

Neuropharmacology of Sleep and Wakefulness

Christopher J. Watson, PhD, Helen A. Baghdoyan, PhD,
Ralph Lydic, PhD*

KEYWORDS

- Neurotransmitters • Receptors • Translational research
- Drug development

Sleep states are comprised of a constellation of physiologic and behavioral traits, and the mechanisms by which sedative-hypnotic medications alter these traits remain unclear. Drugs that enhance states of sleep also alter autonomic physiology, behavior, cognition, and affect. The complexities of brain neurochemistry and the extensive neural circuits regulating levels of behavioral arousal contribute to the present inability to understand exactly how sedative-hypnotics promote sleep. An additional complexity is that many sedative-hypnotic drugs have behavioral state-specific actions. For example, some sedative-hypnotic drugs promote the non-rapid eye movement (NREM) phase of sleep at the expense of decreasing the rapid eye movement (REM) phase of sleep. In spite of the foregoing limitations, there has been progress in developing sleep medications that maximize desired actions such as rapid sleep onset, minimal next day effect, low or no abuse potential, and creation of a drug-induced state that is indistinguishable from physiologic sleep. To date, however, no sedative-hypnotic produces all of these desired effects.

Rational drug design is an approach that has been successful in the development of antibiotic medications. Rational drug development of sedative-hypnotic medications is an approach based on understanding the receptor-binding

properties of a molecule and how a molecule alters ligand binding, neurotransmitter synthesis, release, reuptake, and degradation. All of the foregoing cellular mechanisms can then be interpreted in the context of the overall drug effect. For sedative-hypnotic medications the desired action is, of course, promoting a safe and restorative sleep-like state. This article and **Fig. 1** provide an overview of neurotransmitters and brain regions currently known to modulate states of sleep and wakefulness. This overview of sleep neuropharmacology can be read as a précis of a recent book chapter¹ and interested readers are referred elsewhere for detailed reviews on sleep.^{2–8}

γ -AMINO BUTYRIC ACID

The major inhibitory neurotransmitter in the brain is γ -aminobutyric acid (GABA), and activation of GABA_A receptors causes neuronal inhibition by increasing chloride ion conductance. Because of their powerful inhibitory effects, GABA_A receptors are the targets of most sedative-hypnotic and general anesthetic drugs. GABA_A receptors exist as multiple subtypes⁹ and these subtypes are differentially located throughout the brain.¹⁰ The differences in clinical effects caused by various benzodiazepine (eg, diazepam) and nonbenzodiazepine (eg, eszopiclone) sedative-hypnotics are attributed to the relative selectivity of these drugs

Disclosure Statement: This work supported by National Institutes of Health grants: HL40881, HL65272, HL57120, MH45361, and the Department of Anesthesiology. This work was not an industry-supported study and the authors have no financial conflicts of interest.

Department of Anesthesiology, University of Michigan, 7433 Medical Sciences Building I, 1150 West Medical Center Drive, Ann Arbor, MI 48109-5615, USA

* Corresponding author.

E-mail address: rlydic@umich.edu

Sleep Med Clin 5 (2010) 513–528

doi:10.1016/j.jsmc.2010.08.003

1556-407X/10/\$ — see front matter © 2010 Elsevier Inc. All rights reserved.

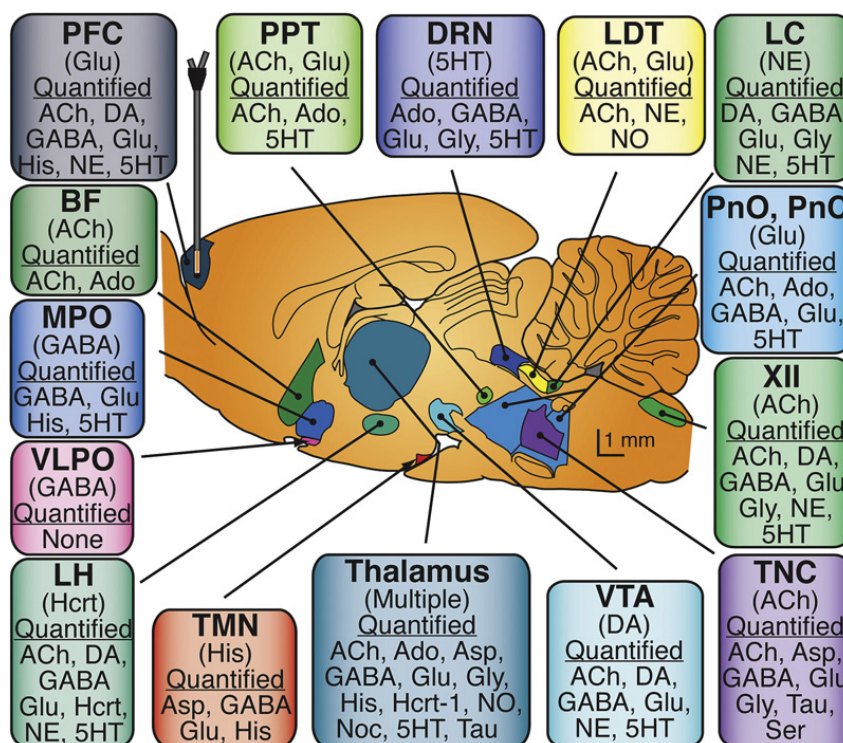


Fig. 1. Brain regions modulating sleep and wakefulness. The rat brain (sagittal view; *Modified from Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 6th edition. New York: Academic Press, 2007.*) schematizes the location, shape, and size of some brain regions that regulate sleep and wakefulness. The microdialysis probe is drawn to scale and is shown sampling from the prefrontal cortex. **Key:** brain region name-bold print; major neurotransmitters used for signaling to other brain regions-in parentheses; neurochemical analytes relevant for arousal-state control that have been measured in that brain region-listed under the header "Quantified". ACh, acetylcholine; Ado, adenosine; Asp, aspartate; BF, basal forebrain; DA, dopamine; DRN, dorsal raphe nucleus; GABA, g-aminobutyric acid; Glu, glutamate; Gly, glycine; His, histamine; Hcrt, hypocretin; 5HT, serotonin; LC, locus coeruleus; LDT, laterodorsal tegmental nucleus; LH, lateral hypothalamus; MPO, medial preoptic area; NE, norepinephrine; NO, nitric oxide; Noc, nociceptin; PFC, prefrontal cortex; PPT, pedunculopontine tegmental nucleus; PnC, pontine reticular formation, caudal part; PnO, pontine reticular formation, oral part; Ser, serine; TMN, tuberomammillary nucleus; TNC, trigeminal nucleus complex; Tau, taurine; VLPO, ventrolateral preoptic area; VTA, ventral tegmental area; XII, hypoglossal nucleus. (*From Watson CJ, Baghdoyan HA, Lydic R. A neurochemical perspective on states of consciousness. In: Suppressing the Mind: Anesthetic Modulation of Memory and Consciousness. Edited by A.G. Hudetz and R.A. Pearce, Springer/Humana Press; 2010. p. 33–80; with permission.*)

for different GABA_A receptor subtypes.¹⁰ The complexity imparted by the numerous GABA_A receptor subtypes is humbling. Although there is detailed knowledge about the many subunit isoforms that comprise GABA_A receptor subtypes,⁹ information is lacking about which of the many possible subtypes actually are expressed in specific brain regions,^{11–13} and which subtypes are localized synaptically versus extrasynaptically.¹⁴ Extrasynaptically localized GABA_A receptors possess a delta subunit and have particular relevance for sleep medicine.^{15,16}

A better understanding of the *in vivo* characteristics and anatomic localization of GABA_A receptor subtypes will contribute to rational drug development. The preclinical studies described in this section illustrate the complexity of the problem and provide examples of how the effects of

GABAergic drugs on behavior vary as a function of brain region. For example, although systemic administration of GABA-mimetic drugs promotes sleep, sedation, or general anesthesia, enhancing GABAergic transmission within the pontine reticular formation actually increases wakefulness and decreases sleep. The pontine reticular formation is part of the ascending reticular activating system and contributes to the generation of REM sleep. Direct administration into the pontine reticular formation of drugs that increase GABAergic transmission increases wakefulness and inhibits sleep.^{17–20} Similarly, pharmacologically increasing the concentration of endogenous GABA within the pontine reticular formation increases the time required for isoflurane to induce general anesthesia.²¹ Consistent with this finding are data showing that endogenous GABA levels

in the pontine reticular formation are greater during wakefulness than during REM sleep²² or during the loss of wakefulness caused by isoflurane (Fig. 2).²¹ Inhibiting GABAergic signaling at GABA_A receptors within the pontine reticular formation causes an increase in REM sleep and a decrease in wakefulness.^{18,19,23,24} Likewise, decreasing extracellular GABA levels in the pontine reticular formation of rat decreases wakefulness and increases sleep,²⁰ and shortens the time required for isoflurane to induce loss of consciousness.²¹ Furthermore, blocking GABA_A receptors in the pontine reticular formation increases time needed to regain wakefulness after isoflurane anesthesia.¹⁸ Considered together, these data demonstrate a wakefulness-promoting role for GABA in the pontine reticular formation.

In brain regions containing neurons that promote wakefulness, GABAergic inhibition has been shown to cause an increase in sleep.²⁵ These brain regions include the dorsal raphe nucleus (see Fig. 1), tuberomammillary nucleus of the posterior hypothalamus (see Fig. 1), medial preoptic area (see Fig. 1), and ventrolateral periaqueductal gray.^{7,25–27}

ACETYLCHOLINE

Acetylcholine is distinguished as being the first identified neurotransmitter. Although the first neurochemical theory of sleep²⁸ correctly posited that acetylcholine plays a primary role in generating the brain-activated states of wakefulness and REM sleep, cholinergic drugs are not part of the standard pharmacologic armamentarium of sleep disorders medicine. Nonetheless, understanding the mechanisms by which cholinergic neurotransmission generates and maintains REM sleep is crucial, because acetylcholine interacts with other transmitter systems that are targets of sleep pharmacotherapy (eg, GABAergic and

monoaminergic). Much of the research on the regulation of sleep by acetylcholine has focused on transmission mediated by muscarinic cholinergic receptors. Five subtypes (M₁–M₅) of the muscarinic receptor have been identified,²⁹ and the M₂ subtype plays a key role in the generation of REM sleep.³⁰

Cholinergic signaling originating from the latero-dorsal tegmental (LDT) and pedunculopontine tegmental (PPT) nuclei and the basal forebrain (see Fig. 1) promotes the cortically activated states of wakefulness and REM sleep.³¹ LDT/PPT neurons can be divided into two populations based on discharge pattern. One population discharges maximally during wakefulness and REM sleep (referred to as Wake-On/REM-On) and another population fires only during wakefulness (Wake-On/REM-Off).² This finding helps explain how acetylcholine can promote both wakefulness and REM sleep. LDT/PPT neurons project to numerous wakefulness-promoting brain regions.² Cholinergic terminals in the pontine reticular formation arise from the LDT/PPT,² and muscarinic receptors are present in the pontine reticular formation.^{30,32,33} Many studies have administered cholinomimetics to the pontine reticular formation and have demonstrated that cholinergic transmission in the pontine reticular formation induces REM sleep.^{2,31} Electrically stimulating the LDT/PPT increases acetylcholine release in the pontine reticular formation³⁴ and increases REM sleep.³⁵ The release of endogenous acetylcholine in the pontine reticular formation is significantly greater during REM sleep than during wakefulness or NREM sleep.^{36–38} Taken together, these data demonstrate that cholinergic projections from the LDT/PPT to the pontine reticular formation promote REM sleep.

Recent in vivo data obtained from normal rats demonstrate that the sedative-hypnotics zolpidem, diazepam, and eszopiclone differentially

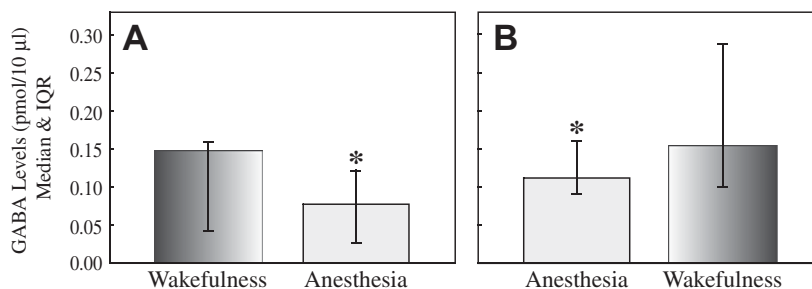


Fig. 2. Pontine reticular formation levels of GABA during wakefulness and isoflurane anesthesia. Consistent results were obtained when measuring GABA during the transition from wakefulness to anesthesia (A) and during the resumption of wakefulness after anesthesia (B). In both cases, GABA was significantly decreased during the loss of wakefulness caused by isoflurane anesthesia. (Reprinted from Vanini G, Watson CJ, Lydic R, et al. γ -aminobutyric acid-mediated neurotransmission in the pontine reticular formation modulates hypnosis, immobility, and breathing during isoflurane anesthesia. *Anesthesiology* 2008;109:978–88; with permission.)

alter acetylcholine release in the pontine reticular formation.³⁹ Intravenous administration of eszopiclone prevented the REM phase of sleep, increased electroencephalogram (EEG) delta power, and decreased acetylcholine release in rat pontine reticular formation (**Fig. 3**).³⁹ These data provide the first functional evidence for a heterogeneous distribution of GABA_A receptor subtypes within the pontine reticular formation. The different effects of GABA_A receptor agonists on sleep have been attributed to brain region-

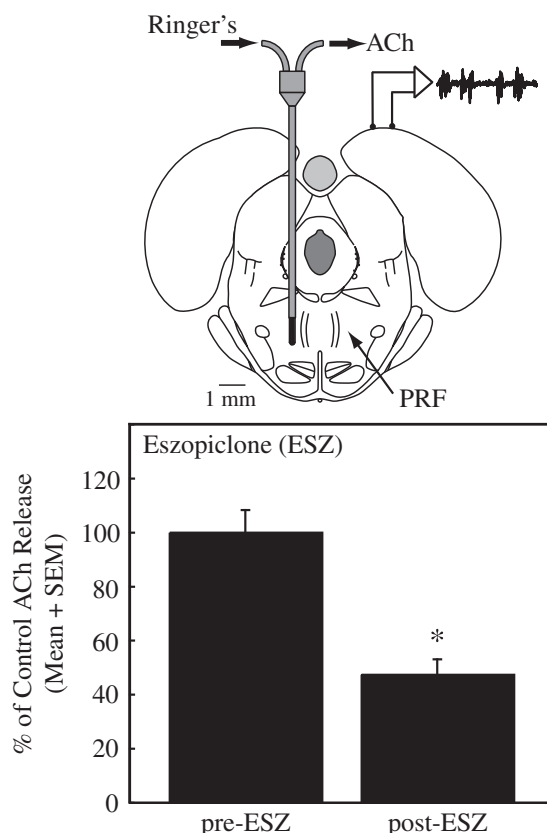


Fig. 3. Intravenous administration of eszopiclone to intact, behaving rats decreases acetylcholine (ACh) release in the pontine reticular formation (PRF). (Top) Coronal section of rat brain stem illustrates placement of a microdialysis probe in the PRF. Ringer's solution is pumped into the probe and samples are collected for quantification of ACh. (Top right) Electrodes and an amplifier for recording the cortical EEG and an EEG activity trace after intravenous administration of eszopiclone. (Bottom) Histograms summarize the significant decrease in ACh release within the PRF caused by intravenous administration of eszopiclone. (From Hambrecht-Wiedbusch VS, Gauthier EA, Baghdoyan HA, et al. Benzodiazepine receptor agonists cause drug-specific and state-specific alterations in EEG power and acetylcholine release in rat pontine reticular formation. *Sleep* 2010;33:909–18; with permission.)

specific distributions of GABA_A receptors and differences in sedative-hypnotic affinities for GABA_A receptor subtypes.⁴⁰ These preclinical data can be contrasted with human psychopharmacology where there has been no study convincingly demonstrating differential GABA_A subtype binding among benzodiazepine and non-benzodiazepine sleeping medications.⁴⁰ To date, the nonbenzodiazepine, benzodiazepine-receptor agonist eszopiclone remains the only sleeping medication for which the long-term (6 months) effects have been characterized.^{41,42}

Cholinergic neurons originating in the basal forebrain project throughout the entire cerebral cortex.⁴³ Acetylcholine release in the basal forebrain is highest during REM sleep, lower during quiet wakefulness, and lowest during NREM sleep.⁴⁴ Cortical acetylcholine release is increased during wakefulness^{43,45,46} and REM sleep⁴⁵ as compared with NREM sleep. These data support the interpretation that cholinergic transmission from the basal forebrain promotes cortical activation during wakefulness and REM sleep.

ADENOSINE

Adenosine is a breakdown product of adenosine triphosphate (ATP). Increases in endogenous adenosine levels in a specific brain region during a period of prolonged wakefulness indicate that the region has been metabolically active. Direct biochemical measures show that ATP levels increase during sleep in areas of the brain that are most active during wakefulness.⁴⁷ This finding provides direct support for the hypothesis that sleep serves a restorative function.⁴⁸

Four subtypes of adenosine receptors, A₁, A_{2A}, A_{2B}, and A₃, have been identified and are distributed widely throughout the brain. Adenosine A₁ and A_{2A} receptors are antagonized by caffeine and the idea that adenosine promotes sleep is supported by the ubiquitous consumption of caffeine to maintain wakefulness and enhance alertness. In humans, oral administration of caffeine before nocturnal sleep increases sleep latency and reduces sleep efficiency.⁴⁹ Furthermore, morning caffeine ingestion has been shown to decrease sleep efficiency and overall sleep during the subsequent night.⁵⁰ No adenosine agonists are presently available to promote sleep. Adenosine, however, is relevant for sleep medicine, as insomnia can be caused by consumption of caffeine or by the respiratory stimulant theophylline. Interestingly, adenosine can have analgesic effects and this action shows promise for clinical use.⁵¹

Adenosinergic transmission in brain regions that regulate sleep and wakefulness has been

extensively investigated.^{2,52–54} Activating adenosine A₁ receptors causes neuronal inhibition, and A₁ is the most abundant adenosine receptor subtype in brain. This section highlights selected studies supporting the interpretation that adenosine promotes sleep, at least in part, by inhibiting neurons in several key wakefulness-promoting brain areas.

Prolonged wakefulness increases adenosine levels selectively in the basal forebrain (see **Fig. 1**) and cortex,⁵⁵ and increases adenosine A₁ receptor binding in human⁵⁶ and rat⁵⁷ brain. Pharmacologically increasing adenosine levels in the basal forebrain⁵⁸ or administering adenosine A₁ receptor agonists to the basal forebrain⁵⁴ causes an increase in sleep. Inactivating adenosine A₁ receptors in the basal forebrain decreases EEG delta power and NREM sleep time,⁵⁹ and immunohistochemical studies reveal that the basal forebrain contains A₁ receptors, but not A_{2A} receptors.⁶⁰ Cholinergic neurons in the basal forebrain project to the cortex and contribute to the EEG activation characteristic of wakefulness and REM sleep. Adenosine directly inhibits cholinergic neurons in the basal forebrain by activating A₁ receptors.⁶¹ Adenosine indirectly inhibits wakefulness-promoting hypocretin (orexin)-containing neurons in the lateral hypothalamus (see **Fig. 1**) by activating A₁ receptors.⁶² Blocking adenosine A₁ receptors in the lateral hypothalamus causes an increase in wakefulness and a decrease in sleep.⁶³ Histaminergic neurons in the tuberomammillary nucleus (see **Fig. 1**) express adenosine A₁ receptors, and activating those receptors increases NREM sleep.⁶⁴ These complementary data suggest that adenosine promotes sleep by inhibiting wakefulness-promoting neurons localized to the basal forebrain, lateral hypothalamus, and tuberomammillary nucleus.

Adenosine also exerts sleep-promoting effects by actions at the level of the prefrontal cortex (see **Fig. 1**) and the pontine reticular formation (see **Fig. 1**). In vivo microdialysis experiments in mouse⁶⁵ have shown that adenosine acting at A₁ receptors in the prefrontal cortex inhibits traits that characterize wakefulness (including acetylcholine release in the prefrontal cortex and activation of the EEG), as well as the state of wakefulness. Activation of adenosine A₁ receptors in the prefrontal cortex also causes a decrease in the release of acetylcholine in the pontine reticular formation. These findings demonstrate that in the prefrontal cortex, adenosine A₁ receptors mediate a descending inhibition of wakefulness-promoting systems. Within the pontine reticular formation, activation of adenosine A_{2A} receptors increases the time needed to recover from general

anesthesia,⁶⁶ increases acetylcholine release,^{66,67} and increases the amount of time spent in NREM sleep⁶⁷ and REM sleep.^{67,68} The increase in REM sleep may be a result of the A_{2A}-mediated increase in acetylcholine release, because coadministration of a muscarinic receptor antagonist with the A_{2A} agonist blocks the REM sleep increase.⁶⁸ Studies examining the effects on sleep of adenosine receptor antagonists are required to conclude that endogenous adenosine within the pontine reticular formation modulates sleep. The finding that clinically used opioids, such as morphine and fentanyl, decrease adenosine levels in the pontine reticular formation⁶⁹ and disrupt REM sleep⁷⁰ suggests the possibility that adenosinergic transmission within the pontine reticular formation participates in REM sleep generation.

BIOGENIC AMINES

The monoamines have long been known to promote wakefulness. Serotonin (5-hydroxytryptamine; 5HT)-containing neurons of the dorsal raphe nucleus (see **Fig. 1**), norepinephrine-containing neurons of the locus coeruleus (see **Fig. 1**), and histamine-containing neurons of the tuberomammillary nucleus (see **Fig. 1**) discharge at their fastest rates during wakefulness, slow their firing in NREM sleep, cease discharging before and during REM sleep, and resume firing before the onset of wakefulness.² Dopaminergic neurons, by contrast, do not show major changes in firing rates across the sleep-wakefulness cycle.

Serotonin

Serotonin release in the dorsal raphe nucleus⁷¹ and preoptic area⁷² of rat is highest during wakefulness. Furthermore, electrical stimulation of the dorsal raphe nucleus increases wakefulness.⁷³ Serotonin receptors are divided into seven families (5HT₁-5HT₇).⁷⁴ Systemic administration of agonists for 5HT_{1A}, 5HT_{1B}, 5HT_{2A/2C} or 5HT₃ receptors causes an increase in wakefulness and a decrease in sleep.⁷⁵ Local administration of a 5HT_{1A} receptor agonist to the dorsal raphe nucleus increases wakefulness in rat⁷⁶ but increases REM sleep in cat.⁷⁷ Microinjection of a 5HT_{2A/2C} receptor agonist into rat dorsal raphe nucleus also decreases REM sleep with no significant effect on wakefulness.⁷⁸ These incongruent findings may be due to species differences, or may indicate that in addition to promoting wakefulness, serotonin plays a permissive role in the generation of REM sleep. Systemic administration of antagonists for the 5HT_{2A} receptor or the 5HT₆ receptor to rat decreases wakefulness, increases NREM sleep, and has no effect on REM sleep.⁷⁹

These data are consistent with the view that serotonin is wakefulness-promoting. Genetically modified mice also have been used to explore the role of serotonin in sleep and wakefulness. Mice lacking the genes for the 5HT_{1A}⁸⁰ or 5HT_{1B}⁸¹ receptor showed an increase in REM sleep. Administration of a 5HT_{1A},^{80,82} a 5HT_{1B},⁸¹ or a 5HT_{2A/2C}⁸³ receptor agonist decreased REM sleep in rodent and human. These data indicate that serotonin acting at 5HT_{1A}, 5HT_{1B}, and 5HT_{2A/2C} receptors plays a role in suppressing REM sleep. The forgoing data underlie the fact that insomnia can be secondary to the use of selective serotonin reuptake inhibitors (SSRI) or serotonin, norepinephrine reuptake inhibitors (SNRI).

Norepinephrine

Noradrenergic cells of the locus coeruleus inhibit REM sleep, promote wakefulness, and project to a variety of other arousal-regulating brain regions (see **Fig. 1**) including the hypothalamus, thalamus, basal forebrain, and cortex.⁸⁴ Noradrenergic receptors include α_1 -, α_2 -, and β -adrenergic subtypes.⁸⁵ Administration of noradrenaline or α - and β -receptor agonists to the medial septal area^{86,87} or the medial preoptic area^{88,89} increases wakefulness. Stimulation of locus coeruleus neurons increases noradrenaline in the prefrontal cortex of anesthetized rat,^{90,91} and contributes to cortical activation. These data are consistent with the view that noradrenaline promotes wakefulness. However, bilateral microinjection of an α_1 -antagonist (prazosin), an α_2 -agonist (clonidine), or a β -antagonist (propranolol) into the PPT increases REM sleep with little to no effect on NREM sleep or wakefulness.⁹² The arousal-regulating effects of noradrenaline are brain-region specific. The treatment of hypertension with blockers of α - and/or β -adrenergic receptors can disrupt normal sleep.

Histamine

Histaminergic cell bodies, which are located in the tuberomammillary nucleus of the posterior hypothalamus have diffuse projections throughout the brain.⁹³ Data from posterior hypothalamic lesion studies and from single unit recordings indicate that the tuberomammillary nucleus promotes wakefulness.⁹³ Three histaminergic receptors, denoted H₁, H₂, and H₃, are present in the brain.⁹⁴ First generation H₁ receptor antagonists, such as diphenhydramine, cause drowsiness (sedation) and impaired performance in humans⁹⁵ and rats.⁹⁶ Newer antagonists that are relatively selective for the H₁ histamine receptor, such as the

potent antagonist doxepin, improve subjective and objective measures of sleep in insomnia patients without causing sedation or psychomotor impairments the next day.⁹⁷ Systemic administration of the H₁ receptor antagonists mepyramine⁹⁸ and cyproheptadine⁹⁹ caused a significant increase in NREM sleep in cat and rat, respectively. Decreasing brain histamine levels by inhibiting synthesis significantly decreases wakefulness and increases NREM sleep in rat^{100,101} and cat.⁹⁸ These data suggest that histaminergic signaling via the H₁ receptor promotes wakefulness. New therapies for sleep disorders and for maintaining vigilance include H₃ receptor antagonists and inverse agonists.^{102–104}

Dopamine

Stimulants such as amphetamine, cocaine, and methylphenidate increase wakefulness and counter hypersomnia by increasing levels of endogenous dopamine.¹⁰⁵ In vivo imaging studies suggest that sleep deprivation increases dopamine levels in human brain.¹⁰⁶ The cell bodies of dopaminergic neurons that regulate arousal reside in the ventral tegmental area (see **Fig. 1**) and the substantia nigra pars compacta.⁷⁵ These dopaminergic neurons project to the dorsal raphe nucleus, basal forebrain, locus coeruleus, thalamus, and LDT.¹⁰⁷ There are also dopaminergic neurons in the ventrolateral periaqueductal gray that are active during wakefulness and have reciprocal connections with sleep-regulating brain areas.¹⁰⁸

Five dopaminergic receptors have been cloned (D1–D5). Dopaminergic neurons of the substantia nigra and ventral tegmental area do not change firing rates as a function of states of sleep and wakefulness.² Dopamine does promote wakefulness and dopamine-transporter-knockout mice display increased wakefulness and decreased NREM sleep compared with controls.¹⁰⁹ Systemic administration of D1 receptor agonists or antagonists causes an increase or decrease, respectively, in wakefulness.¹¹⁰ Intracerebroventricular administration of a D1 or D2 receptor agonist to rat increases wakefulness.¹¹¹ Systemic administration of a D2 receptor agonist causes biphasic effects with low doses decreasing wakefulness and high doses increasing wakefulness.^{112,113} Systemic administration of D-amphetamine to rat increases wakefulness and decreases NREM sleep and REM sleep.¹¹⁴ The mechanisms by which modafinil counters excessive daytime sleepiness remain to be specified. There is evidence that modafinil enhances synaptic release of dopamine and norepinephrine.¹¹⁵

GLUTAMATE

Glutamate is the main excitatory neurotransmitter in the brain and acts at α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate, and *N*-methyl-D-aspartate (NMDA) ionotropic receptors. Surprisingly, little is known about glutamatergic regulation of sleep and wakefulness. Sleep state-dependent changes in levels of endogenous glutamate change differentially across the brain.¹¹⁶ For example, glutamate levels in some areas of rat cortex show increases in concentration during wakefulness and REM sleep, and decreases during NREM sleep.¹¹⁷ Microinjection and electrophysiological studies provide evidence that glutamate acts within the LDT/PPT^{118–120} (see Fig. 1), the pontine reticular formation^{121–123} (see Fig. 1), and medial portions of the medullary reticular formation^{122,124} to modulate traits and states of arousal. Glutamatergic neurons are present in rat pontine reticular formation¹²⁵ and neurons in the pontine reticular formation are capable of synthesizing glutamate for use as a neurotransmitter.¹²⁶ Glutamate elicits excitatory responses from pontine reticular formation neurons,^{127,128} and glutamatergic and cholinergic transmission in the pontine reticular formation interact synergistically to potentiate catalepsy.¹²⁹ Given individually, agonists for AMPA, kainate, and NMDA receptors evoke excitatory responses from pontine reticular formation neurons.¹²¹ Dialysis delivery of the NMDA receptor antagonists ketamine or MK-801 to cat pontine reticular formation decreases acetylcholine release in the pontine reticular formation and disrupts breathing.³⁸

PEPTIDES

Many peptides are known to modulate sleep.¹³⁰ This article focuses on hypocretin (orexin), leptin, and ghrelin because of their relevance for sleep disorders medicine.

Hypocretin-1 and -2

Numerous lines of evidence support a role for hypocretin-1 and -2 (also called orexin A and B) in the maintenance of wakefulness. The cell bodies of hypocretin-producing neurons are localized to the dorsolateral hypothalamus^{131,132} and send projections to all the major brain regions that regulate arousal.^{133,134} Hypocretinergic neurons discharge with the highest frequency during active wakefulness and show almost no discharge activity during sleep.^{135,136} Hypocretin-1 levels in the hypothalamus of cat are greater during wakefulness and REM sleep than during NREM sleep.¹³⁷ Dogs

displaying a narcoleptic phenotype have a mutation of the hypocretin receptor-2 gene,¹³⁸ and hypocretin mRNA and peptide levels are greatly reduced in human narcoleptic patients.^{139,140} Patients presenting with narcolepsy-cataplexy also have greatly reduced levels of hypocretin in their cerebrospinal fluid compared with controls.¹⁴¹ Preclinical studies have demonstrated that selective lesions of hypocretin-containing neurons^{142,143} or genetic removal of the peptide¹⁴⁴ result in a narcoleptic phenotype. By what mechanisms might hypocretin enhance wakefulness?

Two receptors for the hypocretin peptides have been identified. Hypocretin-1 and -2 receptors have been localized to the LDT/PPT, pontine reticular formation, dorsal raphé nucleus, and locus coeruleus.^{145–149} Electrophysiological studies demonstrate that hypocretin-1 or hypocretin-2 excite neurons in these same brain regions.^{150–158} Hypocretin-1 and -2 also excite tuberomammillary neurons^{159,160} and cholinergic neurons of the basal forebrain.¹⁶¹

Intracerebroventricular administration of hypocretin-1 increases wakefulness and decreases NREM sleep and REM sleep in rat.^{162,163} When administered into the lateral preoptic area,¹⁶⁴ the LDT,¹⁶⁵ pontine reticular formation,^{20,166} or basal forebrain,^{167,168} hypocretin-1 causes an increase in wakefulness. In cat, microinjection of hypocretin-1 into the pontine reticular formation increases REM sleep if delivered during NREM sleep,¹⁵² but suppresses REM sleep if delivered during wakefulness.¹⁶⁶ The wakefulness-promoting effect of hypocretin in the pontine reticular formation is further supported by evidence that delivery of antisense oligonucleotides against the hypocretin-2 receptor to the pontine reticular formation of rat enhances REM sleep and induces catalepsy.¹⁶⁹

Measuring the effect of hypocretin-1 on the release of other arousal-regulating transmitters may provide insight into how hypocretin-1 promotes wakefulness. Microinjection of hypocretin-1 into the basal forebrain of rat increases cortical acetylcholine release.¹⁷⁰ Intracerebroventricular delivery of hypocretin-increases histamine in rodent frontal cortex¹⁷¹ and anterior hypothalamus.¹⁷² Microinjection of hypocretin-1 into the ventricles or the ventral tegmental area increases dopamine release in rat prefrontal cortex.¹⁶³ Hypocretin-1 delivered to rat dorsal raphé nucleus increases serotonin release in the dorsal raphé nucleus,¹⁷³ and dialysis delivery of hypocretin-1 to rat pontine reticular formation increases acetylcholine release¹⁷⁴ and GABA levels²⁰ in the pontine reticular formation. The increase in wakefulness produced by microinjecting hypocretin-1

into the pontine reticular formation is prevented by blocking GABA_A receptors.¹⁷⁵ This finding suggests that hypocretin may increase wakefulness, in part, by increasing GABA levels in the pontine reticular formation. Considered together, these data support the classification of hypocretin-1 as a wakefulness-promoting neuropeptide.

An alternative hypothesis is that a primary function of hypocretin is to enhance activity in motor systems and the increase in wakefulness is secondary. This hypothesis is supported by data showing that hypocretin-1 concentrations in the cerebrospinal fluid are significantly greater during active wakefulness with movement than during quiet wakefulness with no movement.¹³⁷ Hypocretinergic neurons also have very low firing rates during quiet wakefulness (without movement) compared with active wakefulness.^{135,136} Oral administration of the hypocretin-1 and -2 receptor antagonist ACT-078573 increases NREM sleep and REM sleep in rat, dog, and human,¹⁷⁶ suggesting a direct, wakefulness-promoting effect of endogenous hypocretin.

Leptin and Ghrelin

Owing to the ongoing epidemic of obesity and the association between metabolic syndrome and sleep disorders, many studies aim to understand the sleep-related roles of leptin and ghrelin. Decreased levels of leptin (a hormone that suppresses appetite) and increased levels of ghrelin (a hormone that stimulates appetite) are associated with short sleep duration in humans.^{177,178} The sleep of *ob/ob* mice (obese mice with reduced levels of leptin) is characterized by an increase in number of arousals and a decrease in the duration of sleep bouts compared with wild type controls.¹⁷⁹ The *ob/ob* mice also have an impaired response to the cholinergic enhancement of REM sleep.¹⁸⁰ Similarly, *db/db* mice (which are also obese but are resistant to leptin) have significant alterations in sleep architecture compared with wild type control mice that include, but are not limited to, increases in NREM sleep and REM sleep during the dark phase and decreases in wakefulness and NREM sleep bout duration.¹⁸¹ Local administration of ghrelin into rat lateral hypothalamus, medial preoptic area, or paraventricular nucleus increases wakefulness, decreases NREM sleep, and increases food intake.¹⁸² Together, these findings suggest that leptin and ghrelin, hormones that are important for appetite regulation, significantly influence sleep and are significantly modulated by sleep.

OPIOIDS

Opioids are the major class of drugs used to treat acute and chronic pain, and one side effect of opioids is sleep disruption. Sleep disruption, in turn, exacerbates pain^{183,184} and increases the dose of opioids required for successful pain management.^{69,70} Clinically relevant doses of opioids given to otherwise healthy humans disrupt sleep.¹⁸⁵ For example, a single intravenous infusion of morphine in healthy volunteers decreases stages 3 and 4 NREM sleep, decreases REM sleep, and increases stage 2 NREM sleep.¹⁸⁶ A nighttime dose of morphine or methadone also decreases stages 3 and 4 NREM sleep while increasing stage 2 NREM sleep.¹⁸⁷ Constant infusion of analgesic doses of remifentanyl overnight decreases REM sleep in healthy volunteers.¹⁸⁸ The cycle of opioid-induced sleep disruption leading to increased pain and increased opioid requirement is recognized as a significant clinical problem that must be addressed at the mechanistic level.¹⁸⁹

Opioid-induced disruption of REM sleep is mediated, at least in part, by decreasing acetylcholine release in the pontine reticular formation.⁷⁰ Opioids also decrease adenosine levels in the basal forebrain and in the pontine reticular formation,⁶⁹ two brain regions where adenosine has sleep-promoting effects. Local administration of morphine into the pontine reticular formation of cat¹⁹⁰ or rat¹⁹¹ increases wakefulness and decreases REM sleep.

FUTURE DIRECTIONS

This selective overview was completed during the summer of 2010, a date also marking the 20th anniversary of the human genome project. The stunning successes—and unmet hopes—of genomic approaches to medicine were highlighted in the June 12th and 14th issues of *The New York Times*.^{192,193} These two articles offer a sobering reminder that taking a molecule from preclinical discovery to commercially available drug typically requires 15 or more years. This time interval is without any mandate to understand the mechanisms of drug action. As a former director of research and development at Wyeth noted “Genomics did not speed up drug development. It gave us more rapid access to new molecular targets.”¹⁹³ Potential molecular targets can be rapidly interrogated with high throughput screening programs that use a cell line transfected to contain a reporter construct. But identifying potential molecular targets leaves unanswered the question of whether the candidate targets will

be druggable in vivo. This complexity is exemplified by sedative-hypnotic medications commonly used in sleep medicine. GABA_A receptors are drug targets that promote a sleep-like state by unknown actions⁴⁰ when they are activated in some brain regions, yet GABA_A receptors enhance wakefulness when activated selectively in the posterior hypothalamus¹⁹⁴ or pontine reticular formation.^{18,19,21} As intricate as Fig. 1 may seem, it barely hints at the complexity of data that must be logically integrated if we are to derive a coherent model of the endogenous neurochemical processes that regulate states of sleep and wakefulness.

Recent progress in understanding the basic neuropharmacology of sleep can be appreciated by comparing the 1990 and the 2005 editions of *Brain Control of Wakefulness and Sleep*.² The incorporation of basic neuropharmacology into sleep disorders medicine is readily apparent by comparing the first and most recent editions of *Principles and Practice of Sleep Medicine*.¹⁹⁵ Future progress is most likely to come from a systems biology approach that seeks to integrate genomic, cellular, network, and behavioral levels of analysis.¹⁹⁶ The focus on sleep medications in the Clinics of North America series demonstrates the cross-cutting relevance of sleep for the practice of medicine. The pressing clinical problem of sleep disorders medicine will continue to stimulate advances in understanding the neurochemical regulation of sleep.

ACKNOWLEDGMENTS

We thank Mary A. Norat and Sarah L. Watson for critical comments on this article.

REFERENCES

1. Watson CJ, Baghdoyan HA, Lydic R. A neurochemical perspective on states of consciousness. In: Hudetz AG, Pearce RA, editors. *Suppressing the mind: anesthetic modulation of memory and consciousness*. New York (NY): Springer/Humana Press; 2010. p. 33–80.
2. Steriade M, McCarley RW, editors. *Brain control of wakefulness and sleep*. New York: Kluwer Academic/Plenum Publishers; 2005. p. 1–728.
3. Datta S, MacLean RR. Neurobiological mechanisms for the regulation of mammalian sleep-wake behavior: reinterpretation of historical evidence and inclusion of contemporary cellular and molecular evidence. *Neurosci Biobehav Rev* 2007;31:775–824.
4. McCarley RW. Neurobiology of REM and NREM sleep. *Sleep Med* 2007;8:302–30.
5. Stenberg D. Neuroanatomy and neurochemistry of sleep. *Cell Mol Life Sci* 2007;64:1187–204.
6. Monti JM, Pandi-Perumal SR, Sinton CM, editors. *Neurochemistry of sleep and wakefulness*. New York: Cambridge University Press; 2008. p. 1–482.
7. Szymusiak R, McGinty D. Hypothalamic regulation of sleep and arousal. *Ann N Y Acad Sci* 2008;1129:275–86.
8. Mallick BN, Pandi-Perumal SR, McCarley RW, et al, editors. *Rapid eye movement sleep: regulation and function*. New York: Cambridge University Press; 2010, in press.
9. Olsen RW, Sieghart W. International union of pharmacology. LXX. Subtypes of γ -aminobutyric acid_A receptors: classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol Rev* 2008;60:243–60.
10. Winsky-Sommerer R. Role of GABA_A receptors in the physiology and pharmacology of sleep. *Eur J Neurosci* 2009;29:1779–94.
11. Fritschy J-M, Möhler H. GABA_A-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. *J Comp Neurol* 1995;359:154–94.
12. Heldt SA, Ressler KJ. Forebrain and midbrain distribution of major benzodiazepine-sensitive GABA_A receptor subunits in the adult C57 mouse as assessed with in situ hybridization. *Neuroscience* 2007;150:370–85.
13. Pirker S, Schwarzer C, Wieselthaler A, et al. GABA_A receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience* 2000;101:815–50.
14. Farrant M, Nusser M. Variations on an inhibitory theme: phasic and tonic activation of GABA_A receptors. *Nat Rev Neurosci* 2005;6:215–29.
15. Orser BA. Extrasynaptic GABA_A receptors are critical targets for sedative-hypnotic drugs. *J Clin Sleep Med* 2006;2:S12–8.
16. Walsh JK, Deacon S, Dijk D-J, et al. The selective extrasynaptic GABA_A agonist, gaboxadol, improves traditional hypnotic efficacy measures and enhances slow wave activity in a model of transient insomnia. *Sleep* 2007;30:593–602.
17. Camacho-Arroyo I, Alvarado R, Manjarrez J, et al. Microinjections of muscimol and bicuculline into the pontine reticular formation modify the sleep-waking cycle in the rat. *Neurosci Lett* 1991;129:95–7.
18. Flint R, Chang T, Lydic R, et al. GABA_A receptors in the pontine reticular formation of C57BL/6J mouse modulate neurochemical, electrographic, and behavioral phenotypes of wakefulness. *J Neurosci* 2010;30:12301–9.
19. Xi M-C, Morales FR, Chase MH. Evidence that wakefulness and REM sleep are controlled by a GABAergic pontine mechanism. *J Neurophysiol* 1999;82:2015–9.

20. Watson CJ, Lydic R, Baghdoyan HA. Pontine reticular formation (PnO) administration of hypocretin-1 increases PnO GABA levels and wakefulness. *Sleep* 2008;31:453–64.
21. Vanini G, Watson CJ, Lydic R, et al. γ -aminobutyric acid-mediated neurotransmission in the pontine reticular formation modulates hypnosis, immobility, and breathing during isoflurane anesthesia. *Anesthesiology* 2008;109:978–88.
22. Vanini G, Wathen BL, Lydic R, et al. GABA levels in cat pontine reticular formation (PRF) are lower during rapid eye movement (REM) sleep and the neostigmine-induced REM sleep-like state (REM-Neo) than during wakefulness [abstract]. *Sleep* 2009;32(Suppl):0011.
23. Sanford LD, Tang X, Xiao J, et al. GABAergic regulation of REM sleep in reticularis pontis oralis and caudalis in rats. *J Neurophysiol* 2003;90:938–45.
24. Marks GA, Sachs OW, Birabil CG. Blockade of GABA, type A, receptors in the rat pontine reticular formation induces rapid eye movement sleep that is dependent upon the cholinergic system. *Neuroscience* 2008;156:1–10.
25. Vanini G, Tortorolo P, McGregor R, et al. GABAergic processes in the mesencephalic tegmentum modulate the occurrence of active (rapid eye movement) sleep in guinea pigs. *Neuroscience* 2007;145:1157–67.
26. Vanini G, Baghdoyan HA, Lydic R. Relevance of sleep neurobiology for cognitive neuroscience and anesthesiology. In: Mashour GA, editor. *Consciousness, awareness, and anesthesia*. New York: Cambridge University Press; 2010. p. 1–23.
27. Vanini G, Lydic R, Baghdoyan HA. GABAergic modulation of REM sleep. In: Mallick BN, Pandi-Perumal R, McCarley RW, et al, editors. *Rapid eye movement sleep—regulation and function*. Cambridge University Press, in press.
28. Jouvet M. The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep-waking cycle. *Ergeb Physiol* 1972;64:166–307.
29. Ishii M, Kurachi Y. Muscarinic acetylcholine receptors. *Curr Pharm Des* 2006;12:3573–81.
30. Baghdoyan HA, Lydic R. M2 muscarinic receptor subtype in the feline medial pontine reticular formation modulates the amount of rapid eye movement sleep. *Sleep* 1999;22:835–47.
31. Lydic R, Baghdoyan HA. Acetylcholine modulates sleep and wakefulness: a synaptic perspective. In: Monti JM, Pandi-Perumal SR, Sinton CM, editors. *Neurochemistry of sleep and wakefulness*. Cambridge (UK): Cambridge University Press; 2008. p. 109–43.
32. Baghdoyan HA. Location and quantification of muscarinic receptor subtypes in rat pons: implications for REM sleep generation. *Am J Physiol* 1997;273:R896–904.
33. DeMarco GJ, Baghdoyan HA, Lydic R. Differential cholinergic activation of G proteins in rat and mouse brainstem: relevance for sleep and nociception. *J Comp Neurol* 2003;457:175–84.
34. Lydic R, Baghdoyan HA. Pedunculopontine stimulation alters respiration and increases ACh release in the pontine reticular formation. *Am J Physiol* 1993;264:R544–54.
35. Thakkar M, Portas C, McCarley RW. Chronic low-amplitude electrical stimulation of the laterodorsal tegmental nucleus of freely moving cats increases REM sleep. *Brain Res* 1996;723:223–7.
36. Kodama T, Takahashi Y, Honda Y. Enhancement of acetylcholine release during paradoxical sleep in the dorsal tegmental field of the cat brain stem. *Neurosci Lett* 1990;114:277–82.
37. Leonard TO, Lydic R. Pontine nitric oxide modulates acetylcholine release, rapid eye movement sleep generation, and respiratory rate. *J Neurosci* 1997;17:774–85.
38. Lydic R, Baghdoyan HA. Ketamine and MK-801 decrease acetylcholine release in the pontine reticular formation, slow breathing, and disrupt sleep. *Sleep* 2002;25:615–20.
39. Hambrecht-Wiedbusch VS, Gauthier E, Baghdoyan HA, et al. Zolpidem, eszopiclone, and diazepam differentially alter EEG delta power and acetylcholine release in the pontine reticular formation of Sprague-Dawley rat. *Sleep* 2010;33:909–18.
40. Krystal AD. In vivo evidence of the specificity of effects of GABA_A receptor modulating medications. *Sleep* 2010;33:859–60.
41. Krystal AD, Walsh JK, Laska E, et al. Sustained efficacy of eszopiclone over 6 months of nightly treatment: results of a randomized, double-blind, placebo-controlled study in adults with chronic insomnia. *Sleep* 2003;26:793–8.
42. Walsh JK, Krystal AD, Amato DA, et al. Nightly treatment of primary insomnia with eszopiclone for six months: effect on sleep, quality of life, and work limitations. *Sleep* 2007;30:959–68.
43. Sarter M, Bruno JP. Cortical cholinergic inputs mediating arousal, attentional processing and dreaming: differential afferent regulation of the basal forebrain by telencephalic and brainstem afferents. *Neuroscience* 2000;95:933–52.
44. Vazquez J, Baghdoyan HA. Basal forebrain acetylcholine release during REM sleep is significantly greater than during waking. *Am J Physiol Regul Integr Comp Physiol* 2001;280:R598–601.
45. Marrosu F, Portas C, Mascia MS, et al. Microdialysis measurement of cortical and hippocampal acetylcholine release during sleep-wake cycle in freely moving cats. *Brain Res* 1995;671:329–32.

46. Materi LM, Rasmusson DD, Semba K. Inhibition of synaptically evoked cortical acetylcholine release by adenosine: an in vivo microdialysis study in the rat. *Neuroscience* 2000;97:219–26.
47. Dworak M, McCarley RW, Kim T, et al. Sleep and brain energy levels: ATP changes during sleep. *J Neurosci* 2010;30:9007–16.
48. Benington JH, Heller HC. Restoration of brain energy metabolism as the function of sleep. *Prog Neurobiol* 1995;45:347–60.
49. Landolt HP, Dijk DJ, Gaus SE, et al. Caffeine reduces low-frequency delta activity in the human sleep EEG. *Neuropsychopharmacology* 1995;12:229–38.
50. Landolt HP, Werth E, Borbely AA, et al. Caffeine intake (200 mg) in the morning affects human and EEG power spectra at night. *Brain Res* 1995;675:67–74.
51. Gan TJ, Habib AS. Adenosine as a non-opioid analgesic in the perioperative setting. *Anesth Analg* 2007;105:487–94.
52. Radulovacki M. Adenosine sleep theory: how I postulated it. *Neurol Res* 2005;27:137–8.
53. Basheer R, Strecker RE, Thakkar M, et al. Adenosine and sleep-wake regulation. *Prog Neurobiol* 2004;73:379–96.
54. Strecker RE, Moriarty S, Thakkar MM, et al. Adenosinergic modulation of basal forebrain and pre-optic/anterior hypothalamic neuronal activity in the control of behavioral state. *Behav Brain Res* 2000;115:183–204.
55. Porkka-Heiskanen T, Strecker RE, McCarley RW. Brain site-specificity of extracellular adenosine concentration changes during sleep deprivation and spontaneous sleep: an in vivo microdialysis study. *Neuroscience* 2000;99:507–17.
56. Elmenhorst D, Meyer PT, Winz OH, et al. Sleep deprivation increases A₁ adenosine receptor binding in the human brain: a positron emission tomography study. *J Neurosci* 2007;27:2410–5.
57. Elmenhorst D, Basheer R, McCarley RW, et al. Sleep deprivation increases A₁ adenosine receptor density in the rat brain. *Brain Res* 2009;1258:53–8.
58. Porkka-Heiskanen T, Strecker RE, Thakkar M, et al. Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness. *Science* 1997;276:1265–8.
59. Thakkar MM, Winston S, McCarley RW. A₁ receptor and adenosine homeostatic regulation of sleep-wakefulness: effects of antisense to the A₁ receptor in the cholinergic basal forebrain. *J Neurosci* 2003;23:4278–87.
60. Basheer R, Halldner L, Alanko L, et al. Opposite changes in adenosine A₁ and A_{2A} receptor mRNA in the rat following sleep deprivation. *Neuroreport* 2001;12:1577–80.
61. Arrigoni E, Chamberlin NL, Saper CB, et al. Adenosine inhibits basal forebrain cholinergic and non-cholinergic neurons in vitro. *Neuroscience* 2006;140:403–13.
62. Liu Z-W, Gao XB. Adenosine inhibits activity of hypocretin/orexin neurons by the A₁ receptor in the lateral hypothalamus: a possible sleep-promoting effect. *J Neurophysiol* 2007;97:837–48.
63. Thakkar MM, Engemann SC, Walsh KM, et al. Adenosine and the homeostatic control of sleep: effects of A₁ receptor blockade in the perifornical lateral hypothalamus. *Neuroscience* 2008;153:875–80.
64. Oishi Y, Huang Z-L, Fredholm BB, et al. Adenosine in the tuberomammillary nucleus inhibits the histaminergic system via A₁ receptors and promotes non-rapid eye movement sleep. *Proc Natl Acad Sci U S A* 2008;105:19992–7.
65. Van Dort CJ, Baghdoyan HA, Lydic R. Adenosine A₁ and A_{2A} receptors in mouse prefrontal cortex modulate acetylcholine release and behavioral arousal. *J Neurosci* 2009;29:871–81.
66. Tanase D, Baghdoyan HA, Lydic R. Dialysis delivery of an adenosine A₁ receptor agonist to the pontine reticular formation decreases acetylcholine release and increases anesthesia recovery time. *Anesthesiology* 2003;98:912–20.
67. Coleman CG, Baghdoyan HA, Lydic R. Dialysis delivery of an adenosine A_{2A} agonist into the pontine reticular formation of C57BL/6J mouse increases pontine acetylcholine release and sleep. *J Neurochem* 2006;96:1750–9.
68. Marks GA, Shaffery JP, Speciale SG, et al. Enhancement of rapid eye movement sleep in the rat by actions at A₁ and A_{2A} adenosine receptor subtypes with a differential sensitivity to atropine. *Neuroscience* 2003;116:913–20.
69. Nelson AM, Battersby AS, Baghdoyan HA, et al. Opioid-induced decreases in rat brain adenosine levels are reversed by inhibiting adenosine deaminase. *Anesthesiology* 2009;111:1327–33.
70. Lydic R, Baghdoyan HA. Neurochemical mechanisms mediating opioid-induced REM sleep disruption. In: Lavigne G, Sessle B, Choinière M, et al, editors. *Sleep and pain*. Seattle (WA): International Association for the Study of Pain Press; 2007. p. 99–122.
71. Portas CM, Bjorvatn B, Fagerland S, et al. On-line detection of extracellular levels of serotonin in dorsal raphe nucleus and frontal cortex over the sleep/wake cycle in the freely moving rat. *Neuroscience* 1998;83:807–14.
72. Python A, Steimer T, de Saint Hilaire Z, et al. Extracellular serotonin variations during vigilance states in the preoptic area of rats: a microdialysis study. *Brain Res* 2001;910:49–54.
73. Houdouin F, Cespuaglio R, Jouvet M. Effects induced by the electrical stimulation of the nucleus

- raphé dorsalis upon hypothalamic release of 5-hydroxyindole compounds and sleep parameters in the rat. *Brain Res* 1991;565:48–56.
74. Fink KB, Gothert M. 5-HT receptor regulation of neurotransmitter release. *Pharmacol Rev* 2007;59:360–417.
 75. Monti JM, Jantos H. The roles of dopamine and serotonin, and of their receptors, in regulating sleep and waking. *Prog Brain Res* 2008;172:625–46.
 76. Monti JM, Jantos H. Dose-dependent effects of the 5-HT_{1A} receptor agonist 8-OH-DPAT on sleep and wakefulness in the rat. *J Sleep Res* 1992;1:169–75.
 77. Portas CM, Thakkar M, Rainnie D, et al. Microdialysis perfusion of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) in the dorsal raphé nucleus decreases serotonin release and increases rapid eye movement sleep in the freely moving cat. *J Neurosci* 1996;16:2820–8.
 78. Monti JM, Jantos H. Effects of activation and blockade of 5-HT_{2A/2C} receptors in the dorsal raphé nucleus on sleep and waking in the rat. *Prog Neuropsychopharmacol Biol Psychiatry* 2006;30:1189–95.
 79. Moriarty S, Hedley L, Flores J, et al. Selective 5-HT_{2A} and 5-HT₆ receptor antagonists promote sleep in rats. *Sleep* 2008;31:34–44.
 80. Boutrel B, Monaca C, Hen R, et al. Involvement of 5-HT_{1A} receptors in homeostatic and stress-induced adaptive regulations of paradoxical sleep: studies in 5-HT_{1A} knock-out mice. *J Neurosci* 2002;22:4686–92.
 81. Boutrel B, Franc B, Hen R, et al. Key role of 5-HT_{1B} receptors in the regulation of paradoxical sleep as evidenced in 5-HT_{1B} knock-out mice. *J Neurosci* 1999;19:3204–12.
 82. Wilson SJ, Bailey JE, Rich AS, et al. The use of sleep measures to compare a new 5HT_{1A} agonist with buspirone in humans. *J Psychopharmacol* 2005;19:609–13.
 83. Monti JM, Jantos H. Effects of the serotonin 5-HT_{2A/2C} receptor agonist DOI and of the selective 5-HT_{2A} or 5-HT_{2C} receptor antagonists EMD 281014 and SB-243213, respectively, on sleep and waking in the rat. *Eur J Pharmacol* 2006;553:163–70.
 84. Berridge CW, Waterhouse BD. The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res Rev* 2003;42:33–84.
 85. Hein L. Adrenoceptors and signal transduction in neurons. *Cell Tissue Res* 2006;326:541–51.
 86. Berridge CW, Foote SL. Enhancement of behavioral and electroencephalographic indices of waking following stimulation of noradrenergic beta-receptors within the medial septal region of the basal forebrain. *J Neurosci* 1996;16:6999–7009.
 87. Berridge CW, Isaac SO, Espana RA. Additive wake-promoting actions of medial basal forebrain noradrenergic α ₁- and β -receptor stimulation. *Behav Neurosci* 2003;117:350–9.
 88. Kumar VM, Datta S, Chhina GS, et al. Alpha adrenergic system in medial preoptic area in sleep-wakefulness in rats. *Brain Res Bull* 1986;16:463–8.
 89. Sood S, Dhawan JK, Ramesh V, et al. Role of medial preoptic area beta adrenoceptors in the regulation of sleep-wakefulness. *Pharmacol Biochem Behav* 1997;57:1–5.
 90. Berridge CW, Abercrombie ED. Relationship between locus coeruleus discharge rates and rates of norepinephrine release within the neocortex as assessed by in vivo microdialysis. *Neuroscience* 1999;93:1263–70.
 91. Florin-Lechner SM, Druhan JP, Aston-Jones G, et al. Enhanced norepinephrine release in prefrontal cortex with burst stimulation of the locus coeruleus. *Brain Res* 1996;742:89–97.
 92. Pal D, Mallick BN. Role of noradrenergic and GABA-ergic inputs in pedunculo-pontine tegmentum for regulation of rapid eye movement sleep in rats. *Neuropharmacology* 2006;51:1–11.
 93. Haas HL, Sergeeva OA, Selbach O. Histamine in the nervous system. *Physiol Rev* 2008;88(3):1183–241.
 94. Haas HL, Panula P. The role of histamine and the tuberomammillary nucleus in the nervous system. *Nat Rev Neurosci* 2003;4:121–30.
 95. Nicholson AN, Stine BM. Antihistamines: impaired performance and the tendency to sleep. *Eur J Clin Pharmacol* 1986;30:27–32.
 96. Kaneko Y, Shimada K, Saitou K, et al. The mechanism responsible for the drowsiness caused by first generation H₁ antagonists on the EEG pattern. *Methods Find Exp Clin Pharmacol* 2000;22:163–8.
 97. Roth T, Rogowski R, Hull S, et al. Efficacy and safety of doxepin 1 mg, 3 mg, and 6 mg in adults with primary insomnia. *Sleep* 2007;30:1555–61.
 98. Lin JS, Sakai K, Jouvet M. Evidence for histaminergic arousal mechanisms in the hypothalamus of cats. *Neuropharmacology* 1988;27:111–22.
 99. Tokunaga S, Takeda Y, Shinomiya K, et al. Effects of some H₁-antagonists on the sleep-wake cycle in sleep-disturbed rats. *J Pharmacol Sci* 2007;103:201–6.
 100. Monti JM, D'Angelo L, Jantos H, et al. Effects of α -fluoromethylhistidine on sleep and wakefulness in the rat. *J Neural Transm* 1988;72:141–5.
 101. Kiyono S, Seo ML, Shibagaki M, et al. Effects of α -fluoromethylhistidine on sleep-waking parameters in rats. *Physiol Behav* 1985;34:615–7.
 102. Parmentier R, Anaclet C, Guhenne C, et al. The brain H₃-receptor as a novel therapeutic target

- for vigilance and sleep-wake disorders. *Biochem Pharmacol* 2007;73:1157–71.
103. Ligneau X, Perrin D, Landais L, et al. BF2.649 [1-[3-[3-(4-Chlorophenyl)propoxy]propyl]piperidine, hydrochloride], a nonimidazole inverse agonist/antagonist at the human histamine H₃ receptor: preclinical pharmacology. *J Pharmacol Exp Ther* 2007;320:365–75.
 104. Le S, Gruner JA, Mathiasen JR, et al. Correlation between ex vivo receptor occupancy and wake-promoting activity of selective H₃ receptor antagonists. *J Pharmacol Exp Ther* 2008;325:902–9.
 105. Boutrel B, Koob GF. What keeps us awake: the neuropharmacology of stimulants and wakefulness-promoting medications. *Sleep* 2004;27:1181–94.
 106. Volkow ND, Wang G-J, Telang F, et al. Sleep deprivation decreases binding of [¹¹C]raclopride to dopamine D₂/D₃ receptors in the human brain. *J Neurosci* 2008;28:8454–61.
 107. Monti JM, Monti D. The involvement of dopamine in the modulation of sleep and waking. *Sleep Med Rev* 2007;11:113–33.
 108. Lu J, Jhou TC, Saper CB. Identification of wake-active dopaminergic neurons in the ventral periaqueductal gray matter. *J Neurosci* 2006;26:193–202.
 109. Wisor JP, Nishino S, Sora I, et al. Dopaminergic role in stimulant-induced wakefulness. *J Neurosci* 2001;21:1787–94.
 110. Monti JM, Fernandez M, Jantos H. Sleep during acute dopamine D₁ agonist SKF 38393 or D₁ antagonist SCH 23390 administration in rats. *Neuropsychopharmacology* 1990;3:153–62.
 111. Isaac SO, Berridge CW. Wake-promoting actions of dopamine D₁ and D₂ receptor stimulation. *J Pharmacol Exp Ther* 2003;307:386–94.
 112. Monti JM, Hawkins M, Jantos H, et al. Biphasic effects of dopamine D-2 receptor agonists on sleep and wakefulness in the rat. *Psychopharmacology (Berl)* 1988;95:395–400.
 113. Monti JM, Jantos H, Fernandez M. Effects of the selective dopamine D-2 receptor agonist, quinpirole on sleep and wakefulness in the rat. *Eur J Pharmacol* 1989;169:61–6.
 114. Anderson ML, Margis R, Frey BN, et al. Electrophysiological correlates of sleep disturbance induced by acute and chronic administration of D-amphetamine. *Brain Res* 2009;1249:162–72.
 115. Minzenberg MJ, Carter CS. Modafinil: a review of neurochemical actions and effects on cognition. *Neuropsychopharmacology* 2008;33:1477–502.
 116. Brevig HN, Baghdoyan HA. Neurotransmitters and neuromodulators regulating sleep and wakefulness. In: Koob GF, Le Moa M, Thompson RF, editors. *Encyclopedia of behavioral neuroscience*, vol. 3: Oxford (UK): Academic Press; 2010. p. 456–63.
 117. Dash MB, Douglas CL, Vyazovskiy VV, et al. Long-term homeostasis of extracellular glutamate in the rat cerebral cortex across sleep and waking states. *J Neurosci* 2009;29:620–9.
 118. Datta S, Patterson EH, Spoley EE. Excitation of the pedunculopontine tegmental NMDA receptors induces wakefulness and cortical activation in the rat. *J Neurosci Res* 2001;66:109–16.
 119. Datta S, Spoley EE, Patterson EH. Microinjection of glutamate into the pedunculopontine tegmentum induces REM sleep and wakefulness in the rat. *Am J Physiol Regul Integr Comp Physiol* 2001;280:R752–9.
 120. Datta S, Spoley EE, Mavanji VK, et al. A novel role of pedunculopontine tegmental kainate receptors: a mechanism of rapid eye movement sleep generation in the rat. *Neuroscience* 2002;114:157–64.
 121. Stevens DR, McCarley RW, Greene RW. Excitatory amino acid-mediated responses and synaptic potentials in medial pontine reticular formation neurons of the rat in vitro. *J Neurosci* 1992;12:4188–94.
 122. Lai YY, Siegel JM. Pontomedullary glutamate receptors mediating locomotion and muscle tone suppression. *J Neurosci* 1991;11:2931–7.
 123. Onoe H, Sakai K. Kainate receptors: a novel mechanism in paradoxical (REM) sleep generation. *Neuroreport* 1995;6:353–6.
 124. Lai YY, Siegel JM. Medullary regions mediating atonia. *J Neurosci* 1988;8:4790–6.
 125. Kaneko T, Itoh K, Shigemoto R, et al. Glutaminase-like immunoreactivity in the lower brainstem and cerebellum of the adult rat. *Neuroscience* 1989;32:79–98.
 126. Jones BE. Arousal states. *Front Biosci* 2003;8:S438–51.
 127. Núñez A, Buño W, Reinoso-Suárez F. Neurotransmitter actions on oral pontine tegmental neurons of the rat: an in vitro study. *Brain Res* 1998;804:144–8.
 128. Greene RW, Carpenter DO. Actions of neurotransmitters on pontine medial reticular formation neurons of cat. *J Neurophysiol* 1985;54:520–31.
 129. Elazar Z, Berchanski A. Glutamatergic-cholinergic synergistic interaction in the pontine reticular formation. Effects on catalepsy. *Naunyn-Schmiedeberg Arch Pharmacol* 2001;363:569–76.
 130. De Lecea L. Neuropeptides and sleep-wake regulation. In: Monti JM, Pandi-Perumal R, Sinton CM, editors. *Neurochemistry of sleep and wakefulness*. New York: Cambridge University Press; 2008. p. 387–401.
 131. de Lecea L, Kilduff TS, Peyron C, et al. The hypocretins: hypothalamus-specific peptides with

- neuroexcitatory activity. *Proc Natl Acad Sci U S A* 1998;95:322–7.
132. Sakurai T, Amemiya A, Ishii M, et al. Orexin and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 1998;92:572–95.
 133. Peyron C, Tighe DK, van den Pol AN, et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci* 1998;18:9996–10015.
 134. Zhang J-H, Sarpogha S, Morales FR, et al. Distribution of hypocretin (orexin) immunoreactivity in the feline pons and medulla. *Brain Res* 2004;995:205–17.
 135. Lee MG, Hassani OK, Jones BE. Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. *J Neurosci* 2005;25:6716–20.
 136. Mileykovskiy BY, Kiyashchenko LI, Siegel JM. Behavioral correlates of activity in identified hypocretin/orexin neurons. *Neuron* 2005;46:787–98.
 137. Kiyashchenko LI, Mileykovskiy BY, Maidment N, et al. Release of hypocretin (orexin) during waking and sleep states. *J Neurosci* 2002;22:5282–6.
 138. Lin L, Faraco J, Li R, et al. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* 1999;98:365–76.
 139. Thannickal TC, Moore RY, Nienhuis R, et al. Reduced number of hypocretin neurons in human narcolepsy. *Neuron* 2000;27:469–74.
 140. Peyron C, Faraco J, Rogers W, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat Med* 2000;6:991–7.
 141. Nishino S, Kanbayashi T. Symptomatic narcolepsy, cataplexy, and hypersomnia, and their implications in the hypothalamic hypocretin/orexin system. *Sleep Med Rev* 2005;9:269–310.
 142. Beuckmann CT, Sinton CM, Williams SC, et al. Expression of a poly-glutamine-ataxin-3 transgene in orexin neurons induces narcolepsy-cataplexy in the rat. *J Neurosci* 2004;24:4469–77.
 143. Murillo-Rodriguez E, Liu M, Blanco-Centurion C, et al. Effects of hypocretin (orexin) neuronal loss on sleep and extracellular adenosine levels in the rat basal forebrain. *Eur J Neurosci* 2008;28:1191–8.
 144. Willie JT, Chemelli RM, Sinton CM, et al. Distinct narcolepsy syndromes in orexin receptor-2 and orexin null mice: molecular genetic dissection of non-REM and REM sleep regulatory processes. *Neuron* 2003;38:715–30.
 145. Bernard R, Lydic R, Baghdoyan HA. Hypocretin-1 causes G protein activation and increases ACh release in rat pons. *Eur J Neurosci* 2003;18:1775–85.
 146. Greco MA, Shiromani PJ. Hypocretin receptor protein and mRNA expression in the dorsolateral pons of rats. *Brain Res Mol Brain Res* 2001;88:176–82.
 147. Hervieu GJ, Cluderay JE, Harrison DC, et al. Gene expression and protein distribution of the orexin-1 receptor in the rat brain and spinal cord. *Neuroscience* 2001;103:777–92.
 148. Marcus JN, Aschenasi CN, Lee CE, et al. Differential expression of orexin receptors 1 and 2 in the rat brain. *J Comp Neurol* 2001;435:6–25.
 149. Brischox F, Mainville L, Jones BE. Muscarinic-2 and orexin-2 receptors on GABAergic and other neurons in the rat mesopontine tegmentum and their potential role in sleep-wake control. *J Comp Neurol* 2008;510:607–30.
 150. Burlet S, Tyler CJ, Leonard CS. Direct and indirect excitation of laterodorsal tegmental neurons by hypocretin/orexin peptides: implications for wakefulness and narcolepsy. *J Neurosci* 2002;22:2862–72.
 151. Takahashi K, Koyama Y, Kayama Y, et al. Effects of orexin on the laterodorsal tegmental neurons. *Psychiatry Clin Neurosci* 2002;56:335–6.
 152. Xi M-C, Fung SJ, Yamuy J, et al. Induction of active (REM) sleep and motor inhibition by hypocretin in the nucleus pontis oralis of cat. *J Neurophysiol* 2002;87:2880–8.
 153. Liu R-J, van den Pol AN, Aghajanian GK. Hypocretins (orexins) regulate serotonin neurons in the dorsal raphe nucleus by excitatory direct and inhibitory indirect actions. *J Neurosci* 2002;22:9453–64.
 154. Soffin EM, Gill CH, Brough SJ, et al. Pharmacological characterization of the orexin receptor subtype mediating postsynaptic excitation in the rat dorsal raphe nucleus. *Neuropharmacology* 2004;46:1168–76.
 155. Brown RE, Sergeeva O, Eriksson KS, et al. Convergent excitation of dorsal raphe serotonin neurons by multiple arousal systems (orexin/hypocretin, histamine and noradrenaline). *J Neurosci* 2002;22:8850–9.
 156. Bourgin P, Huitron-Resendiz S, Spier AD, et al. Hypocretin-1 modulates rapid eye movement sleep through activation of locus coeruleus neurons. *J Neurosci* 2000;20:7760–5.
 157. Hagan JJ, Leslie RA, Patel S, et al. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. *Proc Natl Acad Sci U S A* 1999;96:10911–6.
 158. Horvath TL, Peyron C, Diano S, et al. Hypocretin (orexin) activation and synaptic innervation of the locus coeruleus noradrenergic system. *J Comp Neurol* 1999;415:145–59.
 159. Eriksson KS, Sergeeva O, Brown RE, et al. Orexin/hypocretin excites the histaminergic neurons of the

- tuberomammillary nucleus. *J Neurosci* 2001;21:9273–9.
160. Bayer L, Eggerman E, Serafin M, et al. Orexins (hypocretins) directly excite tuberomammillary neurons. *Eur J Neurosci* 2001;14:1571–5.
 161. Eggerman E, Serafin M, Bayer L, et al. Orexins/hypocretins excite basal forebrain cholinergic neurons. *Neuroscience* 2001;108:177–81.
 162. Piper DC, Upton N, Smith MI, et al. The novel brain neuropeptide, orexin-A, modulates the sleep-wake cycle of rats. *Eur J Neurosci* 2000;12:726–30.
 163. Vittoz NM, Berridge CW. Hypocretin/orexin selectively increases dopamine efflux within the prefrontal cortex: involvement of the ventral tegmental area. *Neuropsychopharmacology* 2006;31:384–95.
 164. Methippara MM, Alam MN, Szymusiak R, et al. Effects of lateral preoptic area application of orexin-A on sleep-wakefulness. *Neuroreport* 2000;11:3423–6.
 165. Xi M-C, Morales FR, Chase MH. Effects on sleep and wakefulness on the injection of hypocretin-1 (orexin-A) into the laterodorsal tegmental nucleus of the cat. *Brain Res* 2001;901:259–64.
 166. Moreno-Balandran ME, Garzon M, Bodalo C, et al. Sleep-wakefulness effects after microinjections of hypocretin-1 (orexin A) in cholinceptive areas of the cat oral pontine tegmentum. *Eur J Neurosci* 2008;28:331–41.
 167. Espana RA, Baldo BA, Kelley AE, et al. Wake-promoting and sleep-suppressing actions of hypocretin (orexin): basal forebrain sites of action. *Neuroscience* 2001;106:699–715.
 168. Thakkar M, Ramesh V, Strecker RE, et al. Microdialysis perfusion of orexin-A in the basal forebrain increases wakefulness in freely behaving rat. *Arch Ital Biol* 2001;139:313–28.
 169. Thakkar M, Ramesh V, Cape EG, et al. REM sleep enhancement and behavioral cataplexy following orexin (hypocretin)-II receptor antisense perfusion in the pontine reticular formation. *Sleep Res Online* 1999;2:113–20.
 170. Dong H-L, Fukuda S, Murata E, et al. Orexins increase cortical acetylcholine release and electroencephalographic activation through orexin-1 receptor in the rat basal forebrain during isoflurane anesthesia. *Anesthesiology* 2006;104:1023–32.
 171. Hong Z-Y, Huang Z-L, Qu W-M, et al. Orexin A promotes histamine, but not norepinephrine or serotonin, release in frontal cortex of mice. *Acta Pharmacol Sin* 2005;26:155–9.
 172. Ishizuka T, Yamamoto Y, Yamatodani A. The effect of orexin-A and -B on the histamine release in the anterior hypothalamus in rats. *Neurosci Lett* 2002;323:93–6.
 173. Tao R, Ma Z, McKenna JT, et al. Differential effect of orexins (hypocretins) on serotonin release in the dorsal and median raphe nuclei of freely behaving rats. *Neuroscience* 2006;141:1101–5.
 174. Bernard R, Lydic R, Baghdoyan HA. Hypocretin (orexin) receptor subtypes differentially enhance acetylcholine release and activate G protein subtypes in rat pontine reticular formation. *J Pharmacol Exp Ther* 2006;317:163–71.
 175. Brevig HN, Watson CJ, Lydic R, et al. Hypocretin and GABA interact in the pontine reticular formation to increase wakefulness. *Sleep* 2010;33:1285–93.
 176. Brisbare-Roch C, Dingemans J, Koberstein R, et al. Promotion of sleep by targeting the orexin system in rats, dogs, and humans. *Nat Med* 2007;13:150–5.
 177. Taheri S, Lin L, Austin D, et al. Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index. *PLoS Med* 2004;1:210–7 (e62).
 178. Spiegel K, Tasali E, Penev P, et al. Brief communication: sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. *Ann Intern Med* 2004;141:846–50.
 179. Laposky AD, Shelton J, Bass J, et al. Altered sleep regulation in leptin-deficient mice. *Am J Physiol Regul Integr Comp Physiol* 2006;290:R894–903.
 180. Douglas CL, Bowman GN, Baghdoyan HA, et al. C57BL/6J and B6.V-LEP^{OB} mice differ in the cholinergic modulation of sleep and breathing. *J Appl Physiol* 2005;98:918–29.
 181. Laposky AD, Bradley MA, Williams DL, et al. Sleep-wake regulation is altered in leptin-resistant (db/db) genetically obese and diabetic mice. *Am J Physiol Regul Integr Comp Physiol* 2008;295:R2059–66.
 182. Szentirmai E, Kapás L, Krueger JM. Ghrelin microinjection into forebrain sites induces wakefulness and feeding in rats. *Am J Physiol Regul Integr Comp Physiol* 2007;292:R575–85.
 183. Roehrs T, Hyde M, Blaisdell B, et al. Sleep loss and REM sleep loss are hyperalgesic. *Sleep* 2006;29:145–51.
 184. Chhangani BS, Roehrs TA, Harris EJ, et al. Pain sensitivity in sleepy pain-free normals. *Sleep* 2009;32:1011–7.
 185. Lavigne G, Sessle BJ, Choinière M, et al, editors. Sleep and pain. Seattle: International Association for the Study of Pain; 2007. p. 1–473.
 186. Shaw IR, Lavigne G, Mayer P, et al. Acute intravenous administration of morphine perturbs sleep architecture in healthy pain-free young adults: a preliminary study. *Sleep* 2005;28:677–82.
 187. Dimsdale JE, Norman D, DeJardin D, et al. The effect of opioids on sleep architecture. *J Clin Sleep Med* 2007;15:33–6.

188. Bonafide CP, Aucutt-Walter N, Divittore N, et al. Remifentanyl inhibits rapid eye movement sleep but not the nocturnal melatonin surge in humans. *Anesthesiology* 2008;108:627–33.
189. Moore JT, Kelz MB. Opiates, sleep, and pain. The adenosinergic link. *Anesthesiology* 2009;111: 1175–6.
190. Keifer JC, Baghdoyan HA, Lydic R. Sleep disruption and increased apneas after pontine microinjection of morphine. *Anesthesiology* 1992;77: 973–82.
191. Watson CJ, Lydic R, Baghdoyan HA. Sleep and GABA levels in the oral part of rat pontine reticular formation are decreased by local and systemic administration of morphine. *Neuroscience* 2007; 144:375–86.
192. Wade N. A decade later, genetic map yields few new cures. *NY Times*. June 12, 2010;2010.
193. Pollack A. Awaiting the genome payoff. *NY Times*. June 14, 2010;2010.
194. Lin JS, Sakai K, Vanni-Mercier G, et al. A critical role of the posterior hypothalamus in the mechanisms of wakefulness determined by microinjection of muscimol in freely moving cats. *Brain Res* 1989; 479:225–40.
195. Kryger MH, Roth T, Dement WC, editors. *Principles and practice of sleep medicine*. 5th edition. Philadelphia (PA): W.B. Saunders; 2010. p. 1–1505.
196. Klipp E, Herwig R, Kowald A, et al. *Systems biology in practice: concepts, implementation and application*. Weinheim (Germany): Wiley-VCH; 2005.