and synaptic inputs and project to diverse downstream targets to coordinate both short-term and long-term feeding. Not surprisingly, destruction of Agrp neurons produces the most dramatic phenotype, aphagia. Similarly, LH Hcrt neurons form a hub in the neural network governing arousal and MeA neurons form a hub for social behaviors. Future tracing and functional studies are needed to map in detail the connections and interactions among different hypothalamic neurons. In doing so, we may be able to identify other hub neurons based on their connectivity and functional characteristics.

Redundancy

The neural network governing feeding or arousal is highly redundant. This is reflected in the fact that while activation of a diverse array of neurons is sufficient to elicit feeding or promote arousal, inactivation or destruction of many of these neurons has little effect, which suggests functional redundancy. One striking example of this redundancy is that a triple lesion of three populations of neurons that independently promote arousal had minimal effects on the sleep/wake cycle. Likewise, the aphagia phenotype caused by the ablation of Agrp neurons can be permanently rescued by infusing GABA into the PBN for a short period, which suggests that the adult feeding circuit has the capacity to compensate for the loss of the "quintessential" Agrp neurons[60].

Functional redundancy in the neural network governing feeding or arousal is implemented *via* parallel neural pathways that transmit similar information to support the behavior. LH Hcrt neurons project to many downstream neurons that independently promote arousal. Likewise, multiple subpopulations of Agrp neurons project in parallel to different downstream targets, many of which can support feeding. Such a "one-to-many" network configuration mitigates noise and ensures the robustness and stability of feeding and arousal, both of which are essential for the survival of an individual. However, it is also possible that these redundant "parallel" pathways confer distinct selective advantages under specific conditions in natural environments. Such subtle differences in function may be difficult to discriminate in a laboratory setting with standardized behavioral paradigms. Nevertheless, it is important in the future to consider such a possibility and diversify behavioral paradigms to match more closely the ethological background of the animal studied.

Antagonist Control

Another interesting feature observed in neural networks governing different innate behaviors is that neighboring populations of neurons antagonistically control a given behavior. For example, Agrp neurons in the arcuate nucleus drive hunger and promote feeding while neighboring POMC neurons inhibit feeding. In a similar manner, Hcrt neurons in the LH promote arousal while adjacent MCH neurons promote sleep, and GABAergic neurons in the MeApd promote social behaviors while glutamatergic MeApd neurons inhibit social behaviors and promote repetitive self-grooming, an asocial behavior. These pairs of neuronal populations typically receive similar inputs and project to many of the same targets, but they exert opposite effects *via* different neurotransmitters (GABA or glutamate) or antagonistic neuropeptides (Agrp *versus* POMC).

Antagonistic control is in fact a prevalent theme in physiology. Sympathetic and parasympathetic branches of the autonomic nervous system innervate all vital organs in parallel, but they control physiological parameters such as heart rate and blood pressure in opposite directions *via* the release of different neurotransmitters, thereby maintaining these parameters within a homeostatic and narrow range. Similarly, the pancreas releases antagonistic hormones (insulin *versus* glucagon) to maintain blood glucose levels within a physiological range, and antagonistic pairs of skeletal muscles (biceps *versus* triceps) ensure postural stability and fine control of motor outputs. It is likely that these antagonistic neural pathways serve similar functions to ensure homeostatic and fine control of innate behaviors. The existence of antagonistic neural pathways may also reflect fundamental principles in the genetic blueprint from which these neural circuits are built. It is tempting to speculate that antagonistic control is a general feature that can be extended to the neural regulation of other complex functions such as reward and motivation[172].

Concluding Remarks

This is an exciting time to study the hypothalamic control of innate behaviors. The availability of genetic and optogenetic tools has made probing the functions of discrete populations of neurons a standard approach that approximates patch clamping or molecular cloning. As we summarize published work, new discoveries are being made each day with these tools. In addition, new generations of tools are being developed that will afford better sensitivity, more power, and will allow finer dissection as well as simultaneous and multiplex manipulation of neural populations. Although there is still a long way to go, we can see the light in understanding the neural circuits governing essential behaviors and perhaps general computational principles in the neural control of behaviors. Such an understanding will serve as a foundation for us to probe how malfunctions of these circuits lead to the pathogenesis of devastating diseases such as insomnia and anorexia.

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