Neuropeptidergic Control of Sleep and Wakefulness

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Abstract

Sleep and wake are fundamental behavioral states whose molecular regulation remains mysterious. Brain states and body functions change dramatically between sleep and wake, are regulated by circadian and homeostatic processes, and depend on the nutritional and emotional condition of the animal. Sleep-wake transitions require the coordination of several brain regions and engage multiple neurochemical systems, including neuropeptides. Neuropeptides serve two main functions in sleep-wake regulation. First, they represent physiological states such as energy level or stress in response to environmental and internal stimuli. Second, neuropeptides excite or inhibit their target neurons to induce, stabilize, or switch between sleep-wake states. Thus, neuropeptides integrate physiological subsystems such as circadian time, previous neuron usage, energy homeostasis, and stress and growth status to generate appropriate sleep-wake behaviors. We review the roles of more than 20 neuropeptides in sleep and wake to lay the foundation for future studies uncovering the mechanisms that underlie the initiation, maintenance, and exit of sleep and wake states.

Contents

INTRODUCTION

Three behavioral criteria define sleep: inactivity, rapid reversibility, and reduced responsiveness to external stimuli (reviewed in Campbell & Tobler 1984, Allada & Siegel 2008). Lower responsiveness distinguishes sleep from inactive wakefulness and indicates an increased arousal threshold, often measured as a lengthened reaction time upon stimulation (reviewed in Allada & Siegel 2008). In mammals, recordings of cortical activity—known as electroencephalograms (EEGs)—complement these behavioral criteria (reviewed in Brown et al. 2012). During non–rapid eye movement sleep (NREM sleep), cortical neurons fire synchronously, producing high-amplitude slow waves [also known as delta waves or depicted as slow-wave activity (SWA)]. In rapid eye movement (REM) sleep, asynchronous cortical activity generates small fast waves, a pattern paradoxically similar to that of waking brains [paradoxical sleep (PS)]. During NREM sleep, muscles partially relax and completely lose their tone during REM sleep. Thus, wake and sleep behaviors consist of substates that can be distinguished by EEG, muscle tone, and behavior (reviewed in Harris & Thiele 2011, Brown et al. 2012).

Anatomy of Sleep-Wake Regulation

Two processes regulate sleep and wakefulness: circadian rhythms and homeostatic drive. Sleep-wake behaviors often oscillate with the rhythm of day and night, whereas sleep length and depth correlate with the amount of preceding wakefulness (reviewed in Brown et al. 2012). These regulatory processes act on specific brain areas, including four major centers that activate the cortex during wake (**Figure 1**) (reviewed in Lin et al. 2011). Cholinergic and monoaminergic nuclei in the brain stem (*a*) activate the posterior hypothalamus (*b*) which then activates the cortex. The brain stem nuclei and the hypothalamus also activate cholinergic neurons in the basal forebrain (*c*) and neurons in the thalamus (*d*) (reviewed in Saper et al. 2010), both of which can activate the cortex.

Additionally, arousal nuclei inhibit sleep-active neurons in the ventral lateral preoptic (VLPO) area (reviewed in Saper et al. 2010). Conversely, neurons in the VLPO send inhibitory γ-aminobutyric acid (GABA)-ergic and galaninergic projections back to arousal centers in the hypothalamus and the brain stem. This mutual inhibition of wake- and sleep-promoting neurons generates a flip-flop switch that is thought to mediate the sharp transitions between sleep and wake (reviewed in Saper et al. 2010). A second switch regulates NREM and REM-sleep transitions through mutual inhibition between REM-activating and REM-suppressing neurons (**Figure 1**) (reviewed in España & Scammell 2011, McCarley 2011).

Finally, the suprachiasmatic nucleus (SCN) generates circadian rhythmicity by translating day–night information from the retina into transcriptional and translational feedback loops of clock genes (**Figure 2**) (reviewed in Welsh et al. 2010, Colwell 2011). Homeostatic sleep regulation relies on sleep-promoting molecules, such as adenosine, which accumulate during wake (reviewed in Brown et al. 2012). Thus the coordinated alternating activity of several brain nuclei and transmitter systems regulates sleep-wake states.

Figure 1

Sleep-wake regulation. (*a*) Wake: Brain stem arousal nuclei (*pink*) containing ACh, DA, 5-HT, or NA activate the thalamus, hypothalamus, spinal cord motor neurons, and the basal forebrain, and inhibit the ventrolateral preoptic area (GABA, galanin); hypothalamic arousal centers [*pink*: HA; *dark purple*: Hcrt] activate the cortex and arousal-related regions in the basal forebrain and brain stem; the thalamus activates the cortex. (*b*) NREM sleep: hypothalamic preoptic area nuclei (*dark blue*), containing GABA and galanin, inhibit brain stem and hypothalamic arousal nuclei; endogenous sleep regulatory substances [adenosine and NO] inhibit basal forebrain arousal nuclei, hypocretin neurons, and TMN neurons; and adenosine activates VLPO neurons. (*c*) REM sleep: REM-active brain stem nuclei, including LDT/PPT/SLD/PC and containing ACh, Glu, or GABA, promote activity in the basal forebrain and cortex and induce muscle atonia and rapid eye movements; hypothalamic neurons, containing MCH, promote REM sleep by suppressing REM-inhibitory brain centers, including vlPAG/LPT/DR/LC. NREM–REM switch: During NREM sleep, serotonergic DR and noradrenergic LC neurons inhibit LDT/PPT neurons. During REM sleep DR/LC neurons become silent, enabling the cholinergic LDT/PPT neurons to generate the hallmarks of REM sleep, including rapid eye movements, EEG activation, and muscle atonia. This reciprocal activity between REM-on (LDT/PPT) and REM-off (DR/LC) neurons drives the cycling between REM and NREM sleep episodes. Additionally, GABAergic neurons participate in the mutual inhibition of REM-activating and REM-suppressing neurons. During REM sleep, SLD/PC neurons use ascending and descending projections to activate the cortex and to promote muscle atonia. During NREM sleep, vlPAG/LPT neurons inhibit SLD/PC neurons. Recently, a pair of novel sleep-wake centers were identified in the brain stem: The glutamatergic MPB in the dorsal pontine tegmentum regulates arousal (Fuller et al. 2011), and the GABAergic PZ in the pontomedullary junction promotes sleep (Anaclet et al. 2012). Figure based on information from the following reviews: Saper et al. (2010), España & Scammell (2011), McCarley (2011), Monti et al. (2013), and Urade et al. (2011). Arrowheads in all figures indicate the effect on target structures and not the nature of the synaptic contact. For exact positions of nuclei, please refer to the primary literature and the Allen Mouse Brain Atlas (Lein et al. 2007, website: ©2014 Allen Institute for Brain Science). Abbreviations: 5-HT, serotonin; ACh, acetylcholine; A, adenosine; DA, dopamine; EEG, electroencephalogram; GABA, γ-amino-butyric acid; Glu, glutamine; HA, histamine; Hcrt, hypocretin; MCH, melanin-concentrating hormone; NA, noradrenaline; NO, nitric oxide.

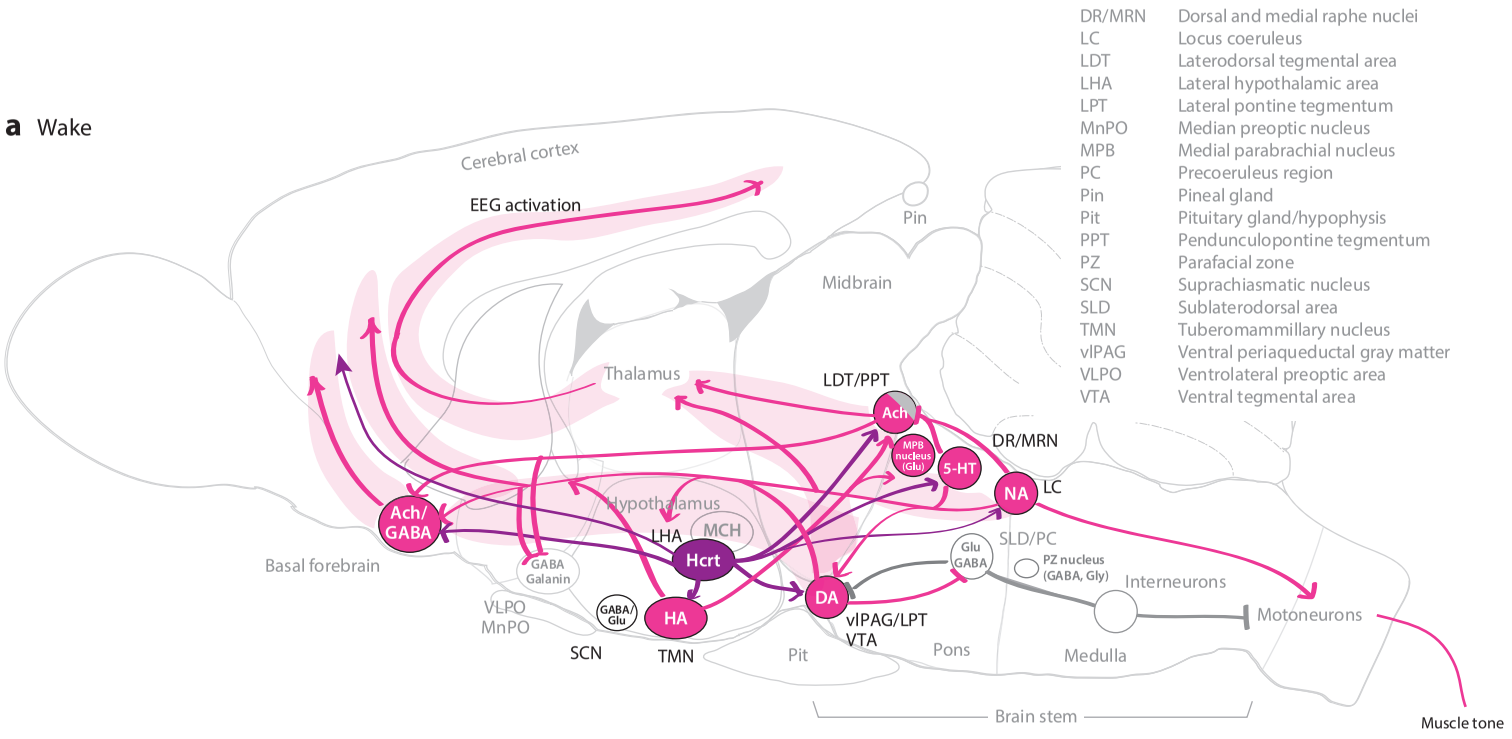

Figure 1

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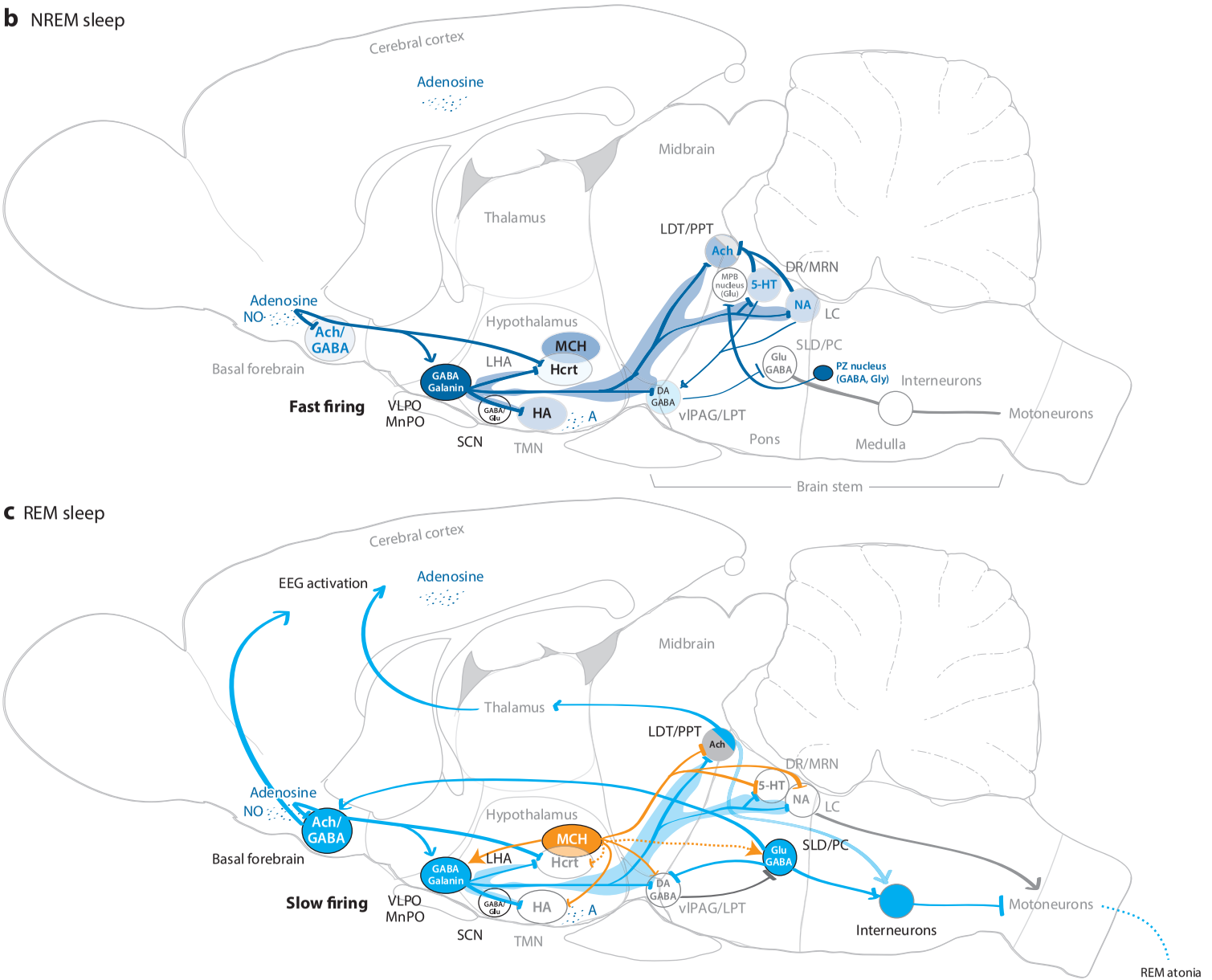
b NREM sleep
Cerebral cortex
Adenosine
Midbrain
Thalamus
LDT/PPT
Ach
DR/MRN
MPB nucleus (Glu)
5-HT
NA
LC
Adenosine
NO
Ach/ GABA
Hypothalamus
MCH
LHA
Hcrt
SLD/PC
Glu GABA
PZ nucleus (GABA, Gly)
Basal forebrain
GABA Galanin
DA GABA
Interneurons
Fast firing
VLPO MnPO
GABA/ Glu
HA
A
vlPAG/LPT
Motoneurons
SCN
TMN
Pons
Medulla
Brain stem
c REM sleep
Cerebral cortex
EEG activation
Adenosine
Midbrain
Thalamus
LDT/PPT
Ach
DR/MRN
5-HT
NA
LC
Adenosine
NO
Ach/ GABA
Hypothalamus
MCH
LHA
Hcrt
SLD/PC
Glu GABA
Basal forebrain
GABA Galanin
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Motoneurons
SCN
TMN
REM atonia

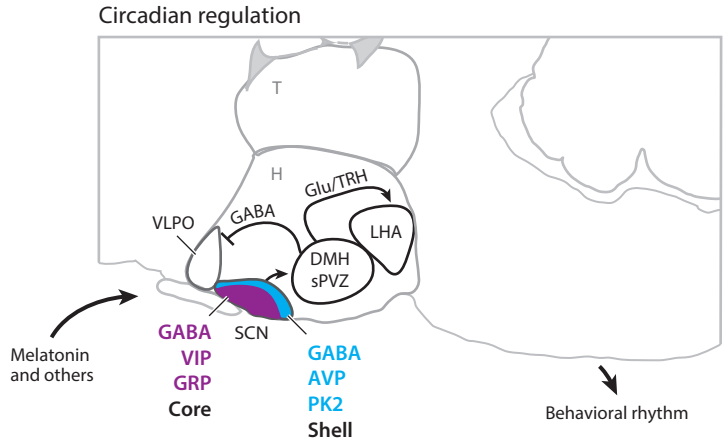


Figure 2

Peptide regulation of circadian rhythm. Circadian regulation: The SCN relays its rhythmic activity via the DMH and the sPVZ to the LHA and the VLPO to promote wake and inhibit sleep, respectively. Input to the SCN originates within the retina, thalamus, and arousal centers and also includes melatonin and other secreted molecules. Figure based on information from the following reviews: Chou et al. (2003), Colwell (2011), Welsh et al. (2010). Abbreviations: AVP, arginine-vasopressin; GRP, gastrin releasing peptide; PK2, prokineticin 2; TRH, thyrotropin releasing hormone; VIP, vasoactive intestinal polypeptide. For anatomic abbreviations, see **Figures 1** and **3**.

Neuropeptide Biogenesis and Signaling

In the mammalian brain, almost 70 different secreted neuropeptides (reviewed in Burbach 2011) complement neurotransmitters and modulate excitability of their targets. After their release, most neuropeptides bind to G protein–coupled receptors (GPCRs) to alter membrane excitability, transcription, and synaptogenesis (reviewed in Ludwig & Leng 2006), enabling neuropeptides to regulate a wide range of behaviors, including sleep-wake states. In contrast with neurotransmitters, neuropeptides are directly encoded by the genome, facilitating their study through genetic approaches.

Neuropeptide activity can be regulated at several levels. After transcription and translation, neuropeptides are processed by peptidases and convertases to generate mature neuropeptides of 3–40 amino acids in length, and these mature peptides often require additional modification for full activity (reviewed in Hook et al. 2008). Neuropeptides can be released from large dense core vesicles along the entire axon, so varying control of their release site(s) can provide another layer of regulation. Neuropeptide release is generally slower and requires stronger neuron activation as compared with the release of neurotransmitters (reviewed in Burbach 2011, Hokfelt 2000). Control of neuropeptide transport and clearance provides an additional layer of regulation. Neuropeptides have half-lives of up to 20 min, allowing them to signal to distant targets, whereas neurotransmitters are cleared from the synapse in milliseconds (reviewed in Lester et al. 1994, Ludwig & Leng 2006). Finally, because neurons often contain several neuropeptides and neurotransmitters, a neuropeptide may have different roles in different neuron populations, depending on its anatomical and biochemical context (reviewed in Bargmann 2012, Schöne & Burdakov 2012, van den Pol 2012).

NEUROPEPTIDERGIC REGULATION OF SLEEP-WAKE CENTERS

Here, we review the roles of more than 20 neuropeptides in sleep-wake regulation (**Table 1**). We focus on signals whose sleep-wake functions have not been discussed extensively and refer

Table 1 Neuropeptides and peptide hormones involved in sleep-wake regulation

Protein name (mouse gene symbol)	Suggested sleep-wake role	Wake	NREM sleep	REM sleep	Selected references	Other roles	Receptors (mouse gene symbol)
Adrenocorticotropic hormone (*Pomc*)	Promotes wake	+	NR	NR	Chastrette et al. 1990, Clow et al. 2010	Stress response Inhibition of feeding Motivation and reward Pain	*Mc1r*, *Mc2r (main)*, *Mc3r*, *Mc4r*, *Mc5r*
Brain-derived neurotrophic factor (*Bdnf*)	May promote NREM sleep Potentially inhibits wake	(−)	(+)	NR	Tononi & Cirelli 2012	Inhibition of feeding Synaptic plasticity	*Trkb*, *Ngfr*
Cholecystokinin (*Cck*)	Promotes wake and NREM sleep	+	+	NR	Obal & Krueger 2003	Inhibition of feeding Pain Anxiety	*Cckar*, *Cckbr*
Cocaine- and amphetamine-regulated transcript (*Cartpt*)	Promotes wake	+	−	−	Keating et al. 2010	Inhibition of feeding Anxiety Antidepressant Conditioned place preference	NA
Corticotropin-releasing hormone (*Crh*)	Promotes wake Modulates REM sleep	+	−	+/−	Kimura et al. 2010, Romanowski et al. 2010	Anxiety Depression Stress	*Crhr1*, *Crhr2*
Cortistatin (*Cort*)	Promotes NREM sleep Inhibits REM sleep	NR	+	−	de Lecea 2008	Inhibition of growth hormone release Memory and learning Pain suppression	*Sstr1-5*, *Ghsra*
Dynorphin (*Pdyn*)	Potentially increases NREM sleep	NR	(+)	NR	Greco et al. 2008	Opioid peptide Promotion of feeding	*Oprk1 (KOR)*
Endomorphin 1/EM1 (NA)	May promote wake	(+)	−	NR	Greco et al. 2008	Pain	*Oprm1*
Epidermal growth factor (*Egf*)	Suppresses locomotion in response to circadian cues	(−)/Timing	Timing	NR	Kushikata et al. 1998, Kramer et al. 2001	Growth stimulation	*Egfr*

(*Continued*)

Table 1 (*Continued*)

Protein name (mouse gene symbol)	Suggested sleep-wake role	Wake	NREM sleep	REM sleep	Selected references	Other roles	Receptors mouse gene symbol
Galanin (*Gal*)	May promote sleep	–	(+)	(+)	Pieribone et al. 1995, Murck et al. 2004, Woods et al. 2014	Pain perception Anxiety Nerve regeneration and neural stem cell proliferation	*Galr1*, *Galr2*, *Galr3*
Ghrelin (*Ghrl*)	Promotes wake	+	–	–	García-García et al. 2014	Promotion of feeding Counteracting leptin	*Ghsra*
Growth hormone (*Gh*)	May inhibit NREM sleep Promotes REM sleep	NR	(–)	+	Obal & Krueger 2003, Steiger 2007	Cell growth	*Ghr*
Growth-hormone-releasing hormone (*Ghrh*)	Promotes NREM sleep	NR	+	NR	Obal & Krueger 2003, Steiger 2007	Stimulation of growth hormone release	*Ghrhr*
Hypocretin (*Hcrt*)	Consolidates wake Inhibits REM sleep	+	–	–	Sakurai 2007, Carter et al. 2013	Promotion of feeding Thermoregulation Mood Reward Energy homeostasis Sensing of external and internal environment	*Hcrtr1*, *Hcrtr2*
Interleukin 1 beta (*Il-1β*)	May promote sleep	–	+	NR	Jewett & Krueger 2012	Inflammatory cytokine Synaptic plasticity	*Il1r1*, *Il1r2*
Leptin (*Lep*)	May promote wake Promotes NREM sleep May inhibit REM sleep	(–)	+	(–)	Sinton et al. 1999, Laposky et al. 2006, Leinninger 2011	Inhibition of feeding Promotion of energy expenditure Reproduction Thermogenesis Synaptic plasticity Neuroprotection	*Lepr*
Melanin-concentrating hormone (*Pmch*)	Promotes sleep	–	+	+	Jego et al. 2013, Konadhode et al. 2013, Monti et al. 2013	Promotion of feeding Memory formation	*Mchr1 (SLC-1)*

Melanocyte-stimulating hormones (*Pomc*)	May promote NREM sleep	NR	(+)	NR	Chastrette et al. 1990	Stress regulation Inhibition of feeding Motivation Pain Reward	*Mc1r*, *Mc3r*, *Mc4r*, *Mc5r*
Neuromedin S (*Nms*)	Promotes lethargus in *C. elegans*	(−)	(+)	NR	Nelson et al. 2013	Circadian rhythm Feeding	*Nmur1*, *Nmur2*
Neuropeptide B (*Npb*)	Promotes NREM sleep	−	+	NR	Hirashima et al. 2011	Feeding Pain sensation	*Npbwr1* (NPBWR2, not in rodents)
Neuropeptide S (*Nps*)	Promotes wake	+	(−)	(−)	Brown et al. 2012	Locomotion Anxiety Pain	*Npsr1*
Neuropeptide Y (*Npy*)	Downregulates CNS excitability Modulates wake and NREM sleep	−/+	+/−	NR	Dyzma et al. 2010	Promotion of feeding Anxiety Epilepsy Addiction Reproduction Immune regulation Neuroprotection	*Npy1r*, *Npy2r*, *Npy4r*, *Npy5r*, *Npy6r*
Neurotensin (*Nts*)	Promotes wake Inhibits NREM sleep May promote REM sleep	+	−	(+)	Cape et al. 2000, Fitzpatrick et al. 2012, Furutani et al. 2013	Anxiety Depression Increase in stress response	*Ntsr1*, *Ntsr2*
Nociceptin (*Pnoc*)	May inhibit wake Modulates REM sleep	(−)	NR	(−/+)	Devine et al. 1996, Xie et al. 2008; Rizzi et al. 2011	Pain sensing	*Oprl1*
Obestatin (*Ghrl*)	Promotes NREM sleep	NR	+	NR	García-García et al. 2014	Counteracting ghrelin	*Gpr39*/NA

(*Continued*)

Table 1 (*Continued*)

Protein name (mouse gene symbol)	Suggested sleep-wake role	Wake	NREM sleep	REM sleep	Selected references	Other roles	Receptors mouse gene symbol
Pituitary adenylyl cyclase-activating polypeptide (*Adcyap1*)	May promote wake Promotes REM sleep	(+)	NR	+	Ahnaou et al. 1999	Circadian rhythms Feeding Stress Memory Pain	*Adcyap1r1 (PAC1)*, *Vipr1 (VPAC1)*, *Vipr2 (VPAC2)*
Prolactin (*Prl*)	Promotes REM sleep	NR	NR	+	García-García et al. 2009	Stress response Reduction in stress response during lactation	*Prlr*
Somatostatin (*Sst*)	Promotes wake and REM sleep Inhibits NREM sleep	+	−	+	Obal & Krueger 2003, Steiger 2007	Inhibition of growth hormone release	*Sstr1-5*
Substance P/ Tachykinin 1 (*Tac1*)	May promote sleep	−	(+)	(+)	Zhang et al. 2004	Pain Neurogenic inflammation Muscle contractility	*Tacr1-3*
Transforming growth factor alpha (*Tgfa*)	Suppresses locomotion in response to circadian cues	(−)/Timing	Timing	NR	Kramer et al. 2001	Expression in cancer Stimulates proliferation	*Egfr*
Tumor necrosis factor (*Tnf*)	May promote sleep	−	+	(+)	Jewett & Krueger 2012	Inflammatory cytokine synaptic scaling Hippocampal neurogenesis	*Tnfrsf1a*, *Tnfrsf1b*
Vasoactive intestinal peptide (*Vip*)	Promotes REM sleep	−	NR	+	Riou et al. 1982, Hu et al. 2011	Inhibition of feeding Circadian rhythm	*Vipr1 (VPAC1)*, *Vipr2 (VPAC2)*

Abbreviations: +, promotes; −, inhibits; NA, not applicable; NR, no role/not known; NREM, non–rapid eye movement; REM, rapid eye movement.

the reader interested in the detailed roles of hypocretin/orexin to the accompanying review by Gao & Horvath (2014) in this volume. Several themes will become apparent in this section. First, there does not appear to be one master regulatory neuropeptide devoted solely to regulating sleep and wakefulness. Even hypocretin, which has a discrete expression pattern and strong effects on sleep and wakefulness, has several additional roles, from feeding to reward, and regulates multiple brain regions. Thus, that neuropeptides have multiple roles makes it impossible for investigators to assign one definitive function. Second, at the molecular level, the same prepropeptide can generate multiple mature peptides with different potencies or functions, and several receptors and receptor isoforms can recognize a given neuropeptide. Third, at the cellular level, the spatial and temporal expressions of signals and receptors are diverse and complex, and several neuropeptides, classic neurotransmitters, and receptors can be coexpressed in the same neuron. Fourth, at the circuit level, neuronal connectivity is highly complex; many brain regions regulate each other at any given time. Fifth, at the physiological level, phenotypic effects can be masked by overlapping roles between related or even unrelated signals and by compensatory feedback mechanisms that maintain homeostasis. Perhaps most important, the effects of neuropeptidergic signaling are extremely context dependent on the behavioral state of the animal, the state of the environment, and the experience of the animal. All these effects undermine attempts at simple classification. Thus, the pictures we paint in the following sections are necessarily incomplete but should provide an overview of the field and help lay the foundation for future studies.

Hypocretin Promotes Wakefulness and Suppresses REM Sleep

We lack molecular understanding of most sleep disorders despite their prevalence (Colten & Altevogt 2006). One exception is narcolepsy, characterized by daytime sleepiness, the premature onset of REM sleep, fragmented nighttime sleep, and cataplexy, the emotionally triggered sudden loss of muscle tone while conscious (reviewed in Sakurai 2007, Sehgal & Mignot 2011).

In humans, narcolepsy results from loss of hypocretin neurons (reviewed in Sakurai 2007, Carter et al. 2013, Gao & Horvath 2014). In rodents, hypocretin promotes wakefulness, suppresses REM sleep, and is important to maintain stable sleep-wake states (reviewed in Gao & Horvath 2014). In zebrafish, a model which complements mammalian models by providing a combination of genetic tractability, high-throughput behavioral analyses, and conserved neuroanatomy, hypocretin increases arousal and inhibits rest (Prober et al. 2006; reviewed in Chiu & Prober 2013).

From their location in the hypothalamus, hypocretin neurons, which are most active during wake, project to the cortex and major arousal centers (**Supplemental Figure 1**) (reviewed in Sakurai 2007, Gao & Horvath 2014). By enhancing activity of arousal nuclei, hypocretin neurons stabilize wake. In addition, hypocretin indirectly represses NREM- and REM-promoting regions (reviewed in Sakurai 2007, Sakurai & Mieda 2011). Hypocretin loss could therefore disinhibit REM nuclei, such as the sublaterodorsal and precoeruleus (SLD/PC) and potentially the laterodorsal and pedunculopontine tegmental nuclei (LDT/PPT) neurons, and thereby sensitize them to triggers of REM sleep (Burgess et al. 2013, Oishi et al. 2013).

Supplemental Material

Hypocretin neurons integrate cues from several physiological systems and thereby respond to changes in energy, mood, and stress (reviewed in Gao & Horvath 2014). Potentially harmful conditions such as fasting or stress excite hypocretin neurons via state-specific signals such as ghrelin or arginine vasopressin (AVP). Conversely, increases in glucose can context-dependently inhibit hypocretin neurons (Venner et al. 2011). In addition, hypocretin neurons receive inputs from stress systems and indirect inputs via the dorsomedial hypothalamus (DMH) from the circadian system (reviewed in Sakurai 2007). Thus hypocretin neurons integrate metabolic, emotional, and circadian signals to match wake levels to environmental needs.

Galanin: A Potential Sleep Promoter?

Some data propose that galanin decreases arousal. Galanin mRNA is found within GABA-positive VLPO neurons (Gaus et al. 2002). The VLPO sends inhibitory projections to the tuberomammillary nucleus (TMN) and to other arousal systems in the brain stem, including the dorsal raphe (DR) and the locus coeruleus (LC) (Sherin et al. 1998). Some of these target areas, the LC for example, express the three galanin receptors: GALR1, GALR2, and GALR3 (O'Donnell et al. 1999, Mennicken et al. 2002). Furthermore, galanin reduces activity of LC neurons in slice preparations (Seutin et al. 1989, Pieribone et al. 1995). In addition, intravenous galanin administration increases REM sleep duration in young men (Murck et al. 2004). In zebrafish, overexpression of *galanin* decreases spontaneous locomotor activity and reduces responsiveness to sensory stimuli (Woods et al. 2014). Altogether, these data suggest that galanin has sedating functions.

Melanin-Concentrating Hormone Counteracts Hypocretin and Promotes REM Sleep

The location and projections of melanin-concentrating hormone (MCH) neurons are remarkably similar to those of hypocretin neurons (reviewed in Monti et al. 2013). MCH neurons target nuclei that promote arousal and REM sleep, including the LC/DR, LDT/PPT, and SLD (reviewed in Torterolo et al. 2011, Monti et al. 2013). However, hypocretin and MCH reside in separate neuron populations, and in contrast to hypocretin neurons, MCH neurons are sleep-active, especially during REM sleep (reviewed in Torterolo et al. 2011). Furthermore, hypocretin and MCH neurons regulate each other: Hypocretin excites MCH neurons, whereas MCH signaling has an inhibitory effect on hypocretin neurons (reviewed in Monti et al. 2013).

Functional evidence supports a role for MCH in sleep. For example, MCH knockout mice exhibit less NREM sleep, are more active, and are more sensitive to wake-promoting stimuli (Willie et al. 2008). MCH injections into the SLD increase REM sleep (reviewed in Torterolo et al. 2011), whereas injections into VLPO neurons increase NREM sleep (Benedetto et al. 2013). Recent optogenetic studies demonstrated that 24 h activation of MCH neurons increased both NREM and REM sleep (Konadhode et al. 2013), and acute activation and inhibition showed that MCH neurons maintain REM sleep by inhibiting arousal nuclei (e.g., TMN) (Jego et al. 2013). Thus MCH may promote sleep and may act as the sleep-active counterpart to the hypocretin and other arousal systems.

Paradoxically, loss of the MCH receptor MCHR1 increases REM sleep, especially after sleep deprivation (Adamantidis et al. 2008), suggesting that MCHR1 promotes wakefulness. This finding is surprising considering the similar effects of receptor and ligand mutations on energy balance and activity, but some explanations have been proposed. For example, differences in genetic background can have major effects on sleep (Tafti 2007). Alternatively, MCHR1 may mediate autoinhibition of MCH neurons directly or indirectly (Chee et al. 2013). Loss of such an inhibitory feedback loop would allow MCH neurons to release other inhibitory cotransmitters, such as GABA, which could promote sleep (Adamantidis et al. 2008; reviewed in Torterolo et al. 2011).

Dynorphin Modulates Hypocretin Neurons and May Affect Sleep

The endogenous opioid prodynorphin is another candidate regulator of sleep, proposed largely on the basis of its coexpression with hypocretin in lateral hypothalamic area (LHA) neurons (Chou et al. 2001). Infusion of dynorphin A(1–17) into the VLPO increases NREM sleep by activating the κ opioid receptor (KOR) (Greco et al. 2008), potentially reflecting its endogenous release from

the parabrachial subnucleus onto VLPO neurons (Khachaturian et al. 1982, Greco et al. 2008). In contrast, systemic infusion of KOR agonists into rats reduces EEG power spectra, indicating CNS activation, yet inhibits movement, potentially via KOR-mediated blockade of dopamine release (Mulder et al. 1984, Coltro Campi & Clarke 1995). To our knowledge, no explicit sleep-wake phenotype has been reported for dynorphin or for KOR knockout mice; instead, these mice exhibit a complex anxiety phenotype that depends on genetic background (Kastenberger et al. 2012).

Thus the physiological significance of dynorphin coexpression in hypocretin neurons is unclear (Chou et al. 2001), although dynorphin and hypocretin can act synergistically to enhance TMN activity: Hypocretin directly activates TMN neurons, and dynorphin inhibits GABAergic inputs from the VLPO (Eriksson et al. 2004). Dynorphin also inhibits hypocretin neurons for short time periods (Li & van den Pol 2006, Williams & Behn 2011). Dynorphin regulation of sleep and arousal may therefore strongly depend on the neuronal context.

PACAP Regulates REM Sleep and Wake

Sleep-wake regulation by the *Adcyap1*-encoded pituitary adenylate cyclase-activating polypeptide (PACAP) is complex owing to its additional functions in anxiety, locomotion, and circadian entrainment (reviewed in Vaudry et al. 2009). Nevertheless, some studies suggest PACAP promotes REM sleep. For example, injection of PACAP into REM nuclei increases REM-sleep duration (Ahnaou et al. 1999). In addition, PACAP is expressed in the SLD, also known as the subcoeruleus area, a REM-active nucleus that blocks muscle tone during REM sleep (Ahnaou et al. 2006). Furthermore, knockout of either PACAP or PAC1-R increases locomotor activity (Hashimoto et al. 2001, Otto et al. 2001), supporting PACAP's sedating role. However, PACAP may also increase arousal. For example, intracerebroventricular (icv) and systemic injections of PACAP into rats enhance activities such as walking and grooming (Masuo et al. 1995). These discrepancies may arise from different genetic backgrounds or differences in the dose or target area of injections. For example, PACAP may promote REM activity of SLD neurons but could also influence other wake-promoting targets, e.g., the LC (Ahnaou et al. 2006).

Functions for PACAP in sleep-wake regulation are further complicated by its involvement in stress- and anxiety-related behaviors (reviewed in Hashimoto et al. 2011). PACAP may simultaneously regulate multiple behaviors via independent action upon discrete brain regions. In zebrafish, for example, genetic overexpression of a PACAP paralog (*adcyap1b*) increased sensory responsiveness without affecting overall levels of locomotor activity (Woods et al. 2014). Studies of PACAP may thus lead to confounding results if induced behaviors influence each other. Dissection of the multiple functions of PACAP will require inhibition or activation of PACAP signaling in specific brain regions.

TGF Alpha and EGF Mediate Circadian Influences on Locomotor Activity

Transforming growth factor alpha (TGF-α) and epidermal growth factor (EGF) signal through receptor protein kinases, in contrast with typical neuropeptides, which signal via GPCRs. Nevertheless, TGF-α and EGF can originate from and act on neurons and can regulate sleep-wake behavior (Kushikata et al. 1998, Kramer et al. 2001, Snodgrass-Belt et al. 2005, Gilbert & Davis 2009). For example, infusion of TGF-α or EGF into the brains of hamsters decreases locomotion, fragments sleep-wake behaviors, and shifts the timing of sleep-wake states. Although overall sleep and wake durations are unaffected, infused hamsters change their usual circadian rhythm to an ultradian rhythm, with 5–6 sleep-wake cycles in 24 h (Kramer et al. 2001, Snodgrass-Belt et al. 2005). Conversely, mice with reduced EGF receptor function are hyperactive and less sensitive

to light-mediated inhibition of activity (Kramer et al. 2001), although this phenotype may vary considerably (Mrosovsky et al. 2005). The expression pattern of TGF-α supports a role in sleep regulation. For example, TGF-α expression in the SCN varies diurnally and decreases during the night, when hamsters are active (Kramer et al. 2001). Collectively, these observations suggest that TGF-α and EGF have conserved roles in suppressing locomotor activity in coordination with the circadian rhythm.

ENERGY HOMEOSTASIS AND SLEEP

Arousal and metabolic state are often inversely correlated: Hunger heightens arousal and satiety promotes rest (Antin et al. 1975, Borbély 1977). This notion suggests cross talk between regulatory regions for feeding and arousal behaviors, potentially via common neuronal pathways (reviewed in Sternson 2013). Indeed, centers regulating both behaviors connect anatomically, reside in the same brain regions (hypothalamus and brain stem), and employ overlapping neuron and transmitter systems (**Figure 3*a***) (reviewed in Smith & Ferguson 2008). The arousal peptide hypocretin, for example, is also a primary regulator of appetite and feeding (reviewed in Gao & Horvath 2014).

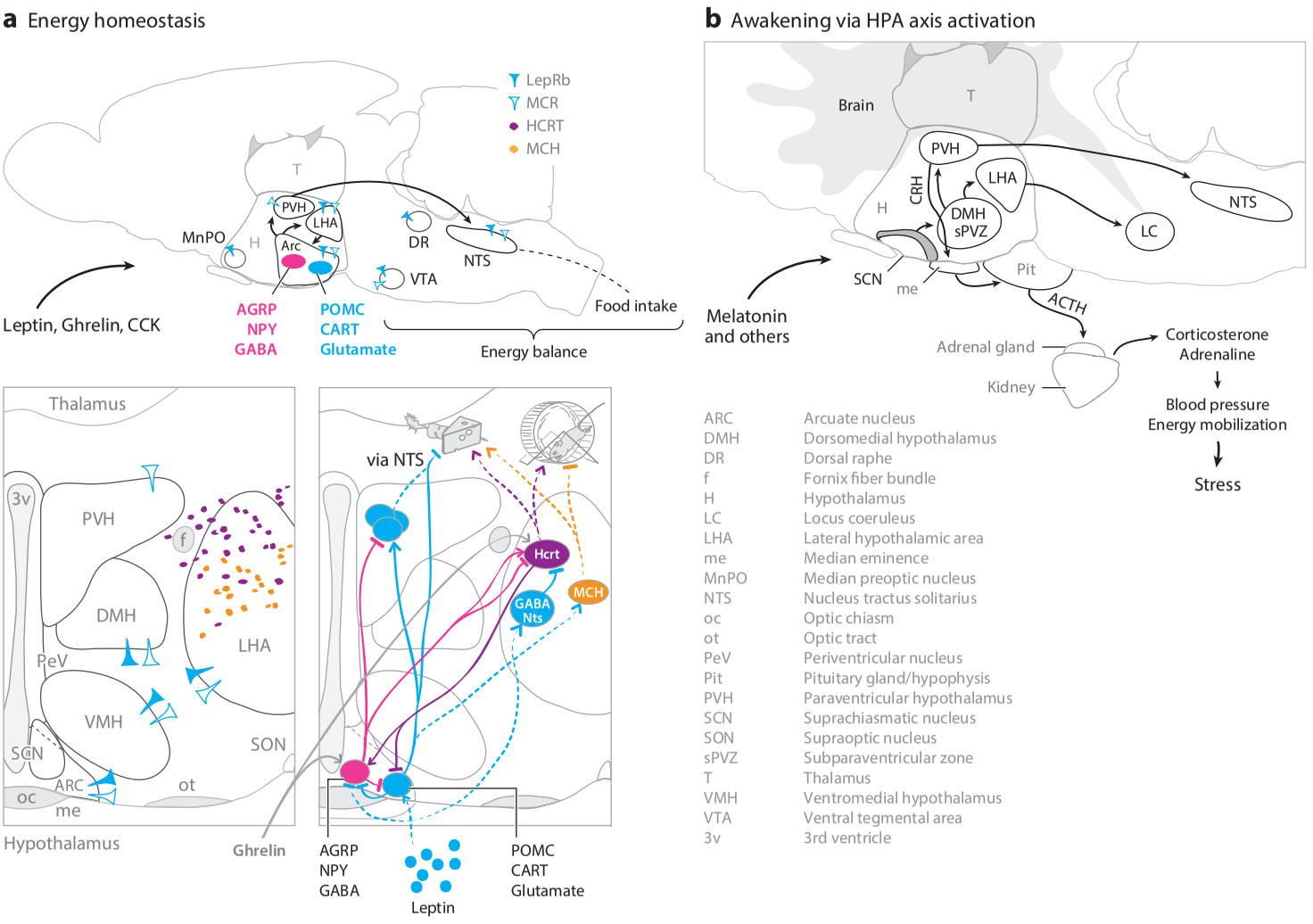
Hypocretin neurons respond to feeding cues such as changing glucose concentrations or the satiety hormone leptin (Yamanaka et al. 2003, Venner et al. 2011; reviewed in Gao & Horvath 2014). Hypocretin also indirectly influences feeding by modulating the mesolimbic dopamine system (reviewed in Tsujino & Sakurai 2013) and directly promotes feeding by activating neuropeptide Y (NPY)/agouti-related protein (AgRP) neurons and inhibiting proopiomelanocortin (POMC)/cocaine and amphetamine-regulated transcript (CART) neurons (reviewed in Adamantidis & de Lecea 2009). Similarly, neuropeptides associated with feeding can modulate arousal systems.

Leptin Can Promote Sleep

Leptin is expressed in the periphery, primarily by fat cells (reviewed in Harvey 2007), whereas its receptor LepRb is expressed in several brain nuclei (Scott et al. 2009). Leptin's primary role is to promote satiety and stimulate energy expenditure (reviewed in Leinninger 2011), but several observations indicate that leptin also regulates sleep and wake. Expression of the leptin receptor

Figure 3

(*a*) Energy homeostasis. Here, we focus on one example feeding circuit, originating in the arcuate nucleus of the hypothalamus (ARC). The ARC is a well-studied integrator of signals relevant to energy homeostasis. Peripheral signals (e.g. leptin, ghrelin, CCK) and central signals from gastrointestinal, gustatory, and arousal regions are integrated within the ARC, which in turn influences locomotion, energy expenditure, and arousal. Feedback among hypothalamic circuits is shown in the square inserts (*frontal views flattened into one plane*). Leptin inhibits feeding through differential regulation of gene expression: leptin enhances POMC expression but lowers AgRP expression (reviewed in Varela & Horvath 2012). LepRb expression in other brain regions indicates the distributed regulation of feeding (reviewed in Sternson 2013). Hypocretin neurons promote feeding and directly influence the Arc. In addition, hypocretin promotes locomotion. MCH neurons promote feeding but inhibit locomotion. Other aspects of feeding are relevant for arousal, including reward and emotional circuits but are beyond the scope of this review. (*b*) Awakening and stress signaling via HPA axis activation: The hypothalamic stress circuit induces central and peripheral changes that contribute to wake. Centrally, stress-responsive PVH neurons activate the NTS to promote energy expenditure and locomotion. Peripherally, PVH neurons stimulate the pituitary gland via CRH to release ACTH, which induces corticosterone secretion from the adrenal glands. Corticosterone elevates blood pressure and mobilizes energy resources. The stress circuitry is also employed during awakening, which is under circadian control by the SCN/DMH. Awakening is supported by the LHA, which promotes arousal via the LC. Abbreviations: ACTH, adrenocorticotropic hormone; AgRP, agouti-related protein; CART, cocaine and amphetamine-regulated transcript; CCK, cholecystokinin; CRH, corticotropin-releasing hormone; HPA, hypothalamic-pituitary-adrenal; LepRb, leptin receptor; NPY, neuropeptide Y; POMC, proopiomelanocortin.



ARC	Arcuate nucleus
DMH	Dorsomedial hypothalamus
DR	Dorsal raphe
f	Fornix fiber bundle
H	Hypothalamus
LC	Locus coeruleus
LHA	Lateral hypothalamic area
me	Median eminence
MnPO	Median preoptic nucleus
NTS	Nucleus tractus solitarius
oc	Optic chiasm
ot	Optic tract
PeV	Periventricular nucleus
Pit	Pituitary gland/hypophysis
PVH	Paraventricular hypothalamus
SCN	Suprachiasmatic nucleus
SON	Supraoptic nucleus
sPVZ	Subparaventricular zone
T	Thalamus
VMH	Ventromedial hypothalamus
VTA	Ventral tegmental area
3v	3rd ventricle

LepRb overlaps with several arousal and sleep nuclei (Elmquist et al. 1998, Scott et al. 2009, Patterson et al. 2011). In the LHA, a subset of leptin-responsive GABAergic neurons projects to hypocretin neurons (reviewed in Leinninger 2011), suggesting that leptin-responsive neurons inhibit hypocretin neurons and thereby lower wakefulness during satiety (Yamanaka et al. 2003, Venner et al. 2011). Mice lacking leptin signaling sleep more but exhibit fragmented sleep and wake (Laposky et al. 2006). Accordingly, exogenous leptin promotes sleep: Systemic leptin increases slow wave and REM sleep (Sinton et al. 1999). These sleep-promoting effects are abolished during fasting, when leptin levels decrease (Sinton et al. 1999). Thus, leptin may induce sleep only above a certain threshold, triggering sleep only when energy resources are high.

Ghrelin, Cholecystokinin, and Neuropeptide Y Have Diverse Roles in Sleep-Wake Regulation

Ghrelin targets brain systems that promote feeding (reviewed in Kojima & Kangawa 2010, García-García et al. 2014). It is mainly produced in the stomach and lowly expressed in the brain (reviewed in Furness et al. 2011). Several lines of evidence support a role for ghrelin in sleep-wake regulation. Injections of ghrelin promote wakefulness and suppress NREM and REM sleep in rats (Szentirmai 2012). Conversely, the ghrelin receptor GHS-R1a, which is expressed in arousal nuclei such as the DR, LDT, and SCN (Zigman et al. 2006), is necessary for the heightened arousal behaviors normally observed upon fasting or introduction to novel environments (Esposito et al. 2012). In addition, ghrelin axons can excite hypocretin neurons (reviewed in Kageyama et al. 2010), which further supports a role for ghrelin in enhancing arousal.

Paradoxically, ghrelin also promotes sleep. Mice lacking preproghrelin have slightly increased wake and REM sleep and fragmented NREM sleep, indicating preproghrelin may promote NREM sleep (Szentirmai et al. 2007). Furthermore, systemic injection of ghrelin increases NREM sleep in young men but not in women (reviewed in García-García et al. 2014). How can one reconcile sleep- and wake-promoting effects of preproghrelin? Preproghrelin also encodes obestatin, which promotes NREM sleep upon injection (Szentirmai & Krueger 2006), and loss of obestatin could increase wakefulness in preproghrelin knockout mice. Alternatively, long-term loss of preproghrelin may activate compensatory mechanisms.

The cholecystokinin (CCK) precursor produces several brain–gut peptides that regulate satiety and also sleep and wakefulness (reviewed in Obal & Krueger 2003). For example, systemic CCK-8S increases NREM sleep (reviewed in Obal & Krueger 2003), and inhibition of the CCKA receptor (CCKAR/CCK1R) blocks the prolonged sleep induced by refeeding after fasting (Shemyakin & Kapás 2001), thus suggesting that CCK promotes sleep.

Paradoxically, CCKAR knockout mice display reduced locomotor activity and no sleep phenotype (Sei et al. 1999), and systemic injections of CCK4 elicit anxiety-like behaviors in humans and rodents (Li et al. 2013), which potentially indicates higher arousal. Similarly, locomotor activity is strikingly increased upon overexpression of *cck* in zebrafish larvae (Woods et al. 2014). Furthermore, the CCK-8S peptide directly activates hypocretin neurons (Tsujino et al. 2005), which express the CCKAR (Dalal et al. 2013), supporting CCK's wake-promoting role.

To reconcile these contradictory observations, investigators have suggested that peripheral and central sources of CCK may affect behavior differently (reviewed in Obal & Krueger 2003). For example, sleep-promotion effects of CCK may result from peripheral activation of the vagal nerve by CCK, whereas central CCK may instead excite hypocretin neurons and counterbalance the postfeeding increases in sleep drive.

NPY is an important regulator of feeding and energy expenditure, but it also affects other behaviors, including sleep (Erickson et al. 1996; reviewed in Dyzma et al. 2010). For example, NPY

can reduce motor activity in rats and can promote sleep-like states in certain genetic backgrounds and shorten sleep latency in young men (reviewed in Dyzma et al. 2010). Furthermore, NPY inhibits hypocretin neurons directly, potentially through the NPY receptors NPY1R and NPY2R (Fu et al. 2004), which are lowly expressed in hypocretin neurons (Dalal et al. 2013). Thus NPY may promote sleep.

In apparent contradiction, central injection of NPY promotes wake in mice if injected at light onset (reviewed in Dyzma et al. 2010), when mice begin to sleep and increase their VLPO activity. This wake-promoting effect may be explained by inhibition of VLPO neurons, which express NPY1R (Kishi et al. 2005), indicating context-dependent activity of NPY neurons and their targets. Given at sleep onset, for example, high NPY may promote wake by inhibiting VLPO neurons, whereas during wake, high NPY may be sedating by inhibiting hypocretin neurons.

CART May Promote Wakefulness

Cocaine and amphetamine-regulated transcript (CART) may enhance arousal. For example, intracerebroventricular CART injection promotes wakefulness in rats (Keating et al. 2010), and larval zebrafish overexpressing *cart* respond more strongly to sensory stimuli (Woods et al. 2014). In addition, CART knockout mice react less to arousing stimuli, such as cocaine or a novel environment (reviewed in Moffett et al. 2006). On the basis of its expression, CART may affect regulation of arousal at multiple levels. For example, in the olfactory bulb and retina, CART may modulate sensory inputs; in mood- and reward-associated nuclei, CART could function in motivation; and in arousal-associated nuclei [e.g., LHA, LC, DR, or periaqueductal gray matter (PAG)], CART could directly regulate arousal (Koylu et al. 1997, 1998). The latter possibility is favorable because CART is coexpressed with several sleep-wake-related neuropeptides in the hypothalamus, including MCH, dynorphin, and neurotensin (Broberger 1999, Elias et al. 2001, Hanriot et al. 2007). CART also increases anxiety-like behaviors, which may also influence arousal behaviors such as locomotion (Chaki et al. 2003). In the future, it will be important to identify the CART receptor, which is still unknown.

STRESS AND SLEEP

Molecules and neuroanatomical structures relevant to stress also affect sleep-wake regulation. Stress signals such as pain or fear activate the hypothalamic-pituitary-adrenal (HPA) axis, which releases hormones that elevate energy metabolism and blood pressure, thereby potentially stabilizing wake states (reviewed in Kalsbeek et al. 2010). Stress also influences REM sleep. In particular, acute anxiogenic stress increases wake in rats and lowers REM sleep, whereas repeated stress reduces wake and increases REM sleep (O'Malley et al. 2013).

Corticotropin-Releasing Hormone Regulates REM Sleep

Corticotropin-releasing hormone (CRH) initiates the stress response (reviewed in Kalsbeek et al. 2010). From the hypothalamus, CRH neurons project to their targets (**Figure 3*b***), including the LDT and PPT, two REM-sleep nuclei that express the receptor CRHR1 (Sauvage & Steckler 2001). Functional relevance of these connections is supported by the observation that the neonatal peak of REM sleep requires CRHR1 (Feng et al. 2007), and long-term overexpression of CRH in mice promotes REM sleep (Kimura et al. 2010). In contrast, acute injections of CRH promote wake at the expense of REM and NREM sleep (Romanowski et al. 2010). Acute effects of CRH on wake and NREM sleep require CRHR1, which is expressed in hypocretin and LC neurons (Sauvage & Steckler 2001, Winsky-Sommerer et al. 2004). However, CRH-mediated suppression

of REM sleep and HPA-axis activation persist in CNS-specific CRHR1 knockout mice, which suggests that CRH may have additional roles. We can therefore distinguish acute and chronic CRH effects: Short-term CRH induces wake and suppresses NREM sleep, potentially via hypocretin and LC activation, whereas long-term CRH increases REM sleep, potentially through activation of LDT/PPT.

Prolactin May Promote REM Sleep

The stress-induced peptide prolactin potentially regulates REM sleep (reviewed in García-García et al. 2009). For example, circadian changes in serum prolactin peak during REM sleep (reviewed in Gan & Quinton 2010); prolactin injections into rodent and cat brains increase REM sleep, whereas mice without prolactin have reduced REM sleep (reviewed in García-García et al. 2009).

Sleep-wake nuclei such as the DR and LC are targets of prolactin neurons in the periventricular nucleus and in the LHA (Siaud et al. 1989; Paut-Pagano et al. 1993). Prolactin is therefore an interesting candidate for REM-sleep regulation, particularly in response to stress.

GROWTH, REPAIR, DEFENSE PEPTIDES, AND SLEEP

The somatotropic axis regulates primarily animal size and metabolism. However, members of this pathway, including growth hormone (GH), growth-hormone-releasing hormone (GHRH), and somatostatin (SST), also regulate sleep-wake behaviors.

GH and GHRH Promote Sleep

Both GH and GHRH can promote sleep centrally. For example, systemic and icv administrations of GHRH enhance NREM sleep (reviewed in Steiger 2007), potentially via direct activation of VLPO and median preoptic nucleus (MnPO) neurons (Peterfi et al. 2010). Similarly, systemic injection of GH increases REM sleep (reviewed in Obal & Krueger 2003), perhaps by affecting DR and LC neurons, which express the GH receptor (reviewed in Hallberg & Nyberg 2012). Loss-of-function studies also support sleep-promoting roles for these peptides. Inhibition of endogenous GHRH prevents spontaneous sleep, and disruption of GHRH signaling in dwarf rats decreases REM and NREM sleep (reviewed in Obal & Krueger 2003). In addition, inhibition of GHRH signaling reduces SWA during NREM sleep, possibly via desynchronization of cortical neurons (Liao et al. 2010; reviewed in Obal et al. 2003). Similarly, loss of GH function decreases REM sleep in rats (Peterfi et al. 2006; reviewed in Obal & Krueger 2003). Expression of GHRH also supports a sleep-promoting role for this peptide: GHRH transcription increases upon sleep deprivation and follows circadian patterns, peaking during NREM sleep (reviewed in Obal & Krueger 2003).

Somatostatin and Cortistatin Have Opposite Actions on Sleep

Both SST and its analog octreotide inhibit sleep, potentially by inhibiting GHRH neurons via the somatostatin receptor 2 (SST2) (reviewed in Obal & Krueger 2003). However, SST also promotes REM sleep in rats (reviewed in Obal & Krueger 2003), suggesting potential GHRH-independent functions for this peptide. Cortistatin (CST) is structurally similar to SST and inhibits GH release and depresses neurons (reviewed in de Lecea 2008, Martel et al. 2012). Icv infusion of CST, however, lowers locomotion, and increases NREM sleep, potentially by enhancing synchrony of cortical neurons (reviewed in de Lecea 2008). Expression of CST is consistent with this function:

mRNA levels peak before sleep onset and increase during sleep deprivation (reviewed in de Lecea 2008).

Brain-Derived Neurotrophic Factor Modulates NREM Sleep

A current hypothesis suggests sleep may renormalize synaptic connections, and increased synaptic strength may correlate with subsequent stronger SWA (reviewed in Tononi & Cirelli 2012). As brain-derived neurotrophic factor (BDNF) mediates activity-dependent synaptic plasticity, among its many functions, and BDNF levels peak after extended wakefulness, BDNF may link plasticity and SWA of NREM sleep (reviewed in Tononi & Cirelli 2012, Bachmann et al. 2012). Cortical injection of BDNF transiently increases subsequent SWA, indicating deeper NREM sleep (reviewed in Tononi & Cirelli 2012). These EEG changes were unilaterally induced, suggesting that BDNF induces local sleep, similar to GHRH and Cst. Conversely, reduced neuron activity-dependent BDNF secretion in humans, caused by heterozygosity for a single-nucleotide polymorphism (SNP) in the BDNF gene, decreased SWA during sleep (Bachmann et al. 2012); likewise, mice without BDNF function develop stress-induced hyperactivity, indicating that BDNF may be sedating (Rios et al. 2001). Taken together, these data suggest a role for BDNF in promoting NREM-sleep depth.

TNF and IL-1β Promote Sleep

Increased sleepiness during illnesses, such as influenza and bacterial infection, suggests that the immune system influences arousal state (reviewed in Zielinski & Krueger 2011). Accordingly, cytokines such as tumor necrosis factor (TNF) and interleukin 1 beta (IL-1β) can enter the brain directly during an immune response and, among other things, induce sleep-like states (reviewed in Majde & Krueger 2005). Expression of these cytokines suggests they may also regulate sleep under normal conditions. For example, their expression varies diurnally; levels of IL-1β increase centrally during sleep deprivation; and glial cells release TNF and IL-1β in response to ATP, which accumulates extracellularly during wake (reviewed in Jewett & Krueger 2012). Both IL-1β and TNF promote NREM sleep, potentially by inhibiting wake-active DR or LC neurons, activating preoptic sleep-active neurons respectively, and stimulating release of sleep-inducing substances such as prostaglandin D2 (PGD2), adenosine, and GHRH (reviewed in Obal & Krueger 2003, Huang et al. 2007, Krueger et al. 2008, Urade & Hayaishi 2011, Jewett & Krueger 2012). Loss-of-function studies support this sleep-promoting function. For example, disruption of TNF signaling reduces REM and NREM sleep and lowers SWA locally (Kapás et al. 2008; reviewed in Jewett & Krueger 2012). In addition, TNF can change neuronal excitability by altering the distribution of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and adenosine receptors at the cell surface (reviewed in McCoy & Tansey 2008, Jewett & Krueger 2012). Moreover, unilateral injection of TNF enhances IL-1β and SWA locally in the injected hemisphere (reviewed in Krueger 2008). Thus these cytokines induce sleep locally by altering cortical network states and promoting the release of sleep-inducing substances (reviewed in Jewett & Krueger 2012) and globally by influencing sleep-wake regulatory brain centers.

CONCLUSIONS AND PROSPECTS

The studies discussed here reveal how numerous neuropeptides regulate sleep-wake behaviors, and these data establish tantalizing connections with other behavioral states such as hunger, stress, anxiety, and infection. Despite this progress, it is still unclear how neuropeptides and their

receptors generate stable behavioral states and transitions. Some of these challenges arise from differences in experimental settings. For example, the site, dose, and timing of neuropeptide administration can cause varying effects, and diverse assays such as EEG, locomotion, and feeding are difficult to compare. More standardized manipulations and assays may help to clarify some of the current controversies. Moreover, it is unclear whether all the major neuropeptidergic regulators of sleep have been discovered. It therefore remains unclear whether some of the currently described effects are mediated by additional signals.

Several technical advances promise to overcome these limitations: Genetic and optical methods are so sophisticated that one can now genetically manipulate signals and receptors in specific neurons in combination with optical methods to change the activity of neuron subsets. Standardized behavioral assays and careful measurements of neural activity will provide a better understanding of neuropeptide networks and will facilitate the generation of computational models for sleep-wake regulation.

If the insights gained from simpler neuromodularity systems are any indication, our analyses of sleep-wake states will continue to be extraordinarily diverse and complex. For example, classic studies of the crustacean stomatogastric ganglion have revealed how a circuit consisting of only ~30 neurons can be regulated by more than one dozen neuromodulators to generate many different outputs (reviewed in Marder 2012). The impressive progress in this system has been due to a combination of connectivity mapping, activity mapping, molecular studies, and computational modeling and provides a blueprint for the analysis of sleep-wake states; however, the complexity of this seemingly simple circuit is humbling when considering the much-more-complicated mammalian brains and behaviors.

The recent emergence of nonmammalian model systems in sleep research also promises novel insights. Systems such as *Drosophila*, *Caenorhabditis elegans*, and zebrafish have sleep-like states (Shaw et al. 2000, Cho & Sternberg 2014; reviewed in Crocker & Sehgal 2010, Chiu & Prober 2013, Nelson & Raizen 2013, Rihel & Schier 2013). For example, both *Drosophila* and zebrafish display the fundamental behavioral properties associated with sleep, including reduced locomotion and decreased arousal, which are regulated by circadian and homeostatic mechanisms. Moreover, neurotransmitters that regulate mammalian sleep also affect *Drosophila* and zebrafish sleep-like states. For example, a large-scale drug screen in zebrafish uncovered that many neuromodulators induce sleep-wake phenotypes in zebrafish, similar to phenotypes observed in mammals, ranging from the noradrenaline, serotonin, dopamine, GABA, glutamate, histamine, adenosine, and melatonin systems to numerous modulators of NF-κB, which is a likely integrator of cytokine, prostaglandin, and adenosine signals (Rihel et al. 2010).

Neuropeptidergic regulation is also conserved. For example, overexpression of hypocretin in larval zebrafish leads to increased wakefulness at the expense of rest, and adult zebrafish with mutations in the hypocretin receptor exhibit sleep fragmentation (reviewed in Chiu & Prober 2013). In vivo measurements of hypocretin neural activity reveal that these hypothalamic neurons are maximally active during episodes of spontaneous locomotor activity and are inactive during rest (reviewed in Chiu & Prober 2013), consistent with results obtained in mammals (reviewed in Gao & Horvath 2014). These studies reveal that key aspects of the neuropeptidergic regulation of sleep-wake states are preserved in nonmammalian model systems.

Many nonmammalian model systems also have the advantage that large numbers of animals can be analyzed simultaneously in well-defined and reproducible conditions. For example, in one screen of nearly 6,000 small molecules, several hundred structures were identified that alter the locomotor behavior of larval zebrafish (Rihel et al. 2010). The large data sets generated by such screens can be used to organize compounds by their multidimensional phenotypic output using clustering algorithms (Rihel et al. 2010). Importantly, the parallel comparison of different

molecules in standardized settings generates behavioral profiles that organize compounds into clusters of similar phenotypes and can identify novel relationships between behavioral modulators. The same logic has been recently applied to the neuropeptidergic regulation of arousal in zebrafish by quantifying spontaneous locomotor behaviors and responsiveness to sensory stimuli (Woods et al. 2014). Phenotypic clustering revealed both shared and divergent features of neuropeptidergic functions and revealed surprising relationships between hypocretin and calcitonin gene-related peptide, between galanin and nociceptin, and between CART and PACAP. These assays can now be used in genetic screens to identify novel regulators of sleep and wakefulness.

Model systems with translucent bodies and fewer neurons (302 in *C. elegans*; ~100,000 in zebrafish larva) also facilitate the analysis and manipulation of circuit activity. For example, a recent study dissected the generation of roaming and dwelling states in *C. elegans* using genetic and optical tools and demonstrated that serotonin signaling and its associated circuitry lead to dwelling states, whereas neuropeptidergic pigment-dispersing factor (PDF) signaling generates roaming states (Flavell et al. 2013). These studies begin to outline how the entry into long-lasting but reversible behavioral states can be triggered by activation of distinct signaling pathways and neurons (reviewed in Schier 2013).

Traditional model systems will help to identify molecular and neuronal regulators of sleep and wakefulness, but they cannot explain the remarkable diversity of sleep-related behaviors in different animals (reviewed in Siegel 2011, Tobler 2011). Although sleep/wake states across species can be generalized behaviorally by activity levels, rapid transitions, and changes in arousal threshold, the particulars of sleep behaviors in different animals can vary widely (reviewed in Siegel 2011). For example, sleep-wake periods are strikingly diverse across different species, ranging from 3–4 h per day in elephants to 18–20 h per day in opossums (reviewed in Siegel 2005). Circadian regulation of sleep can also vary. For example, most lab rat strains are diurnal, whereas the unstriped Nile grass rat is nocturnal (Martinez et al. 2002). These behavioral differences likely reflect adaptations to diverse lifestyles and habitats (reviewed in Siegel 2009), but it is unclear how these variations are generated. Neuropeptides may contribute to the diversity of sleep and wake behaviors, similar to the generation of morphological diversity by changes in the expression of conserved developmental regulators (reviewed in Carroll 2008).

In addition, sleep behaviors can also differ dramatically within single organisms. For example, Finnish bats are nocturnal from June to September but shift to diurnal cycles in spring (reviewed in Saper et al. 2005), and sleep length decreases over the human lifetime. Analogously, disease states can induce specific changes in sleep regulation. For example, in Alzheimer's patients, who often suffer from fragmented sleep, increased sleep-wake fragmentation correlates with lower levels of hypocretin (Friedman et al. 2007; reviewed in Zeitzer 2013). Conversely, sleep-wake changes influence disease states. For instance, sleep disorders often accompany psychological conditions. Insomnia, for example, increases by fivefold the risk of developing a depression (Colten & Altevogt 2006). Furthermore, sleep deprivation correlates with inflammation, increased levels of Alzheimer's-associated amyloid beta, and impaired immune system function (Zager et al. 2007, Kang et al. 2009; reviewed in Krueger 2008). Additionally, some sleep-associated neuropeptides are neuroprotective, including galanin, dynorphin, and leptin (Hobson et al. 2008, Wang et al. 2012; reviewed in Signore et al. 2008). Thus, the study of neuropeptides is important not only to solve the fundamental mystery of sleep-wake regulation, but also to understand the contributions of sleep and wakefulness to health and disease.

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