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Different Neuronal Phenotypes in the Lateral Hypothalamus and Their Role in Sleep and Wakefulness

Dmitry Gerashchenko* and Priyattam J. Shiromani

West Roxbury VA Medical Center and Harvard Medical School, 1400 VFW Parkway, West Roxbury, MA 02132

Abstract

The sleep disorder narcolepsy is now linked with a loss of neurons containing the neuropeptide hypocretin (also known as orexin). The hypocretin neurons are located exclusively in the lateral hypothalamus, a brain region that has been implicated in arousal based on observations made by von Economo during the viral encephalitic epidemic of 1916–1926. There are other neuronal phenotypes located in the lateral hypothalamus that are distinct and separate from the hypocretin neurons. Here the authors identify these neurons based on peptides and neurotransmitters that they express and review roles of these neurons in sleep. Given the heterogeneity of the neuronal phenotypes in the lateral hypothalamus, it is likely that hypocretin neurons, as well as other types of neurons in the lateral hypothalamus, influence sleep and provide state-dependent regulation of physiological functions.

Index Entries: Sleep; lateral hypothalamus; neuropeptide; neurotransmitter; hypocretin-saporin; lesions.

Introduction

The lateral hypothalamus (LH) has long been associated with feeding and energy metabolism. However, recently it was also implicated in regulating sleep as a result of the discovery that the sleep disorder narcolepsy was associated with the loss of neurons containing the peptide hypocretin *(1–3).* The hypocretin-containing neurons are located only in the LH from where they project to diverse areas in the brain and spinal cord, especially innervating areas responsible for arousal *(4–6).* A number of recent reviews have discussed how the HCRT neurons might regulate sleep through interaction with the brain areas responsible for arousal *(7,8).* However, the LH is a complex anatomical structure

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*Author to whom all correspondence and reprint requests should be addressed. E-mail: dmitri_gerachtchenko@ hms.harvard.edu

containing many neuronal populations that differ from each other in the neurotransmitter type and afferent and efferent connections with other brain areas *(9).* Neurons of one particular type do not usually assemble in clusters but are rather scattered throughout the LH *(9).* In this review, the authors advance the hypothesis that the HCRT neurons and other neurons within the LH regulate sleep through their mutual interactions within the LH, and via projections to other brain regions. This study starts by reviewing the LH lesion data that first suggested the importance of this region in sleep regulation. The various phenotypes of neurons present in the LH are identified and the evidence of their role in sleep behavior reviewed. Finally, the authors demonstrate how LH neurons can regulate sleep through projections to other brain areas implicated in sleep and wakefulness.

Sleep After LH Lesions

The first evidence that the posterior and lateral hypothalamus were important for arousal was presented by von Economo *(10),* who published his report on inflammatory lesions produced by encephalitis lethargica. Nauta tested von Economo's observations by making bilateral incisions at different levels of the hypothalamus *(11)* and concluded that the caudal hypothalamic region was indeed important for maintaining the waking state during the absence of external stimuli ("waking center"). More importantly, he concluded that the lateral hypothalamic area was more important for waking compared to the inner areas of the hypothalamus *(11).* Similar conclusions were reached by other studies conducted at that time *(12,13).* In these early studies, the somnolence was based on behavioral observations since the EEG was not recorded and REM sleep had not yet been discovered. McGinty *(14)* recorded the EEG and found hypersomnolence following large electrolitic lesions surpassing the lateral and posterior hypothalamus. Shoham and Teitelbaum *(15)* made extensive electrolytic lesions of the LH and observed what we now know to be sleep-onset REM sleep periods. The shortcoming of the early electrolytic lesions and incision studies *(11–15)* is that they destroy fibers passing through the LH, which makes it difficult to conclude whether loss of neurons of the LH or loss of projections from the ascending reticular activating system in the brainstem were responsible for the hypersomnolence. An important fiber system, the medial forebrain bundle (MFB) *(16),* traverses the LH, and therefore, is destroyed by incisions or electrolytic lesions within the LH.

Later studies utilized excitotoxic aminoacids *(17,18)* since the advantage of this method destroys neurons and spares fibers of passage *(19).* Excitotoxic lesions within the lateral and posterior hypothalamus produced transient somnolence in some studies *(17),* but excitotoxic as well as electrolytic lesions failed to produce sleepiness in other studies *(20–22).* Such inconsistent effects of the lateral hypothalamic lesions on sleep may derive from the neuronal complexity of the LH, differences in the lesion size, and non-selective character of the electrolytic or exitotoxic lesions.

Due to the heterogenous organization of the LH *(9),* it is to be expected that only vast lateral hypothalamic lesions would destroy sufficient number of neurons to produce significant changes in the sleep–wake behavior, whereas smaller lesions in the LH might not significantly change sleep amounts. The electrolytic or exitotoxic lesions performed in previous studies were non-specific because they indiscriminately destroyed neurons. A loss of one of the neuronal populations may be associated with sleepiness, whereas a loss of other may not. To produce more specific lesions, the authors *(23)* created a new neurotoxin by conjugating the ribosomal inactivating protein, saporin (SAP) *(24),* to the hypocretin receptor-binding ligand, hypocretin-2. Extensive studies have shown that when SAP is coupled with antibodies or ligands that recognize cell-surface antigens or receptors, the conjugate binding is specific and initiates apoptosis in

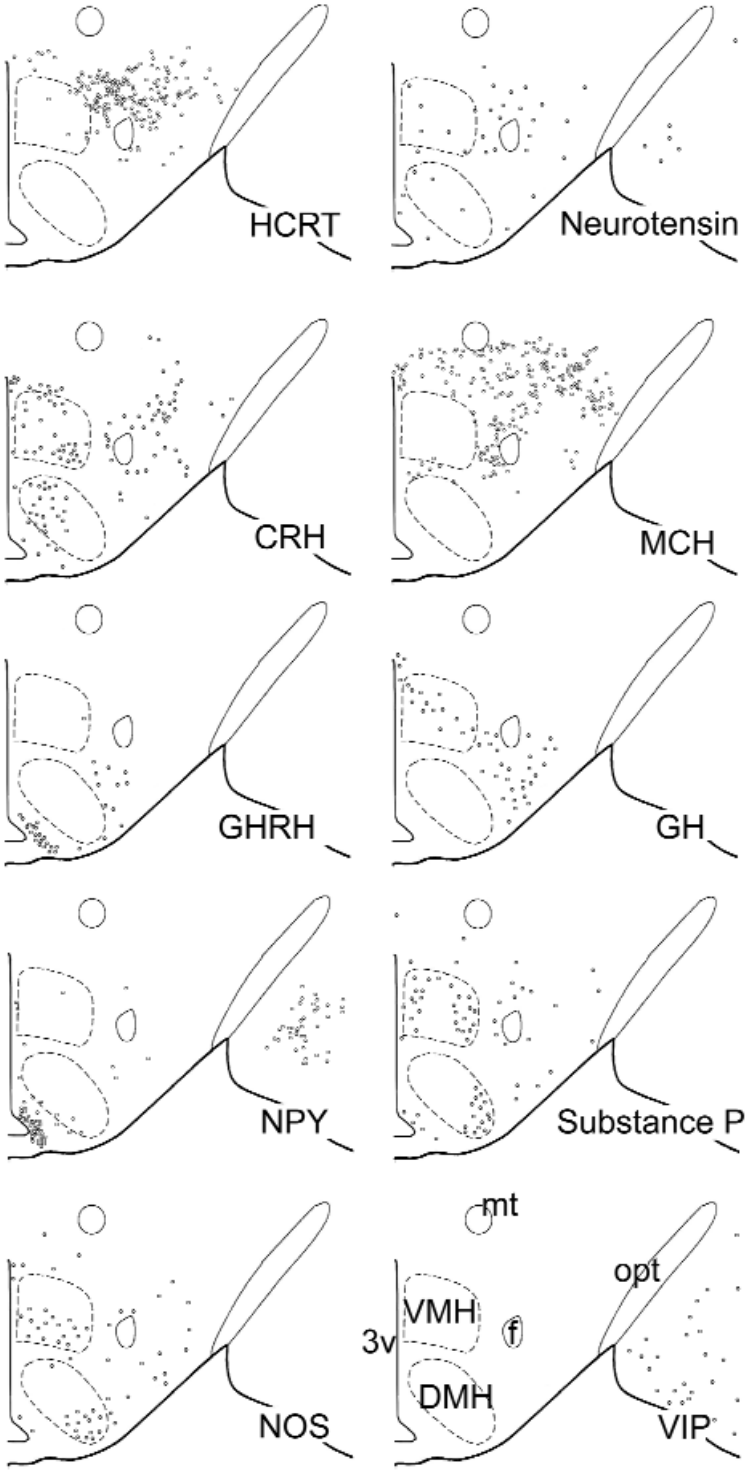

Fig. 1. Localization of some phenotypes of LH neurons implicated in the regulation of sleep/wakefulness. The figure represents a schematic distribution of some known neuronal types in the LH; the actual number of neurons may be different from those shown on the figure. Hypocretin (HCRT), melanin-concentrating hormone (MCH), and neuropeptide Y (NPY) neurons were plotted based on the immunohistochemical staining performed in the authors' laboratory. Distribution of other neurons was adapted from the following articles: neurotensin *(35);* corticotropin-releasing hormone (CRH) *(36);* growth-hormone-releasing hormone (GHRH) *(37);* growth hormone (GH) *(38);* substance P *(39);* neuronal nitric oxide synthase (NOS) *(40);* vasoactive intestinal polypeptide (VIP) *(41).*

targeted cells *(25,26).* The hypocretin2-SAP toxin was therefore designed to selectively destroy cells expressing hypocretin receptor *(23).* Hypocretin receptor-containing cells were chosen as a target because of importance of hypocretin in the sleep–wake regulation *(7).*

Administration of hypocretin2-SAP into the LH produced narcoleptic-like symptoms in both albino (Sprague-Dawley) *(23)* and pigmented rats (Long-Evans) *(27).* The symptoms included sleep fragmentation, excessive sleepiness, increase in REM sleep, and sleep-onset REM sleep periods. At present, narcoleptic-like behavior has been shown to be associated with the dysfunction of hypocretin system in humans *(2,3,28,29),* dogs *(1),* and mice *(30,31).* Therefore, the authors looked for a relationship between severity of narcoleptic symptoms and number of remaining hypocretin neurons, and found that the hypersomnia and sleep-onset REM sleep periods were negatively correlated with the loss of hypocretin neurons *(23).* This finding demonstrates a connection between a defect in the LH and narcoleptic-like sleep in rats. However, this result does not necessarily mean that hypocretin neurons are the only phenotype that is damaged in human narcoleptic patients. New evidence is beginning to suggest that there might be other neurons lost in narcolepsy. For instance, in about 10% of human narcoleptics CSF levels of hypocretin are similar to normal individuals *(32).* Moreover, in human narcolepsy there is considerable variability in the degree of severity of the symptoms, and a clinical diagnosis can be made if the patient manifests unexplained sleepiness, sleep-onset REM sleep periods, sleep paralysis, and hypnagogic hallucinations without any cataplexy *(29).* Such variations in symptom expression could be related to a dysfunction of other non-hypocretin-containing cells in the LH.

Neuronal Phenotypes in the LH

The LH is a complex anatomical structure in which neurons of one particular type do not usually assemble in clusters but are rather scattered throughout the LH *(9).* The LH contains a variety of peptidergic neurons, but catecholamine, acetylcholine, dopamine- or serotonin-containing neurons are not present in the LH. However, fibers containing these classical neurotransmitters are distributed robustly in the LH *(33,34).*

Hypocretin (Orexin)

The hypocretins, also known as orexins, are recently discovered peptides with a discrete localization in the LH *(4–6).* A single gene encodes hypocretin, which is cleaved by proteolytic processing into two smaller peptides, hypocretin-1 (orexin A) and hypocretin-2 (orexin B) *(4,6).* Hypocretin-containing neurons also co-express glutamate *(42),* dynorphin *(43),* secretogranin II *(43),* and neuronal activity-regulated pentraxin *(44).* These neurons project to the entire brain and spinal cord, providing especially heavy innervation to forebrain and brainstem neuronal populations implicated in wakefulness *(5).* Hypocretin has been implicated in the human sleep disorder, narcolepsy based on the findings that canines with narcolepsy possess a mutation in the hypocretin-2 receptor *(1).* Transgenic mice with a deletion of the hypocretin gene *(30)* or mice with a gene-specific ablation of the hypocretin neurons *(31)* exhibit symptoms of narcolepsy. In human narcolepsy, there is a massive loss of hypocretin neurons *(2,3),* and consistent with such a neuronal loss, levels of hypocretin-1 are undetectable in the cerebrospinal fluid of human narcoleptic patients *(28).*

Hypocretins have mainly excitatory effects on neuronal activity in all major brain areas implicated in arousal. Hypocretins strongly excite serotonergic neurons in the dorsal raphe *(45),* noradrenergic neurons of the locus coeruleus *(46),* histaminergic neurons in the tuberomammillary nucleus *(47),* cholinergic neurons in the basal forebrain *(48),* both cholinergic and noncholinergic neurones in the laterodorsal tegmental nucleus (LDT) *(49),* as well as thalamocortical projecting neurons in

Table 1
Electrophysiological Characteristics of the Lateral Hypothalamic Neurons

Type of neurons	Number of neurons and percent from total number of recorded neurons			
Wake/REM-related	56 (53%)	6 (27%)	26 (43%)	28 (32%)
Wake-related	40 (38%)	3 (14%)	31 (52%)	31 (36%)
REM-related	10 (9%)	11 (50%)	3 (5%)	19 (22%)
State-indifferent				7 (8%)

Recordings *(56)* were done at the level of the tuberomammillary nucleus in the posterior lateral hypothalamus. All other recordings were done in the perifornical region of the lateral hypothalamus *(53–55).*

the centromedial nucleus and rhomboid nuclei of the thalamus *(50).* In the ventral tegmental area (VTA), hypocretins excite all GABAergic cells and a subset of dopaminergic neurons *(51).* Both hypocretin-1 and -2 evoke a strong excitatory response from hypocretin neurons, but it is mediated indirectly by local excitatory glutamatergic neurons in synaptic contact with hypocretin neurons *(52).*

A few studies have examined the activity of neurons in the perifornical as well as posterior lateral hypothalamic areas and found that most neurons in these areas are active during wakefulness and/or REM sleep (*see* Table 1) *(53–56).* Unfortunately, these studies are limited due to the fact that the phenotype of the neurons cannot be determined. A recent microdialysis study where HCRT levels were measured in relationship to sleep–wake states suggests that HCRT neurons behave as most other brain neurons in that they have highest discharge rate during wakefulness and REM sleep *(57).*

Neurotensin

Neurotensin is an endogenous polypeptide that exerts potent effects in the central nervous system (CNS) including hypothermia, antinociception, modulation of dopamine neurotransmission, and stimulation of anterior pituitary hormone secretion *(58).* Microinjections of neurotensin into the VTA increase locomotion that is associated with an increase in extracellular dopamine concentrations *(59).* Since a large population of neurotensin projections to the VTA arises from the rostral part of the LH *(60),* behavioral hyperactivity associated with the activation of the mesolimbic dopamine system may be produced by excitation of neurotensinergic neurons in the LH. Neurotensin-containing LH neurons may also have an effect on sleep/wakefulness via innervation of the basal forebrain *(61).* Neurotensinergic neurons comprise only a minor population of all LH neurons that send projections to the basal forebrain *(61),* but they appear to be unique in their capacity to modulate cholinergic basal forebrain neurons in a highly selective manner *(62).* Microinjection of neurotensin into the basal forebrain of freely moving rats produces a dose-dependent decrease in non-REM sleep and increase in a quiet wakefulness with low EMG and high theta activity *(62).* There is also evidence for an involvement of the serotonergic system in mediating some arousal effects of neurotensin *(63),* but these effects are probably mediated by neurotensin-containing neurons located outside of the LH, such as those in the lateral parabrachial nucleus *(64).* LH neurotensinergic input to the dorsal raphe nucleus has not yet been demonstrated.

Overall, neurotensin-containing neurons in the LH are in a position to induce both active wakefulness via projections to the dopamine neurons in the VTA and quiet wakefulness via input to the cholinergic neurons in the basal

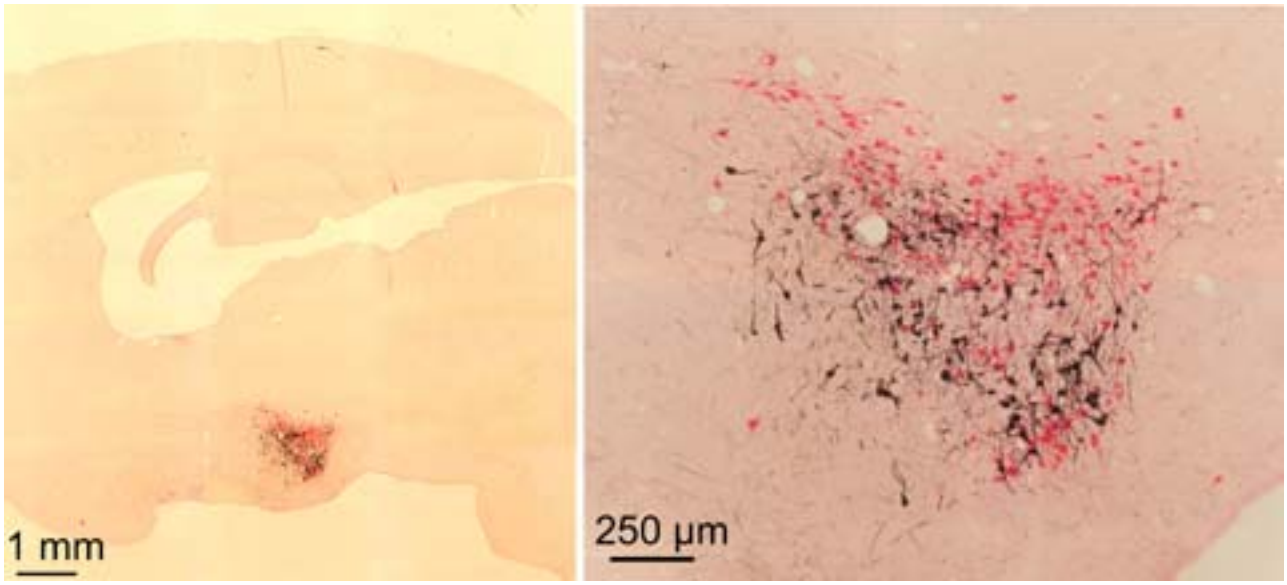

Fig. 2. Distribution of hypocretin-containing neurons (black) and melanin-concentrating hormone-containing neurons (red) in the rat lateral hypothalamus. Immunohistochemical staining was performed in 40-µ*m* brain section cut in a sagittal plane. Close up of the lateral hypothalamus is shown on the right. LV, lateral ventricle; opt, optic tract.

forebrain. Lesion of these neurons could decrease wakefulness and lead to hypersomnia.

Corticotropin-Releasing Hormone (CRH)

CRH is one of the most widely distributed neuropeptides in the CNS. High expression of the mRNA encoding CRH has been demonstrated in the LH *(65).* CRH may mediate stressor-induced increase in waking as well as contribute to the regulation of spontaneous waking in the absence of stressors *(66).* Thus, loss of CRH neurons could produce hypersomnia. A potential mechanism by which CRH regulates waking is via action on the immunomodulatory cytokine interleukin-1 *(66).* Hypocretin-1 was shown to stimulate CRH and NPY release from hypothalamic explants in vitro and ACTH and corticosterone release in vivo *(67,68).*

CRH mRNA-containing neurons in the LH are activated by cellular dehydration and provide a significant input to the pontine parabrachial nucleus *(36).* Through this pathway, CRH neurons in the LH could possibly play a role in the integration of waking mechanisms with the central processing of autonomic information.

Melanin-Concentrating Hormone (MCH)

MCH-containing neurons are located in close proximity to cells containing hypocretin *(5),* but MCH and hypocretin do not colocalize *(5,43).* At present, MCH and hypocretin neurons are the only known phenotypes that are localized almost exclusively within the lateral hypothalamus (*see* Fig. 2). MCH neurons may be separated into functionally different subgroups on the basis of their projections, chemical phenotype, and time of birth *(69).* MCH neurons projecting to the cerebral cortex or spinal cord are born on a different time schedule and differentially express the NK3 receptor *(69).* Some MCH neurons project to both the cerebral cortex and the median eminence and

posterior pituitary *(70).* Based on this observation, it has been suggested that this group of neuroendocrine MCH neurons could directly modulate cortical activity and thus play a role in arousal in correlation with specific goal-oriented behaviors such as feeding or reproduction *(70).*

Projections to the locus coeruleus and dorsal raphe nucleus, as well as diffuse projections to the cerebral cortex suggest involvement of MCH-containing neurons in arousal. However, recent genetic and physiologic studies provide little evidence to support such a role. For instance, amounts of time spent in nonREM sleep, REM sleep, and wakefulness are not different between MCH-overexpressing and wild-type mice *(71).* Moreover, intracerebroventricularly infused MCH does not affect spontaneous motor activity during either the light or the dark cycle *(72),* and does not reduce non-REM sleep amounts compared to vehicle controls *(73).*

MCH-expressing neurons contain the product of alternate splicing of the MCH, neuropeptide glutamic acid-isoleucineamide (NEI). NEI bears epitopes recognized by antibodies directed against α-MSH *(74)* and has a sequence similarity to α-MSH at the C terminus (Pro-Ile-NH2 as compared to Pro-Val-NH2 of α-MSH). The immunoreactivity of NEI to α-MSH antibodies along with the structural similarities between NEI and α-MSH suggest that NEI may exhibit some of its physiological effects via melanocortin receptors. Activation of melanocortin receptors may have some effects on sleep–wakefulness. For example, the 4–9 fragment of ACTH, that is identical to melanocyte stimulating hormone, MSH-(4–9), increases waking during the first 3 h of the sleep period *(75).*

MCH-deficient mice that have a deletion of the entire coding region of the MCH gene do not express both functional MCH and NEI *(76).* These mice are lean and hypophagic and show an increase in metabolic rate *(76).* Unfortunately, distribution of sleep–wake states in these mice has not yet been determined.

Growth Hormone-Releasing Hormone (GHRH) and Growth Hormone (GH)

The majority of the GHRH-immunoreactive cell bodies are found in the arcuate nucleus and the medial perifornical region of the LH *(37).* GHRH regulates secretion of the pituitary GH *(77)* as well as expression of the brain GH messenger RNA that is predominantly detected in the LH *(78).* Sleep may be promoted by GHRHergic neurons residing outside of the arcuate nucleus, in the neurons in the periventromedial area of the LH and/or in the paraventricular nucleus, which regulate activity of the preoptic basal forebrain neurons *(79).* In the extra-arcuate neurons, GHRH displays a diurnal variation with low levels in the morning, gradual increases in the afternoon, and decreases at night *(80).*

Both ICV *(81)* and systemic *(82)* GHRH administration induces non-REM sleep, whereas GH administration increases REM sleep *(83).* Dwarf (dw/dw) rats with a defective GHRH signaling mechanism in the pituitary have a reduction in REM sleep during the light period and non-REM sleep during both the light and dark periods *(84),* whereas transgenic mice producing excess rat GH have moderate increase in the time spent in spontaneous non-REM sleep and a large increase in the time spent in REM sleep during the light period *(85).*

GH secretion is altered in patients with the sleep disorder, narcolepsy. Narcoleptics were found to secrete approx 50% of their total production of GH during the daytime, whereas controls secreted only 25% of GH during the day *(86).* It was proposed that hypocretin deficiency disrupted the circadian distribution of hypothalamic GHRH release in narcoleptic patients to simultaneously cause daytime GH release and promote their propensity to fall asleep during the day *(86).*

The balance between the GHRH and CRH is thought to be critical in sleep regulation *(87).* CRH reduces delta EEG activity in rats *(88),* nonREM sleep amounts, and the nocturnal GH surge in humans *(89).* GHRH has opposite effects, such as a promotion of non-REM sleep

and the sleep-associated GH surge in animals *(81)* and humans *(90).* During both aging and acute depression, the GHRH:CRH ratio is changed in favor of CRH, resulting in disturbances in sleep and endocrine function *(87).*

Neuropeptide Y (NPY)

NPY is one of the most abundant peptides in the mammalian nervous system *(91).* It is highly expressed in the hypothalamus of humans and rats *(91).* The largest group of NPY neurons is localized within the arcuate nucleus of the hypothalamus *(91),* whereas few NPY-containing neurons are located in the LH *(33,92).* The LH is heavily innervated by a dense network of NPY-immunoreactive fibers *(93).* Sleep-promoting properties of NPY have been demonstrated in rats *(94)* and humans *(95).* The effects induced by NPY, peptide YY, and pancreatic polypeptide (they all belong to the NPY hormone family) are mediated by at least six different receptor subtypes. They belong to the large superfamily of G protein-coupled receptors and are denoted as the Y1-, Y2-, Y3-, Y4-,Y5- and Y6-receptors. It is likely that at least some NPY effects on sleep can be mediated via Y1 and Y2 receptors, because injections of NPY into the lateral or posterior hypothalamus increased the sedative effect of a GABA agonist, and this effect was absent in Y1 knockout mice *(96)* and enhanced in Y2 knockout mice *(97).* Sleep has not been recorded in these knockout mice.

Delta Sleep-Inducing Peptide (DSIP) and Substance P (SP)

DSIP is a nonapeptide that is synthesized in the hypothalamus and targets multiple sites in the brain *(98).* DSIP-positive neurons have a rather widespread distribution in the brain *(99,100).* They are mostly scattered throughout several brain areas, but are grouped into clusters in the LH *(99).* DSIP promotes sleep in rabbits, mice, rats, cats, and humans *(100).* NonREM sleep induced by DSIP is characterized by an increase in the delta rhythm of the EEG. DSIP may exert its action by blocking the excitatory effect of glutamate on LH neurons *(101).* Since glutamate stimulates HCRT neurons, DSIP may block the glutamate-induced stimulation, thereby promoting sleep.

Some effects of DSIP can be mediated by substance P (SP), since administration of DSIP significantly increases the content of SP in the hypothalamus *(102).* SP is significantly lower in narcoleptic patients *(103)* and higher in untreated patients with the sleep apnea syndrome *(104).* Both SP and DSIP normalize sleep in chronically stressed rats with hyposomnia *(105).*

Nitric Oxide (NO)

The LH contains population of neurons expressing nNOS, and these neurons are not colocalized with hypocretin-containing neurons *(106).* The involvement of nitric oxide (NO) in sleep–wake regulation has been shown in a number of studies *(107–110).* ICV injection of NO donors, such as 3-morpholinosydnonimine and *S*-nitroso-*N*-acetylpenicillamine, increases time spent in nonREM sleep as well as nonREM sleep intensity in rats *(107).* Production of NO is higher during wakefulness than during non-REM sleep in the cerebral cortex *(110)* and thalamus *(109).* NO is produced by nitric oxide synthase *(NOS),* which has several isoforms, such as an inducible NOS (type II), an endothelial NOS (type III), and a neuronal NOS (nNOS, type I). All of these isoforms may be involved in sleep–wake regulation, but a specific role of each isoform is not clear *(108).*

Vasoactive Intestinal Peptide (VIP)

When administered intracerebroventricularly, VIP promotes REM sleep in the rat *(111),* cat *(112)* and rabbit *(113),* whereas VIP antagonist decreases REM sleep *(114).* The effect of VIP on REM sleep may be due to its action in the dorsal raphe nucleus *(115)* and pontine reticular formation *(116).* Moderate to low den-

sity of VIP-like immunoreactive fibers is found in the LH, mainly in the area located dorsally to the fornix *(41).* Neurons containing VIP are present in the neocortex, in the suprachiasmatic nucleus of the hypothalamus, and in the central gray of the midbrain *(41).* Some VIP-like immunoreactive neurons are found in the LH in the chick *(117),* little brown bat *(118),* and cat *(119).* VIP-containing neurons are not found in the LH of rats and mice *(41),* except for a few neurons in the area of LH adjacent to the supramammillary region *(120).* VIP-containing neurons of the supramammillary region and the adjacent LH innervate the central amygdaloid nucleus *(120),* but their projections to the sleep/wake-related structures, including brainstem structures, have not been demonstrated. Additional studies are needed to identify whether these VIP-like immunoreactive neurons can regulate REM sleep through input to the dorsal raphe nucleus or pontine reticular formation.

Prolactin

Prolactin is a 23 kD protein secreted by lactotrophic cells of the anterior pituitary. At present, prolactin has been linked to more than 300 separate actions due to the quasi-ubiquitous distribution of its receptor *(121).* Prolactin-like immunoreactive cell bodies have been found in the LH area surrounding the fornix, especially dorsolateral to it *(122).* However, later studies revealed that an ovine prolactin antiserum used to detect prolactin actually recognized an epitope carried by the 104–109 fragment of the preprohypocretin *(123).* Thus, there are probably no prolactin-containing cells in the LH, whereas prolactin receptor is distributed within the hypothalamic nuclei *(124).*

Among other functions *(121),* prolactin has been linked to the regulation of REM sleep *(125).* Subcutaneous or intracerebroventricular administration of prolactin decreases REM sleep duration when injected during the dark period and increases it when injected during the light period *(126).* Microinjection of prolactin into the rat dorsolateral hypothalamus has similar effect on REM sleep duration and has no effect on non-REM sleep duration irrespective of injection time *(127).* It has been suggested that hypothalamic prolactin may be involved in the mediation of the REM sleep-promoting activity of VIP *(125).* Role of prolactin in the regulation of REM sleep has been reviewed elsewhere *(125).*

Glutamate

Glutamate is the principal excitatory neurotransmitter in brain. There are three families of ionotropic receptors named after their preferred agonists (*N*-methyl-D-aspartate [NMDA], α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA] and kainate), and three groups of metabotropic, G protein-coupled glutamate receptors *(128).* The recently discovered vesicular glutamate transporters (VGLUT) have been used as definitive markers of glutamatergic neurons. VGLUT2 mRNA is abundantly expressed throughout the LH region, and scattered VGLUT1 mRNA-positive cells are also observed in the LH *(129).* Hypocretin may increase glutamate transmission in the LH leading to increases in alertness or arousal *(52).* It is, therefore, very likely that glutamate in the LH increases wakefulness.

γ-Aminobutyric Acid (GABA)

GABA is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS). It is believed to be involved in the induction and the maintenance of nonREM sleep through interactions with $GABA_A$ receptor subtypes *(130).* A large number of drugs commonly used in medical practice (barbiturates, neuroactive steroids, benzodiazepines, and nonbenzodiazepine hypnotics) affect sleep via modulation of $GABA_A$ receptor functioning *(130).* Messenger RNA for the glutamic acid decarboxylase (GAD), the GABA biosynthetic enzyme, is widely expressed within the LH *(131).* Recent studies demonstrate the existence of GABAergic interneuronal synapses in

in vitro preparations of rat LH *(132).* In the LH, ATP and GABA may be costored within the same synaptic vesicle, because recordings from identified pairs of presynaptic and postsynaptic neurons revealed that ATP and $GABA_A$ receptor-mediated postsynaptic currents arose from the coordinate release of both ATP and GABA from individual neurons *(133).* It was further shown that such coordinate release of ATP and GABA was linked to coordinate modulation of transmission by cholinergic modulators *(134),* in which cholinergic modulators exerted independent control over GABA vs ATP transmission in the LH circuits. Cholinergic neurons from the pedunculopontine and laterodorsal tegmental nuclei project to the LH *(135)* and thus may control LH neurons by selective enhancement of excitatory or inhibitory pathways in the GABA/ATP cotransmission system.

Histamine

There are no neurons containing histamine in the LH, as the whole population of histaminergic neurons is located in the tuberomammillary nucleus (TMN) in the posterior hypothalamus. Loss of these neurons, in addition to loss of lateral hypothalamic neurons, could account for sleepiness observed by von Economo in patients suffering from the viral encephalitis at the beginning of 20th century *(10).* TMN neurons have been implicated in the arousal mechanisms *(136).* Knockout mice lacking the histidine decarboxylase gene show a deficit of wakefulness at lights-off period and fail to remain awake when placed in a new environment *(137),* and hypocretin-1 increase wakefulness in wild-type mice, but not in histamine H1 receptor knockout mice *(138).* Among other potential mechanisms, histaminergic neurons may regulate wakefulness via reciprocal connections with the lateral hypothalamic neurons. Role of histamine in the brain has been discussed in several recent reviews *(136,139).*

In summary, at least three neuropeptides (hypocretin, neurotensin, and CRH) located in the lateral hypothalamic neurons have been implicated in arousal. In addition, glutamate-containing neurons in the LH may also play a role in maintenance of arousal by activating other neurons both within and outside of the LH. Such complex organization of arousal neuronal network in the LH may reflect a redundancy of waking system. Indeed, it would be very unusual if such important function in the brain as maintenance of wakefulness were executed only by a limited number of neurons of few neurotransmitter phenotypes. It is, however, more likely, that the complex arousal network of neurons in the LH not only reflects redundancy of the system, but also serves for a fine regulation of different components of arousal system. Each neurotransmitter type of neurons in the LH may be responsible for a separate component of arousal system (keeping an animal awake during feeding, maintenance of wakefulness in a novel environment, awakening an animal after receiving a signal from some components of the circadian clock, etc.). Identification of such specific functions of different neuronal types in the LH would require production of inducible gene knockouts in future studies, since such models would allow investigation of a gene function by ceasing its expression not only in the whole organism, but also locally in defined area of the LH.

Efferent and Afferent Connections of the LH With Other Brain Areas Implicated in Sleep Regulation

The efferent fibers of the LH neurons are widely distributed throughout the brain *(140,141),* and all parts of the LH contribute ascending and descending fibers to the MFB *(140).* Neuronal connections between the LH and other specific brain areas implicated in the regulation of sleep and wakefulness depend on both location of the neurons within the LH, and type of the neurons. The anterior and lateral parts of the LH project substantially to the

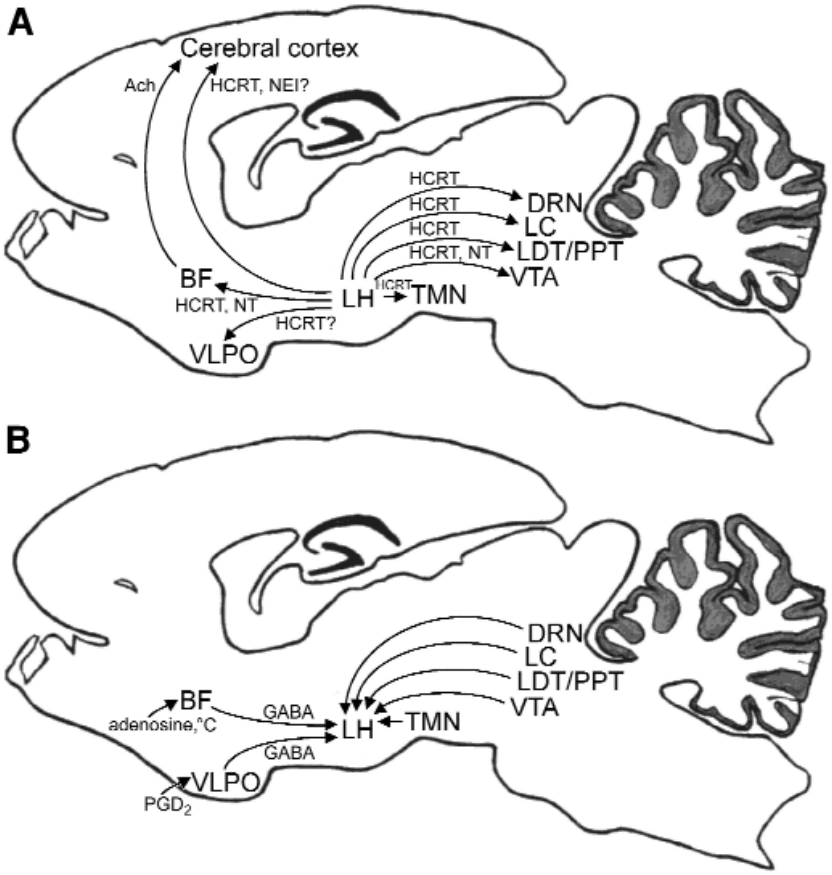

Fig. 3. Schematic illustration of efferent **(A)** and afferent **(B)** connections of the lateral hypothalamus with other brain areas implicated in sleep regulation. Lateral hypothalamic neurons maintain wakefulness via an excitatory influence on all major brain areas implicated in arousal (A). The arousal areas in turn also project to the LH and may excite LH neurons thereby promoting wakefulness (shown in B). Prostaglandin D2, adenosine, or preoptic area warming inhibit lateral hypothalamic neurons via GABAergic projections and initiate sleep (B). Ach, acetylcholine; BF, basal forebrain; DRN, dorsal raphe nucleus; GABA, γ-aminobutyric acid; HCRT, hypocretin; LC, locus coeruleus; LH, lateral hypothalamus; LDT/PPT, laterodorsal tegmental nucleus/ pedunculopontine tegmental nucleus; NEI, neuropeptide glutamic acid-isoleucineamide; NT, neurotensin; PGD_2, prostaglandin D_2; TMN, tuberomammillary nucleus; VLPO, ventrolateral preoptic nucleus; VTA, ventral tegmental area.

anterior hypothalamic area *(140),* including the ventrolateral preoptic nucleus (VLPO) *(142),* an area important for sleep onset *(143).* Cells in the tuberal and posterior parts of the LH give projections to monoaminergic populations implicated in arousal, such as the serotonergic dorsal raphe and noradrenergic locus coeruleus *(140)* (*see* Fig. 3A).

Efferent pathways to the cerebral cortex originating in the LH are organized in the medial and lateral pathway. The medial pathway runs through the MFB, traverses the medial septal area, enters the fornix and the cingulate bundle, and reaches the hippocampus and medial cortical fields *(141).* The lateral pathway runs through the lateral part of the

MFB, and then through the substantia innominata of the basal forebrain, before entering the cortical fields *(141).* The LH provides dense innervation of the caudal basal forebrain *(61),* raising the possibility that fibers of the lateral pathway send collaterals to wake-active neurons in the basal forebrain. The basal forebrain acetylcholine-synthesizing neurons constitute one of the major sources of subcortical afferents to the cerebral cortex and thus could play a role in the regulation of the sleep–wakefulness *(144).* The basal forebrain neurons can also regulate arousal by providing a feedback mechanism via GABAergic projections to the LH *(145).* The lateral hypothalamic neurons could thus effectively maintain arousal in a reciprocal fashion through projections to the basal forebrain as well as through direct input to the cortex.

The LH also receives input from other brain areas implicated in sleep and wakefulness (*see* Fig. 3B). Because of reciprocal connections between the LH and other arousal populations it is not clear which area is primarily responsible for wakefulness. There are afferent projections to the LH from the raphe nucleus *(146),* locus coeruleus *(147),* tuberomammillary nucleus *(148),* laterodorsal and pedunculopontine tegmental nuclei *(135).* Fibers of noradrenergic neurons are rather sparsely distributed throughout the lateral and posterior hypothalamus, whereas many serotonin-immunoreactive fibers are present in both these hypothalamic areas and they are noticeably more dense in the lateral than posterior hypothalamus *(33).* High concentrations of histaminergic fibers are found in the hypothalamic nuclei and MFB *(148).* Projections from the preoptic area (POA) of the anterior hypothalamus to the LH may be particularly important for interaction between sleep- and wake-active brain areas, since POA warming promotes sleep by increasing activity of warm-sensitive neurons *(149).* The POA, in turn, inhibits activity of wake-active LH neurons in the perifornical area *(55).* Importantly, suppression of the neuronal discharge in the LH in this study coincided with timing of POA warming while there was no significant difference in the EEG delta and theta bands during the warming, and levels of behavioral activity were similar for baseline and warming periods *(55).* This suggests that sleep-active neurons in the POA including those in the VLPO may inhibit wake-active neurons in the LH directly, and thereby promote sleep onset.

Conclusion

As discussed previously, the LH can promote arousal via direct projections to the cerebral cortex as well as indirect connections with other sleep/wake-related areas in the brain. At least three neuropeptides (hypocretin, neurotensin, and CRH) located in the lateral hypothalamic neurons have been implicated in arousal. The LH neurons also contain neurotransmitters/neuromodulators that have been implicated in the regulation of nonREM sleep and REM sleep. Given the heterogeneity of the neuronal phenotypes in the LH, it is likely that hypocretin neurons as well as other types of neurons in the LH influence sleep and provide state-dependent regulation of physiological functions.

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