CHAPTER

57

The Neurochemistry of Sleep and Wakefulness

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OUTLINE

SLEEP PHENOMENOLOGY AND FUNCTION: THE SEARCH FOR NEUROCHEMICAL SUBSTRATES

The daily cycle of sleep and wakefulness is one of the most fundamental aspects of human biology

Sleep occurs as two states, rapid eye movement (REM) and non-rapid eye movement (NREM), and these states are as distinct from each other as each one is from wakefulness. NREM sleep and REM sleep oscillate with an ultradian frequency (<< 24 hours). Alternations between sleep and wakefulness are modulated by the circadian system (~24 hours) and by homeostatic processes, which regulate recovery from sleep restriction and deprivation. The neurochemical bases of homeostatic mechanisms regulating sleep remain to be discovered.

States of sleep and wakefulness are defined by a constellation of traits that also provide objective measures used to study underlying mechanisms and diagnose disorders. Figure 57-1 schematizes a night of sleep in a normal young adult and shows some of the state-dependent changes in brain metabolism. Cognitive function, motor control, sensory input and autonomic regulation change significantly during sleep (Pace-Schott & Hobson, 2002), and are negatively impacted by sleep deprivation. One of the best predictors of daytime performance is the quality of the prior night of sleep.

All placental, terrestrial mammals have sleep states and traits that are similar to those of humans. Thus, animal models are extremely useful for providing insights into the neurochemical and neurophysiological mechanisms that generate sleep and wakefulness. For example, studies in animals provided the first demonstration that the brain is as active during REM sleep as it is during wakefulness (Steriade & McCarley, 2005), a finding that was subsequently demonstrated in humans using noninvasive brain imaging (Nofzinger, 2005). Sleep disorders medicine continues to be advanced by animal models that make it possible to elucidate the neurochemical mechanisms regulating sleep, as well as the mechanisms by which a variety of medications alters sleep.

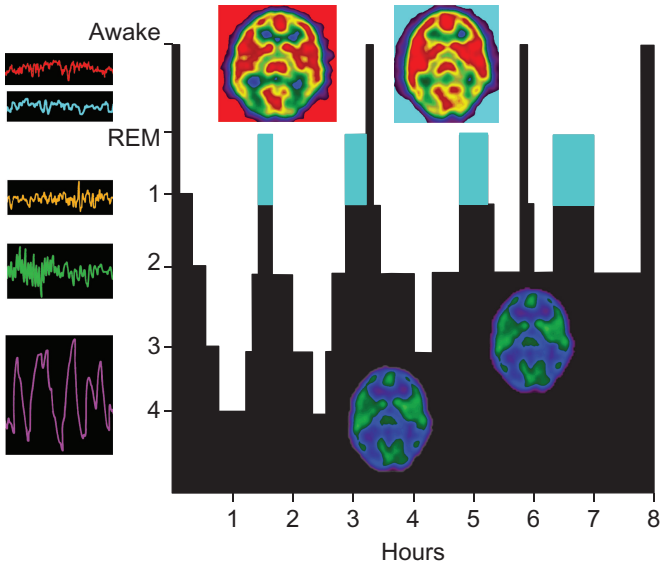

FIGURE 57-1 **Montage relating cortical EEG traces and scans of brain energy metabolism to a plot of sleep architecture depicting sleep/wake states (ordinate) as a function of time (abscissa).** EEG traces are shown for wakefulness (red), REM sleep (aqua), Stage 1 NREM sleep (tan), Stage 2 NREM sleep (green) and Stage 3/4 NREM sleep (purple). Brain scans are in the horizontal plane with the front of the brain at the top and the back of the brain at the bottom of each image. The brain scans are aligned relative to an 8-hour plot of a normal sleep cycle in a young, healthy adult to illustrate high levels of glucose metabolism (red, orange, yellow) during states of wakefulness and REM sleep and lower levels of brain energy metabolism (green, blue, purple) during epochs of NREM sleep. Note the similarity between energy metabolism and EEG activity during wakefulness and REM sleep. The brain is as metabolically active during REM sleep as it is during wakefulness. Brain scans and hypnogram were kindly provided by Dr. Eric Nofzinger and are used with permission.

The functions of sleep remain enigmatic

Numerous theories about the functions of sleep have been proposed, but none has gained universal acceptance. Commonsense notions of sleep providing rest have been difficult to prove and are contradicted by findings that the brain is metabolically highly active during REM sleep (Fig. 57-1). Theories about function span from sleep as a state of dormancy that regulates behavior when activity could be maladaptive, to sleep being essential for memory consolidation, to sleep playing a key role in brain development, and to maintain synaptic homeostasis (Cirelli & Tononi, 2008; Siegel, 2009). Many current theories are quite provocative, but because they lack substantial support this chapter focuses

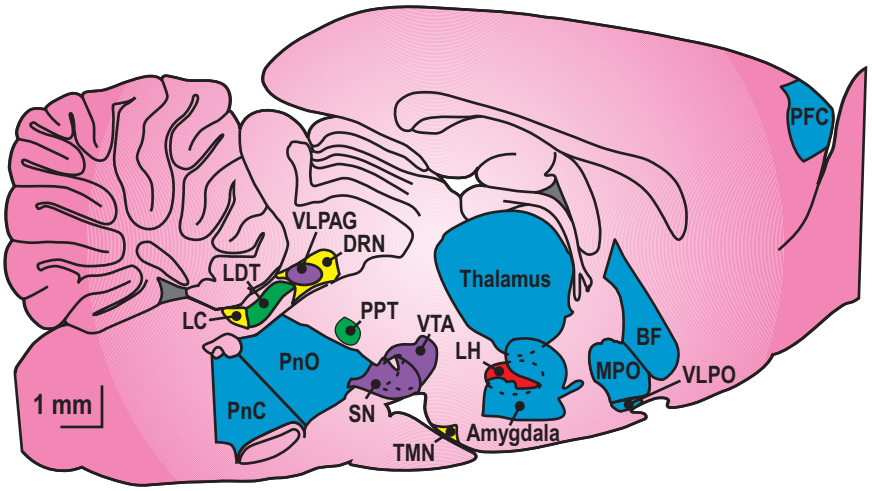

FIGURE 57-2 **Sagittal schematic of the rat brain (rostral to right) showing sleep related brain regions**. Abbreviations: BF, basal forebrain; DRN, dorsal raphé nucleus; LC, locus ceruleus; LDT, laterodorsal tegmental nucleus; LH, lateral hypothalamus; MPO, medial preoptic area; PFC, prefrontal cortex; PPT, pedunculopontine tegmental nucleus; PnC, pontine reticular formation, caudal part; PnO, pontine reticular formation, oral part; SN, substantia nigra; TMN, tuberomammillary nucleus; VLPAG, ventrolateral periaqueductal gray; VLPO, ventrolateral preoptic area; VTA, ventral tegmental area. (Modified from Watson et al., 2010.)

on neurochemical mechanisms regulating sleep. This chapter places particular emphasis on neurotransmitter and neuromodulator systems that, when altered, cause disorders of sleep. Neural systems that evolved to generate sleep are now recognized to play a role in the loss of wakefulness caused by anesthetic drugs (Lydic & Baghdoyan, 2005; Brown et al., 2010; Vanini et al., 2010c), and recent advances concerning shared neurochemical regulation of sleep and anesthesia are selectively reviewed.

There are more neurotransmitters that promote wakefulness than those that produce sleep

This may be because wakefulness is such a heterogeneous state, and because the awake organism must be capable of many complex behaviors. The major wakefulness-promoting neurotransmitters discussed here (and the brain regions containing the neurons that release these neurotransmitters) (Figure 57-2) include serotonin (dorsal raphé nucleus); norepinephrine (locus ceruleus); histamine (tuberomammillary nucleus of the posterior hypothalamus); dopamine (ventral tegmental area, substantia nigra pars compacta, ventral periaqueductal gray); glutamate (basal forebrain, laterodorsal and pedunculopontine tegmental nuclei, pontine reticular formation); hypocretin/orexin (lateral hypothalamus); and acetylcholine (basal forebrain, laterodorsal and pedunculopontine tegmental nuclei). No single neurotransmitter or brain region regulates states of sleep and wakefulness. Some neurotransmitters regulate multiple states. Acetylcholine, for example promotes REM sleep in addition to wakefulness. Figure 57-3 schematizes the sleep-state–dependent nature of monoaminergic, cholinergic and GABAergic neurotransmission and emphasizes that these neurotransmitter systems influence multiple brain regions. NREM sleep-promoting neurotransmitters include GABA (medial and ventral preoptic areas of the anterior hypothalamus) and the neuromodulator adenosine. Sleep and wakefulness occur as a result of complex interactions between these various neurotransmitter systems and brain regions. Because of these elaborate network interactions, new medications developed to enhance sleep or maintain wakefulness will be most effective if they act on multiple neurotransmitter systems. This complexity also underlies the fact that many medications taken to treat other medical conditions impact sleep negatively. A complete understanding of the neurochemical control of sleep requires knowledge about changes in neurotransmitter release across the sleep–wakefulness cycle and differences in those patterns of release between brain regions. Factors that affect state-dependent changes in neurotransmitter release include inputs received by the neurons that release each neurotransmitter, the receptors expressed by those neurons, and the receptor-activated signal transduction cascades. When these neurochemical control mechanisms are disrupted, the result is disordered sleep.

DEVELOPMENT OF SLEEP DISORDERS MEDICINE AND SLEEP NEUROBIOLOGY

Compared to other medical specialties, sleep disorders medicine has a very short history

An initial barrier to the development of sleep disorders medicine was the absence of a knowledge base relevant to

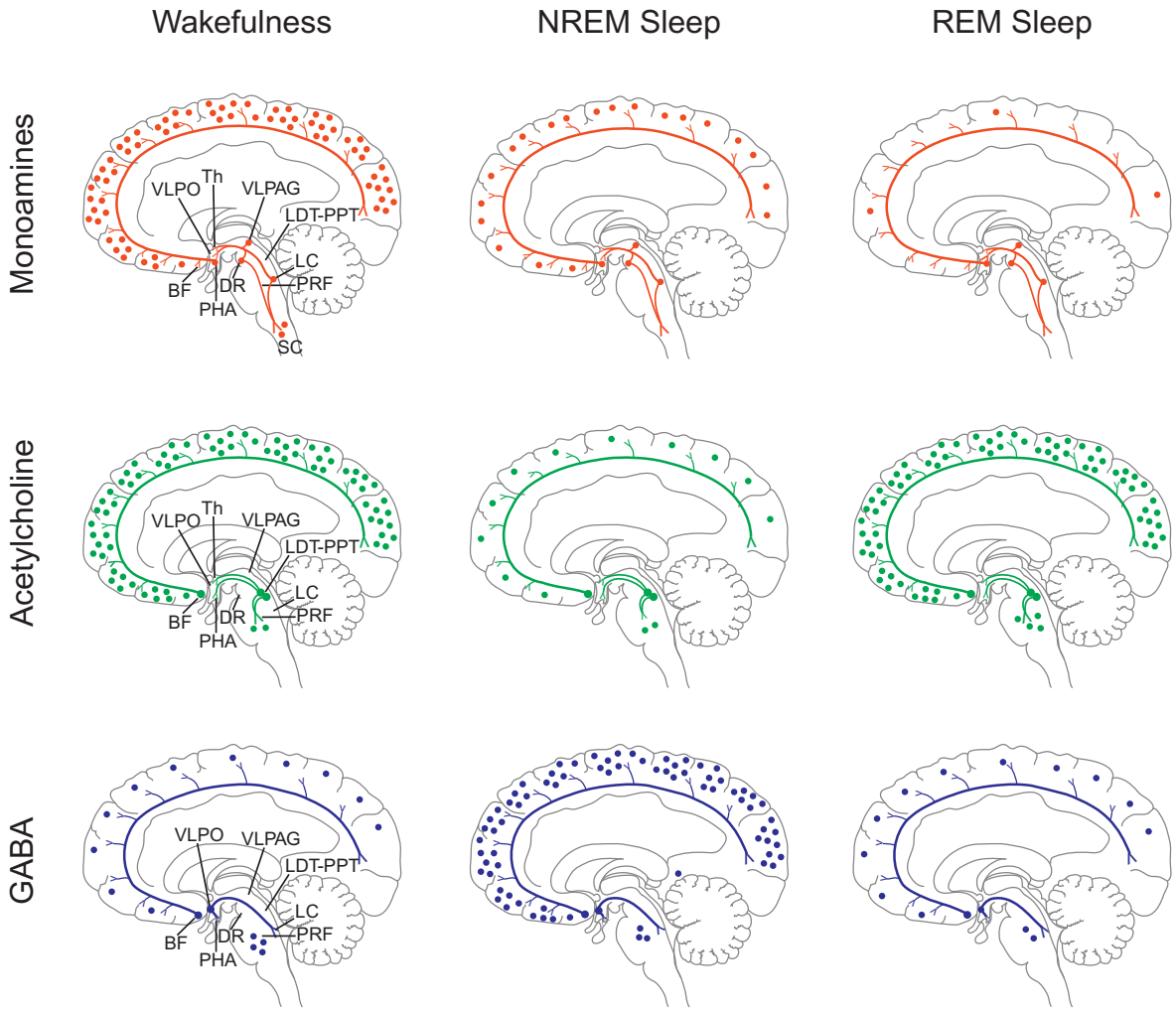

FIGURE 57-3 Drawings schematize state-dependent changes in monoaminergic, cholinergic, and GABAergic neurotransmission in the cerebral cortex and pontine reticular formation. Colored lines represent chemically coded projections from monoaminergic (red), cholinergic (green), and GABAergic (blue) neurons. Density of colored dots surrounding the schematized neuronal terminals indicates that neurotransmitter release varies as a function of sleep and wakefulness. State-dependent changes in cell discharge activity have been confirmed by multiple laboratories and provide the foundation for the current cellularly based, mathematical model of sleep cycle control that has predictive power (Steriade & McCarley, 2005). Abbreviations: DR, dorsal raphé nucleus; PHA, posterior hypothalamic area; PRF, pontine reticular formation; SC, spinal cord; Th, thalamus. (Modified from Hobson, 1999; Vanini et al., 2010a.)

clinical practice. Sleep disorders medicine in the United States began in the early 1960s. The first sleep disorders center was established at Stanford University in 1970. The first textbook on sleep disorders medicine, now in its fifth edition (Kryger et al., 2011), was published in 1989. In 1993 the NIH established a National Center for Sleep Disorders Research. In 1996, sleep disorders medicine was recognized as a specialty by the American Medical Association. Today, the American Academy of Sleep Medicine has more than 8,000 members and accredits 2,075 sleep disorders centers and laboratories. The significant public health impact of disordered sleep is recognized by the NIH (Lenfant, 2003) and by the Institute of Medicine (Colten et al., 2006).

Understanding the neurochemical regulation of sleep is essential for advancing sleep disorders medicine

The foregoing 50-year outline of sleep disorders medicine relative to the 30,000-year history of *Homo sapiens* helps explain why a neurochemical approach to sleep disorders is perceived as one of the most exciting additions to neuroscience. Although sleep disorders medicine is making great progress, there is a mismatch between the large burden of disease represented by sleep disorders and the relatively small amount of available information concerning the neurochemical regulation of sleep. For example, approximately 30% of the general

population reports one or more symptoms of insomnia (Roth et al., 2010), and an estimated 31 million Americans currently suffer from insomnia, the most common sleep disorder (NIH Consensus Panel Report, 2005). In 2005 approximately 42 million prescriptions for sleeping pills were filled (Saul, 2006), yet the neurochemical mechanisms through which these drugs achieve desired (and undesired) effects are not well understood. Neurochemical data are critical for advancement of sleep pharmacology beyond the current, state-of-the-art practice of symptom relief.

MONOAMINES

Serotonin, norepinephrine and histamine are major components of the ascending reticular activating system, and each of these neurotransmitters plays a unique role in shaping the multifaceted behavioral state of wakefulness

These monoaminergic cell groups are discussed here together. Decreases in firing rate precede the onset of sleep, and resumption of firing occurs prior to the onset of wakefulness. Because a cause must precede the event it is causing, the timing of the changes in firing rates relative to changes in behavioral state is the basis for inferring a causal role for these neuronal firing patterns in the control of sleep and wakefulness (Steriade & McCarley, 2005). The "Wake-On/REM-Off" discharge pattern of monoaminergic neurons is consistent with the pattern of neurotransmitter release that, in all brain regions so far studied, is greatest during wakefulness and lowest during REM sleep (Brevig & Baghdoyan, 2010). Dopamine is also a sleep-related monoamine but is discussed in a separate section because dopaminergic neurons do not show this "Wake-On/REM-Off" discharge pattern.

Norepinephrine promotes arousal during normal wakefulness, and augments arousal during periods of stress and in response to psychostimulant drugs

Most of the norepinephrine in the brain is released from neurons of the locus ceruleus and exerts its effects by activating α_1, α_2 and β receptors (see Ch. 14). Using *in vivo* microdialysis, norepinephrine has been collected across the sleep–wakefulness cycle from the locus ceruleus, amygdala, nucleus accumbens and prefrontal cortex (Figs. 57-2 and 57-3). Extracellular norepinephrine levels are greater during wakefulness than during sleep in each of these regions (Brevig & Baghdoyan, 2010), consistent with a role for norepinephrine in generating different aspects of wakefulness. Wakefulness-promoting and EEG-activating effects of norepinephrine are mediated, in part, by α_1 and β adrenergic receptors in the medial septal area and medial preoptic area of the hypothalamus. Cholinergic stimulation of the locus ceruleus using muscarinic receptor agonists also causes activation of the cortical EEG (Berridge, 2008).

Agonists of α_2 adrenergic receptors such as xylazine and dexmedetomidine are used, respectively, in veterinary and human medicine to produce short-term sedation. Activation of α_2 adrenergic receptors inhibits locus ceruleus neurons, and the increase of endogenous norepinephrine within the locus ceruleus during wakefulness is thought to be part of an autoinhibitory mechanism that turns off locus ceruleus neurons and permits the onset of REM sleep (Steriade & McCarley, 2005). Genetically modified mice that cannot synthesize norepinephrine and have a total absence of brain norepinephrine show a decrease in wakefulness but, surprisingly, also show a decrease in REM sleep. This finding suggests that at least some norepinephrine must be present in order to generate REM sleep. These same mice show a hypersensitivity to the inhaled anesthetic isoflurane, such that lower concentrations of isoflurane cause a loss of consciousness compared to wild-type mice, and more time is required for resumption of wakefulness when delivery of isoflurane is discontinued. The phenotypes for loss and recovery of consciousness in these mice are eliminated when normal brain levels of norepinephrine are restored (Friedman et al., 2010). Taken together, these data provide strong support for the hypothesis that norepinephrine contributes to the maintenance of wakefulness. The hypothesis that norepinephrine plays a permissive role in REM sleep generation is interesting but remains to be confirmed.

Serotonin has a biphasic effect on sleep

Unlike the other neurochemical components of the ascending reticular activating system, serotonergic neurons are not located bilaterally but instead form a series of nuclei that lie along the midline of the brainstem. Serotonin acts on at least 14 receptor subtypes classified into families named 5-HT_1 – 5-HT_7 (see Ch. 15). Studies from the early 1960s indicated that serotonin is critical for the generation of sleep (Jouvet, 1969). However, the discovery in the 1970s that putative serotonergic neurons in the dorsal raphé nucleus (Fig. 57-2, DRN) show a "Wake-On/REM-Off" discharge pattern led to the revised hypothesis that serotonin is important for generating wakefulness and inhibiting REM sleep (Steriade & McCarley, 2005). The dorsal raphé nucleus has been the most extensively studied serotonergic cell group with respect to regulation of sleep and wakefulness. Serotonin levels have been measured across the sleep–wakefulness cycle in sleep-related areas that receive input from the dorsal raphé nucleus, including the cortex, hippocampus and many brainstem nuclei (Fig. 57-3). Extracellular serotonin levels in these brain regions are greatest during wakefulness, intermediate during NREM sleep and lowest during REM sleep, consistent with the "Wake-On/REM-Off" firing pattern of dorsal raphé neurons (Brevig & Baghdoyan, 2010). Within the dorsal raphé nucleus, 5-HT_{1A} receptors function as autoreceptors to regulate serotonin release. Delivering a 5-HT_{1A} receptor agonist directly into the dorsal raphé nucleus causes a decrease in local serotonin levels and a concomitant three-fold increase in REM sleep (Steriade & McCarley, 2005). Genetically modified mice lacking either 5-HT_{1A} or 5-HT_{1B} receptors have an increased amount of

REM sleep (Landolt & Wehrle, 2009). These finding are compatible with the interpretation that serotonin inhibits REM sleep.

Serotonin is now thought to have a biphasic effect on sleep, producing an initial phase of wakefulness and triggering the subsequent onset of NREM sleep (Ursin, 2002; Landolt & Wehrle, 2009; Kryger et al., 2011). The release of serotonin during wakefulness may inhibit the activity of other wakefulness-promoting neurons in the basal forebrain and hypothalamus (Datta & MacLean, 2007). Serotonin is well known to modulate the release of acetylcholine, norepinephrine, dopamine and GABA (Fink & Göthert, 2007). Increased serotonin levels during wakefulness also have been suggested to stimulate the production of endogenous sleep factors (Jouvet, 1999).

Histamine levels are greater during wakefulness than during sleep, consistent with the fastest firing rates of histamine-containing neurons occurring during wakefulness

Histaminergic neurons are localized to the tuberomammillary nucleus of the posterior hypothalamus (Fig. 57-2, TMN), and send extensive projections throughout the central nervous system (Hass et al., 2008). Of particular relevance for the regulation of sleep and wakefulness are histaminergic projections to the cortex, thalamus, preoptic area of the anterior hypothalamus, laterodorsal tegmental nucleus, dorsal raphé nucleus and locus ceruleus. Histamine release in the prefrontal cortex and anterior hypothalamus is greater during wakefulness than during sleep (Brevig & Baghdoyan, 2010). Histamine exerts its effects by actions at four G-protein–coupled receptors, H_1–H_4 (see Ch. 16). Mice that cannot synthesize histamine due to a knockout of the synthetic enzyme histidine decarboxylase do not show a normal increase in wakefulness when placed in a novel environment, providing support for the hypothesis that histamine in the brain functions to maintain wakefulness during environmentally imposed challenges (Parmentier et al., 2007).

Sleep disorders and depression are linked by monoamines

Selective serotonin reuptake inhibitors and serotonin–norepinephrine reuptake inhibitors enhance the actions of serotonin and norepinephrine in the brain and are widely used for the treatment of depression (see Ch. 60). Insomnia and sleep disruption are common side effects of these monoamine reuptake inhibitors (Landolt & Wehrle, 2009), consistent with the wakefulness-promoting role of these monoamines. Patients with major depressive disorders frequently have coexisting insomnia and although antidepressant therapy improves affect, patients often continue to struggle with insomnia symptoms. Co-administering fluoxetine (a selective serotonin reuptake inhibitor) with the sedative/hypnotic eszopiclone (a benzodiazepine receptor agonist) increases the antidepressant response and improves sleep (Fava et al., 2006). This finding suggests that actively treating insomnia may be an important approach to the treatment of some affective disorders.

First-generation antihistamines, such as diphenhydramine, block histamine H_1 receptors and produce profound drowsiness. Doxepin, which at low doses selectively blocks H_1 receptors, improves sleep in individuals diagnosed with primary insomnia (Roth et al., 2007). The risk and severity of insomnia increases with age, and doxepin is also effective in treating insomnia in the elderly (Krystal et al., 2010). Histamine H_3 receptors function as autoreceptors to inhibit the release of histamine, and are considered to be targets for sleep-disorder–related pharmacotherapy (Sander et al., 2008). H_3 receptor antagonists increase the release of histamine and increases wakefulness, whereas H_3 agonists decrease histamine release and increase sleep (Parmentier et al., 2007).

Modafinil is a stimulant indicated for the treatment of excessive daytime sleepiness associated with narcolepsy. The mechanisms by which modafinil increases wakefulness are not known, but involve activation of many wakefulness-promoting neurotransmitter systems. Modafinil activates adrenergic and serotonergic receptors, and decreases GABA release in the cortex via a monoaminergic mechanism. Modafinil increases the activity of histaminergic neurons and increases the release of histamine in the anterior hypothalamus. However, the actions of modafinil do not depend upon histamine, as modafinil promotes wakefulness in mice that do not synthesize histamine (Parmentier et al., 2007).

ACETYLCHOLINE

Acetylcholine contributes significantly to the generation of REM sleep and wakefulness

Two major clusters of cholinergic projection neurons are responsible for the arousal-promoting effects of acetylcholine (Steriade et al., 2005; Stenberg, 2007; Lydic et al., 2008). One cluster is located in the rostral portion of the pontine brainstem and is referred to as the laterodorsal and pedunculopontine tegmental nuclei (LDT/PPT) (Fig. 57-2). The LDT/PPT are briefly described here as a unit; details of the differences between the LDT and PPT nuclei have been reviewed (Datta, 2010). Cholinergic LDT/PPT neurons project to the dorsal raphé nucleus and the locus ceruleus where ACh inhibits the wakefulness-promoting discharge of these monoaminergic cell groups. LDT/PPT neurons send projections to the thalamus, where they inhibit the generation of EEG spindles and slow waves, and induce cortical activation. Cholinergic LDT/PPT neurons also innervate the pontine reticular formation, where release of ACh triggers and maintains REM sleep. The second major group of cholinergic projection neurons is localized in the basal forebrain. These neurons project to the entire neocortex and hippocampus, and contribute to the activation of the EEG that is characteristic of REM sleep and wakefulness (Fig. 57-3), as well as to behavioral arousal, selective attention, and learning and memory (Steriade & McCarley, 2005). Nicotinic and muscarinic receptors (see Ch. 13) contribute to the regulation of sleep and wakefulness by acetylcholine.

Early neurochemical studies that focused on the sleep-related role of ACh were performed by Michel Jouvet and colleagues, who demonstrated that blocking muscarinic cholinergic receptors (mAChRs) with systemically administered atropine inhibited REM sleep, and that enhancing cholinergic transmission with cholinesterase inhibitors could, in some cases, increase REM sleep. Jouvet's localization of the neuronal machinery necessary for generating REM sleep to the rostral pontine brainstem encouraged other investigators to microinject muscarinic cholinergic agonists directly into the pontine reticular formation. These investigators found that cholinomimetics caused large increases in REM sleep, supporting the idea that pontine cholinergic neurotransmission is important for REM sleep generation. These preclinical studies, in turn, inspired pioneering work with healthy human volunteers. Domino showed that administering the mAChR antagonist scopolamine to humans delayed the onset of REM sleep. Gillin and colleagues made the fascinating discovery that human REM sleep can be increased by intravenous infusion of cholinomimetics, such as physostigmine or arecoline, if the drugs are given during NREM sleep. However, the same dose of physostigmine administered during REM sleep caused the sleeping subjects to awaken (Lydic & Baghdoyan, 2005; Brown et al., 2010; Vanini et al., 2010c). The state-dependent effects of cholinomimetics on sleep have been subsequently demonstrated in animals (López-Rodríguez et al., 1994; Xi & Chase, 2010) and are likely to result from the interactions of multiple neurotransmitters. A classic finding in sleep neurobiology is the phenomenon of reticular response reversal, in which a change in behavioral state from wakefulness and/or NREM sleep to REM sleep is accompanied by a change from excitation to inhibition of motor systems (Chase et al., 1976; Kryger et al., 2011). Response-reversal, during which synaptic sign can change from excitation to inhibition and vice versa, has profound implications for interpreting the results of neurochemical studies that are conducted across the sleep/wake cycle.

Evidence that pontine cholinergic neurotransmission promotes the generation of REM sleep comes from many studies using a wide range of approaches

The release of endogenous ACh in the pontine reticular formation is greater during REM sleep than during wakefulness or NREM sleep. Electrical stimulation of cholinergic LDT/PPT neurons causes an increase in ACh release in the pontine reticular formation and an increase in REM sleep. Lesions that selectively destroy cholinergic neurons in the LDT/PPT cause a long-lasting decrease in REM sleep that is proportional to the number of neurons lost. Microinjection of the cholinesterase inhibitor neostigmine into the pontine reticular formation causes a concentration-dependent increase in REM sleep that is blocked by atropine, indicating mediation by endogenous ACh acting at mAChRs. Studies using relatively subtype-selective mAChR antagonists indicate a prominent role for the M2 subtype in REM sleep generation (Lydic et al., 2008).

Two populations of LDT/PPT neurons have been described with respect to discharge patterns during sleep and wakefulness (Datta, 2010). One group fires maximally during REM sleep and discharges infrequently during wakefulness and NREM sleep. By releasing ACh into the pontine reticular formation, these "REM-On" neurons drive REM sleep. Another population fires fastest during wakefulness and REM sleep, and discharges minimally during NREM sleep. These neurons with a "WAKE-On/REM-On" discharge pattern contribute to the EEG activation characteristic of both wakefulness and REM sleep by providing the cholinergic input to the thalamus that inhibits the EEG slow waves and spindles of NREM sleep. ACh release in the thalamus is greater during wakefulness and REM sleep than during NREM sleep.

Cholinergic neurons in the basal forebrain also show a "WAKE-On/REM-On" discharge pattern, and ACh release in the cortex is relatively high during wakefulness and REM sleep compared with during NREM sleep. Early studies by Krnjevic showed that ACh activates cortical neurons, and cortical ACh is known to be important for focused attention. ACh release is also greater in the hippocampus during REM sleep and wakefulness than during NREM sleep, consistent with the discharge pattern of basal forebrain cholinergic neurons. ACh generates oscillations of 4–10 Hz in the hippocampus, known as theta activity. The theta rhythm is important for spatial learning and is prominent during REM sleep.

Acetylcholine, depression, REM sleep and pain

Depression affects about 10% of the US population, and hyperactivity in cholinergic transmission has been suggested to contribute to some types of depression. Some depressed patients show a reduced REM sleep latency, and REM sleep is more easily triggered by cholinergic stimulation in patients with depression. Some tricyclic antidepressant drugs have anticholinergic properties, and these drugs cause an increase in REM sleep latency that precedes the onset of mood improvement (Nofzinger, 2008). Opioids are the most widely used drugs for the treatment of acute and chronic pain, and although these drugs are effective for pain relief they suppress normal sleep architecture (Fig. 57-1) and suppress REM sleep (Lydic & Baghdoyan, 2007). Preclinical studies have demonstrated that opioids decrease ACh release in the pontine reticular formation, which contributes to REM sleep suppression. Opioids also decrease ACh release in the prefrontal cortex, cause slowing of the cortical EEG and impair cognitive function during wakefulness. Basal forebrain cholinergic neurons degenerate in Alzheimer's disease, and these patients show sleep disruptions that include less REM sleep and fewer rapid eye movements during REM sleep. Finally, the unconsciousness caused by the intravenous anesthetic propofol can be reversed by physostigmine. This finding supports the interpretation that propofol produces loss of consciousness, in part, by decreasing cholinergic transmission (Lydic & Baghdoyan, 2005; Brown et al., 2010; Vanini et al., 2010c).

DOPAMINE

Unlike other monoaminergic neurons, dopaminergic cells do not cease firing during REM sleep

The dopaminergic neurons most studied with respect to sleep are found in the ventral tegmental area (VTA) and the

substantia nigra pars compacta (SN) of the midbrain (Fig. 57-2). These neurons project to, and receive input from, many sleep-related nuclei including the dorsal raphé nucleus, locus ceruleus, laterodorsal and pedunculopontine tegmental nuclei, lateral hypothalamus, thalamus and basal forebrain (Datta & MacLean, 2007; Monti & Monti, 2007; Stenberg, 2007). In the early 1980s, *in vivo* recordings from single neurons in the VTA and SN revealed relatively stable firing rates across the sleep–wakefulness cycle. This is in contrast to the discharge patterns of other catecholaminergic neurons that fire fastest during wakefulness and cease discharging during REM sleep. Measures of endogenous dopamine made during sleep and wakefulness show that in some brain areas, such as the locus ceruleus and amygdala, dopamine levels are stable across the sleep–wake cycle. These discharge rate and release data have lent support to the idea that dopamine contributes to motor aspects of waking behavior, rather than to wakefulness itself. However, a bursting discharge pattern of dopaminergic neurons is associated with increased dopamine release, and bursting is increased during wakefulness. Furthermore, in the prefrontal cortex and nucleus accumbens, dopamine levels are greater during wakefulness than during NREM sleep (Brevig & Baghdoyan, 2010). Despite the lack of a clear sleep-state–dependent discharge pattern in dopaminergic neurons, numerous lesion and pharmacological studies as well as the use of genetically modified mice provide support for dopamine as a wakefulness-promoting neurotransmitter (Monti & Monti, 2007). Compelling support also comes from the clinical finding that patients with Parkinson's disease are excessively sleepy. In addition to the VTA and SN, there is a cluster of dopaminergic neurons in the ventral periaqueductal gray (VLPAG) (Fig. 57-2) that may contribute to the regulation of wakefulness. Whether the VLPAG dopaminergic neurons show a state-dependent discharge profile is not yet known.

Dopamine receptors exist as multiple subtypes (see Ch. 14) and the effects of dopamine on sleep and wakefulness are nuanced, depending upon the receptor subtype(s) that are involved. Studies of the sleep–wake related roles of dopamine receptors are complicated by the difficulty in distinguishing between primary effects on wakefulness, locomotor activity, behavioral arousal, and/or EEG activation. In general, D1 and D2 receptor activation increases wakefulness and related behaviors, whereas D3 activation decreases locomotion and may increase sleep (Monti & Monti, 2007). Dopamine transporter (DAT) knockout mice have increased levels of extracellular dopamine, an increase in wakefulness and a decrease in NREM sleep. In addition, DAT knockout mice are insensitive to the wakefulness-promoting effects of amphetamine and modafinil (Stenberg, 2007).

Restless legs syndrome, Parkinson's disease and sleep

Restless legs syndrome (RLS) is a circadian-related sleep disorder characterized by an unpleasant urge to move the legs that worsens at night (Ferini-Strambi et al., 2009; Trenkwalder & Paulus, 2010). RLS has a genetic component and has been estimated to affect up to 10% of Caucasians. One cause of sleep disruption in RLS is periodic limb movements during sleep. RLS is referred to as a somatosensory network disorder because many regions of the central nervous system, from the motor cortex to the spinal cord, are involved. The mechanisms are poorly understood, but are known to involve altered dopaminergic neurotransmission. Dopamine receptor agonists, such as ropinirole, pramipexole, and rotigotine, are the first-line treatment (Ch. 49). L-dopa is also effective but use is limited due to side effects. Patients with Parkinson's disease have a loss of dopaminergic neurotransmission and often suffer from excessive daytime sleepiness. Their sleepiness, paradoxically, can be exacerbated by treatment with dopamine receptor agonists (Chaudhuri et al., 2009).

HYPOCRETINS/OREXINS

The discovery of hypocretins (orexins) provides an excellent example of how preclinical studies using animal models provided powerful tools for gaining mechanistic insights into human disease processes

In the late 1990s advances in understanding the neurochemical control of wakefulness and sleep were provided by the discovery of the excitatory peptides hypocretin-1 and hypocretin-2, also called orexin A and orexin B (de Lecea, 2010; Sakurai et al., 2010). Two laboratories independently discovered these peptides. One group named the peptides hypocretins by combining the location of their cell bodies (hypothalamus) with the similarity of their amino acid sequence to a gut peptide (secretin). Another laboratory selected the name orexins, from the Greek word for appetite, because the region of the hypothalamus containing the cell bodies contributes to the central regulation of feeding behavior and energy homeostasis. One remarkable aspect of this discovery is the rapidity with which deficits in hypocretinergic neurotransmission in animals were identified to be the underlying cause of the human sleep disorder narcolepsy (Nishino et al., 2010). Hypocretins are now known to contribute to the regulation of normal sleep and wakefulness. These peptides are also important for the integration of physiological signals relevant for energy homeostasis, reward, and the coordination of motor activity and behavioral arousal (Tsujino & Sakurai, 2009).

Neurons that synthesize hypocretin are localized bilaterally in the tuberal region of the hypothalamus, which includes the perifornical nucleus, the dorsomedial hypothalamic nucleus, and the dorsal and lateral hypothalamic areas (Kilduff & Peyron, 2000). This chapter uses the term lateral hypothalamus to describe the location of the hypocretinergic cell bodies (Fig. 57-2, LH). The total number of hypocretinergic neurons has been estimated to be between 3,000–4,000 in rat and approximately 70,000 in human. One striking feature of these few hypocretinergic neurons in the lateral hypothalamus is the wide-ranging extent of their projections throughout the brain and all levels of the spinal cord. Of particular note is that all major wakefulness-promoting regions of the brain receive hypocretinergic input (Kilduff & Peyron, 2000). Hypocretins cause neuronal excitation by signaling through two G-protein–coupled receptors, Hcrt-r1 and Hcrt-r2, also named OX1 and OX2. The distribution of hypocretin

receptors follows that of the neuronal projections, and the postsynaptic neurons that express hypocretin receptors are neurochemically diverse. Hypocretinergic neurons receive considerable arousal-related input arising from the limbic, circadian, and reward systems. Hypocretinergic neurons also express receptors for metabolic signals, such as the appetite suppressant leptin, the appetite stimulant ghrelin and glucose (Sakurai, 2007).

Hypocretins promote normal wakefulness

The insight that one physiological role of hypocretins is to promote wakefulness initially arose from anatomical findings demonstrating that hypocretinergic neurons project to every major arousal-promoting nucleus in the brain (Kilduff & Peyron, 2000), and from electrophysiological data showing that hypocretins activate monoaminergic and cholinergic wakefulness-promoting neurons (Sakurai, 2007). The locus ceruleus contains the greatest density of hypocretin receptors, and microinjection of hypocretin-1 into the locus ceruleus increases wakefulness. Hypocretinergic neurons discharge maximally during active wakefulness when animals are moving (Lee et al., 2005; Mileykovskiy et al., 2005), and hypocretin-1 levels in the cerebrospinal fluid are greater during active waking than during periods of quiet wakefulness (Kiyashchenko et al., 2002). Hypocretin-1 levels in the lateral hypothalamus and basal forebrain are greater during wakefulness and REM sleep than during NREM sleep, and do not show state-dependent changes in the locus ceruleus. These data support the interpretation that hypocretins promote wakefulness and contribute to central motor activation (Kiyashchenko et al., 2002). A wakefulness-promoting role for hypocretins is also supported by data showing that genetically modified mice with impaired hypocretinergic signaling require additional time to recover from general anesthesia, and by case reports of narcoleptic patients who require prolonged time to regain consciousness after receiving general anesthesia (Kelz et al., 2008).

Hypocretin-1 increases the release of many wakefulness-promoting transmitters *in vivo* (Watson et al., 2010). Administering hypocretin-1 into the basal forebrain increases ACh release in the cortex. Hypocretin-1 delivered into the pontine reticular formation increases local ACh release and local GABA levels. Intracerebroventricular administration of hypocretin-1 increases histamine and dopamine in prefrontal cortex, and delivering hypocretin-1 to the dorsal raphé nucleus increases local serotonin levels. Increasing the levels of wakefulness-promoting neurotransmitters and neuromodulators in different brain regions is likely to be a crucial neurochemical mechanism by which hypocretins contribute to the maintenance of wakefulness.

Loss of hypocretinergic neurons underlies the human sleep disorder narcolepsy and contributes to other neurological disorders that show sleep abnormalities

Initial preclinical findings revealed that canine narcolepsy is caused by a mutation in the hypocretin receptor-2 gene, and hypocretin knockout mice show a narcoleptic phenotype (Fronczek et al., 2009; Nishino et al., 2010). Hypocretins are not detectable in the cerebrospinal fluid of human narcoleptics with cataplexy, and postmortem brains of human narcoleptics show a reduced number of hypocretinergic neurons. Transgenic mice that lose hypocretin-containing neurons during development show a phenotype similar to narcolepsy, including periods of behavioral arrest, REM sleep onset periods, fragmented sleep, and late-onset obesity. Symptoms of narcolepsy in these transgenic mice are reversed by ectopic production of hypocretin or by intracerebroventricular administration of hypocretin-1. Hypocretin replacement is under consideration as a therapeutic option for the treatment of narcolepsy, and requires the development of stable hypocretin receptor agonists that can cross the blood–brain barrier (Nishino et al., 2010). Hypocretin receptor antagonists are in development for the treatment of insomnia, and clinical trials show promising results for an orally active dual Hcrt-r1 and Hcrt-r2 antagonist (Herring et al., 2010).

AMINO ACIDS

γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain, and drugs that enhance transmission at $GABA_A$ receptors are used clinically to produce sleep, sedation, or general anesthesia

Activation of $GABA_B$ receptors (See Ch. 18.) also produces sleep. Preclinical studies, however, show that the effects of GABA on sleep and wakefulness are brain-region–specific. In the pontine reticular formation, for example, GABA actually increases wakefulness and inhibits sleep. These preclinical findings are important because they suggest a mechanism by which benzodiazepines produce sleep patterns that are similar, but not identical, to spontaneously occurring sleep (Flint et al., 2010).

Several groups of GABAergic projection neurons are relevant for sleep regulation. A relatively large population of GABAergic neurons in the basal forebrain (Fig. 57-2, BF) sends axons to the cerebral cortex. GABA levels in the cortex (Fig. 57-3) are greater during NREM sleep than during wakefulness or REM sleep (Vanini et al., 2010b). Basal forebrain GABAergic neurons also innervate the pontine reticular formation (Rodrigo-Angulo et al., 2008), where GABAergic transmission promotes wakefulness and inhibits REM sleep (Vanini et al., 2011). GABAergic input to the pontine reticular formation also arises from the posterior lateral hypothalamus and reticular nucleus of the thalamus (Rodrigo-Angulo et al., 2008). GABAergic neurons in the medial preoptic nucleus (MPO, Fig. 57-2) and the ventrolateral preoptic area of the anterior hypothalamus (VLPO, Fig. 57-2) project to many wakefulness-promoting nuclei, including the dorsal raphé nucleus, locus ceruleus and tuberomammillary nucleus of the posterior hypothalamus. These GABAergic neurons increase their discharge rates during NREM sleep and are thought to promote sleep by inhibiting neurons that drive wakefulness. There is a column of GABAergic cells running through the pontine and midbrain reticular formation that

projects to the region of the pontine reticular formation where cholinomimetics or $GABA_A$ receptor antagonists cause an increase in REM sleep (Liang & Marks, 2009).

The effects of GABA on sleep and wakefulness vary as a function of brain region

Within the pons, GABA can have opposite effects on sleep and wakefulness. In the dorsal raphé nucleus and locus ceruleus (Fig. 57-2, DRN, LC), GABAergic inhibition of wakefulness-promoting neurons increases sleep. However, within the pontine reticular formation (Fig 57-2, PnO, PnC), which contains effector neurons for generating REM sleep, enhancing GABAergic neurotransmission increases wakefulness and inhibits REM sleep. By contrast, GABAergic transmission in the ventrolateral part of the periaqueductal gray (Fig. 57-2, VLPAG) increases REM sleep. Opposite effects of GABA on sleep and wakefulness also occur within the hypothalamus. In the tuberomammillary region of the posterior hypothalamus (Fig. 57-2, TMN), which contains wakefulness-promoting neurons, GABA acts to increase NREM sleep. In preoptic and anterior hypothalamic areas, which contain NREM sleep-promoting neurons, enhancing GABAergic transmission inhibits sleep and increases wakefulness. The main point to emerge from these preclinical studies that administer drugs into discrete brain regions is that the effects of GABAergic inhibition on sleep and wakefulness depend on the functional role of the neurons being inhibited. GABAergic inhibition of sleep-promoting neurons causes increased wakefulness, whereas GABAergic inhibition of wakefulness-promoting neurons enhances sleep.

GABAergic transmission in the pontine reticular formation contributes to the regulation of sleep and wakefulness

Extracellular levels of endogenous GABA in the pontine reticular formation are greater during wakefulness than during the loss of consciousness caused by the general anesthetic isoflurane or during naturally occurring sleep (Vanini et al., 2008). Increasing levels of endogenous GABA in the pontine reticular formation by direct administration of nipecotic acid, which blocks uptake of GABA into nerve terminals and glia, causes an increase in wakefulness and a decrease in sleep. Similarly, decreasing the concentration of endogenous GABA in the pontine reticular formation by the synthesis-blocking drug 3-mercaptopropionic acid causes an increase in sleep and a decrease in wakefulness. A related finding is that nipecotic acid also increases the time needed for isoflurane to induce loss of consciousness, whereas 3-mercaptopropionic acid decreases anesthesia induction time (Watson et al., 2010). Receptor antagonists are also extremely useful tools for determining the role of endogenous GABA in regulating sleep and wakefulness. Large increases in REM sleep are produced by microinjecting the $GABA_A$ receptor antagonists bicuculline or gabazine into the pontine reticular formation (Watson et al., 2010). These data support the interpretation that GABAergic transmission in the pontine reticular formation is inhibitory to REM sleep. Consistent with this interpretation is the finding that levels of endogenous GABA in the pontine reticular formation are greater during wakefulness than during REM sleep (Vanini et al., 2011).

Clinical implications of GABAergic transmission for sleep

Benzodiazepine receptor agonists have binding sites on $GABA_A$ receptors and exert their effects by enhancing GABAergic transmission (Fig. 18-3). These drugs are effective for the treatment of insomnia, but the sleep they produce is qualitatively different from sleep that occurs spontaneously. For example, some benzodiazepine receptor agonists alter the temporal organization of sleep (Fig. 57-1) (Flint et al., 2010). These drugs tend to increase the time it takes to enter the first REM sleep period and decrease the deeper stages of slow-wave sleep, while increasing the lighter stages of NREM sleep. The fact that GABAergic transmission in certain areas of the brain, such as the pontine reticular formation, causes an increase in wakefulness might explain why some benzodiazepine receptor agonists enhance lighter, rather than deeper, stages of NREM sleep. These drugs are administered orally or intravenously for clinical use and thus simultaneously exert their effects on wakefulness-promoting and sleep-promoting regions throughout the brain. The finding that intravenous delivery of eszopiclone to rat decreases the release of ACh in the pontine reticular formation and prevents REM sleep is consistent with the foregoing interpretation (Hambrecht-Wiedbusch et al., 2010). The eszopiclone-induced inhibition of ACh release is likely to be mediated by GABA.

Narcolepsy is characterized by excessive daytime sleepiness that results, in part, from disrupted nighttime sleep. One of the most effective treatments for increasing and consolidating sleep in narcoleptics is gamma-hydroxybutyric acid (GHB), also known as sodium oxybate (Carter et al., 2009; Mamelak, 2009). GHB is an endogenous metabolite of GABA that promotes deep NREM sleep and REM sleep. The effects of GHB are mediated by specific GHB receptors and by $GABA_B$ receptors, although the mechanisms by which GHB increases sleep and produces its other behavioral effects are poorly understood. GHB is also a drug of abuse and its distribution for medical use is highly regulated by the U.S. Food and Drug Administration. The effectiveness of GHB for increasing sleep is consistent with a sleep-promoting role for GABA.

Glutamate is the major excitatory neurotransmitter in the brain, yet elucidating the role of glutamate in regulating sleep and wakefulness has been challenging

Glutamate is a precursor of GABA, and glutamate participates in many neurochemical reactions involving intermediary metabolism and protein synthesis (see Ch. 17). Thus it has been difficult to immunohistochemically identify neurons that use glutamate as a neurotransmitter, and mapping glutamatergic neurons in regions of the brain that regulate sleep and wakefulness has lagged behind the mapping of monoaminergic and cholinergic neurons. Labeling

cells for the presence of phosphate-activated glutaminase (the enzyme that converts glutamine to glutamate in neurons) and vesicular transporters for glutamate (markers for transmitter uptake and release) has revealed the presence of glutamatergic neurons in the basal forebrain that project to several areas of the cortex (Henny & Jones, 2008) and to the lateral hypothalamus (Henny & Jones, 2006). Glutamatergic neurons with sleep–wake activity patterns also are present in the pontine reticular formation (Kaneko et al., 1989), LDT/PPT (Wang & Morales, 2009), and thalamus (Steriade & McCarley, 2005). Glutamatergic input from the pontine brainstem to the thalamus contributes to cortical activation by inhibiting the thalamocortical system that generates the EEG spindles and slow-wave activity characteristic of sleep (Steriade & McCarley, 2005). Changes in the release of glutamate across the sleep–wakefulness cycle have been measured in several brain regions, and in most of those regions levels of glutamate are greater during the cortically activated states of wakefulness and/or REM sleep than during NREM sleep (Brevig & Baghdoyan, 2010). When microinjected into specific sleep-related brain regions, glutamate receptor agonists cause an increase in wakefulness or REM sleep (Datta & MacLean, 2007).

Effects of glutamate on sleep and wakefulness vary as a function of brain region

As is the case with GABA, the role of glutamate in regulating sleep and wakefulness differs across brain regions. Glutamate appears to promote either wakefulness or REM sleep, both of which are states characterized by cortical activation. Glutamatergic neurons in the basal forebrain discharge rhythmically in association with cortical EEG activation (Jones, 2005), and extracellular glutamate levels in the cortex increase progressively during wakefulness and REM sleep (Dash et al., 2009). Limbic and paralimbic areas associated with emotion are activated during REM sleep in humans (Nofzinger, 2008), and glutamate levels in rat orbitofrontal cortex, which is part of the paralimbic system, are greater during REM sleep than during wakefulness or NREM sleep (López-Rodríguez et al., 1994).

Glutamate may contribute to maintaining wakefulness by activating hypocretin-containing neurons in the lateral hypothalamus (Li et al., 2002). Hypocretinergic neurons give rise to numerous collaterals within the lateral hypothalamus, yet hypocretin has no direct effect on hypocretinergic neurons. Rather, hypocretin within the lateral hypothalamus activates glutamatergic interneurons that depolarize hypocretinergic cells (Li et al., 2002). An additional source of glutamatergic input to hypocretinergic neurons is the basal forebrain, which sends glutamatergic projections to the lateral hypothalamus (Henny & Jones, 2006). Direct administration of glutamate into the lateral hypothalamus increases wakefulness (Alam & Mallick, 2008).

Glutamatergic projections to the tuberomammillary nucleus of the posterior hypothalamus arise from the lateral hypothalamus (Torrealba et al., 2003), and glutamate levels in the tuberomammillary nucleus increase during active wakefulness (John et al., 2008). This finding implies that glutamate also promotes wakefulness by exciting histaminergic neurons. The sleep-related role of glutamate in the posterior hypothalamus is complex, however, as glutamate levels also increase with the onset of REM sleep and begin to decline prior to the end of REM sleep episodes (John et al., 2008). Histaminergic neurons in the tuberomammillary nucleus discharge during wakefulness but are inactive during REM sleep (Takahashi et al., 2006).

Extracellular recordings obtained from glutamatergic neurons in the basal forebrain show that almost half of cells sampled discharge at their fastest rates during wakefulness and REM sleep, consistent with a role for glutamate in causing cortical activation (Hassani et al., 2009). However, other glutamatergic neurons in the basal forebrain show different state-related discharge patterns. Some fire fastest during slow wave sleep, others during REM sleep, and still others discharge fastest during wakefulness but not during REM sleep (Hassani et al., 2009). Thus, the role of glutamate in regulating cortical activity and wakefulness is nuanced and brain-region specific.

Microinjection studies that deliver glutamatergic drugs directly into specific brain regions reveal that differential effects of glutamate on sleep and wakefulness are mediated by different receptor subtypes and by interactions with other transmitters. NMDA and AMPA receptor activation in the basal forebrain increases wakefulness and decrease NREM sleep, but only AMPA receptor activation also decreases REM sleep (Manfridi et al., 1999). Most neurons in the reticular formation are glutamatergic (Kaneko et al., 1989), and the LDT/PPT nuclei also contain a population of glutamatergic neurons (Wang & Morales, 2009). Activation of kainate receptors in the LDT/PPT increases REM sleep (Datta & MacLean, 2007), presumably by increasing the release of acetylcholine into the pontine reticular formation (Lydic & Baghdoyan, 2008). Blocking NMDA receptors in the pontine reticular formation with ketamine decreases ACh release in the pontine reticular formation and inhibits REM sleep (Lydic & Baghdoyan, 2002).

The increase in wakefulness caused by microinjecting glutamate into the basal forebrain is accompanied by an increase in adenosine and followed by an increase in NREM sleep and EEG slow wave activity (Wigren et al., 2007). Extracellular concentrations of glutamate and adenosine are regulated, in part, by astrocytes, and data now exist to support a role for glia in sleep regulation (Halassa et al., 2009a; Halassa et al., 2009b).

Glutamate modulates the interaction between sleep, depression and pain

Short-term sleep deprivation has long been known to acutely improve mood in some depressed patients, and excessive glutamate-induced excitation is thought to play a role in certain types of depression (Benedetti & Smeraldi, 2009). Neuroimaging work has shown that sleep deprivation therapy altered glutamate metabolism in the anterior cingulate cortex, which is part of the limbic system. The implication of this finding is that a decrease in glutamate may contribute to the mood improvement caused by sleep deprivation.

Glutamate is an important transmitter in pain pathways, and pain-induced sleep disruption is a significant clinical problem (Lavigne et al., 2007). In humans with fibromyalgia, a chronic pain condition, glutamate levels are elevated in brain regions that process pain information (Harris et al., 2009). One exciting direction for future studies is to determine whether increased glutamatergic neurotransmission in brain regions regulating pain comprises part of the mechanisms by which pain disrupts sleep (see also Chs. 54 and 60).

ADENOSINE

Adenosine is an endogenous sleep factor that mediates the homeostatic drive to sleep

Adenosine is an endogenous sleep factor that mediates the homeostatic drive to sleep (Basheer et al., 2004; Landolt, 2008; Halassa et al., 2009a). Caffeine, which blocks adenosine receptors, is highly effective and used worldwide for increasing wakefulness and improving performance on psychomotor tasks (Sebastião & Ribeiro, 2009). Adenosine is a byproduct of cellular metabolism (see Ch. 19), which explains why adenosine levels in many areas of the brain are greater during wakefulness than during sleep (Porkka-Heiskanen et al., 2000). The basal forebrain is the only brain region where adenosine levels have been shown to increase progressively during periods of extended wakefulness, providing evidence that adenosine in the basal forebrain mediates the homeostatic drive to sleep (Porkka-Heiskanen et al., 2000). Endogenous adenosine levels also increase in the basal forebrain of rats subjected to a sleep fragmentation protocol (McKenna et al., 2007). Studies in humans show that sleep fragmentation causes daytime sleepiness, detriments in psychomotor performance, cognitive dysfunction, and mood impairments that are similar to those caused by sleep deprivation (Stepanski, 2002). Infusing adenosine into the basal forebrain of rats causes an increase in NREM sleep, cortical slow-wave activity, and ATP levels in areas of the brain that are active during wakefulness, such as the cortex and hippocampus (Dworak et al., 2010). The finding of a sleep-dependent surge in ATP levels in wake-active brain areas provides support for the view that one function of sleep is energy restoration (Scharf et al., 2008). Adenosine levels are regulated, in part, by astrocytes, and adenosine released from astrocytes acts at adenosine A_1 receptors to increase homeostatic sleep drive (Halassa et al., 2009b). The mechanisms by which adenosine increases sleep are the subject of vigorous investigation using neurochemical, biochemical and electrophysiological approaches.

Adenosine inhibits wakefulness and promotes sleep via multiple mechanisms

The sleep-producing effects of adenosine are mediated by adenosine A_1 and A_{2A} receptors localized to multiple brain regions (Huang et al., 2007; Szymusiak et al., 2007; Sebastião & Ribeiro, 2009; Van Dort et al., 2009). Activating inhibitory adenosine A_1 receptors localized on wakefulness-promoting neurons is one mechanism by which adenosine causes sleep. Cholinergic neurons in the basal forebrain and LDT express adenosine A_1 receptors and are inhibited by adenosine. Pharmacologically increasing or decreasing neurotransmission at adenosine A_1 receptors in the basal forebrain or LDT causes an increase or decrease, respectively, in sleep (Basheer et al., 2004). Histaminergic neurons in the tuberomammillary nucleus of the posterior hypothalamus also express adenosine A_1 receptors, and activating those receptors increases NREM sleep (Oishi et al., 2008). Hypocretinergic neurons of the lateral hypothalamus express adenosine A_1 receptors (Thakkar et al., 2002) and are inhibited by adenosine (Liu & Gao, 2007), and blocking adenosine A_1 receptors in the lateral hypothalamus causes an increase in wakefulness (Thakkar et al., 2008) (see also adenosine receptors in Ch. 19).

Neurochemical studies have provided insights into sleep generating mechanisms by using *in vivo* microdialysis to deliver adenosine receptor agonists and antagonists while measuring endogenous transmitters and quantifying sleep. Activation of excitatory adenosine A_{2A} receptors in the ventrolateral and medial preoptic areas of the anterior hypothalamus increases GABA release in the posterior hypothalamus, where GABA inhibits wakefulness-promoting histaminergic neurons (Hong et al., 2005). Adenosine A_1 receptors in the prefrontal cortex inhibit the release of ACh in the prefrontal cortex, and comprise part of a descending system that inhibits wakefulness (Van Dort et al., 2009). In the pontine reticular formation, adenosine acting at A_1 receptors inhibits ACh release and prolongs recovery time from general anesthesia (Tanase et al., 2004), whereas activation of adenosine A_{2A} receptors increases ACh release in the pontine reticular formation and increases REM sleep (Marks et al., 2003; Coleman et al., 2006). Taken together, these studies illustrate the key point that elucidating neurotransmitter–neurotransmitter and neuromodulator–neurotransmitter interactions between brain regions is required for a mechanistic understanding of sleep and wakefulness.

Adenosine is a link between opioid-induced sleep disruption and pain

Sleep can be disrupted by many factors, one of which is pain (Lavigne et al., 2007). Opioids are widely used to treat both acute and chronic pain, but these drugs also cause sleep disruption (Lydic & Baghdoyan, 2007). Specifically, opioids decrease the deeper stages of slow wave sleep and suppress REM sleep. Opioids disrupt sleep even in the absence of pain, and sleep disruption alone increases the perception of pain in healthy, normal people (Chhangani et al., 2009). Increased pain, in turn, increases the need for opioids. Adenosine is sleep promoting and has been well studied for use as an adjuvant agent for pain management. Adenosine is antinociceptive and can reduce the requirement for opioids (Eisenach et al., 2003; Zhang et al., 2005).

Neurochemical studies have provided some insights into the mechanisms underlying the interactions between sleep, pain and opioids. Adenosine levels in the basal forebrain and the pontine reticular formation are decreased by the

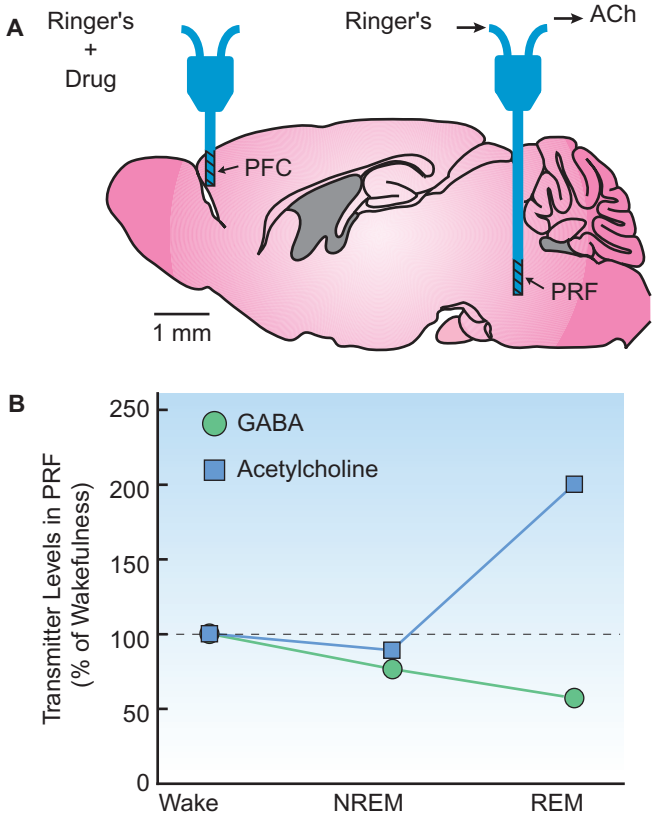

FIGURE 57-4 **Continued advances in understanding the neurochemical regulation of sleep will come from simultaneous measurement of multiple analytes in multiple brain regions. A.** Even the brain of a 25-g mouse can accommodate stereotaxic placement of multiple microdialysis probes and/or chemical sensors. The leftmost probe was positioned in the prefrontal cortex (PFC) for dialysis delivery of drugs while the rightmost probe was used to measure the effect on acetylcholine release in the pontine reticular formation (PRF). In spite of the limited spatial and temporal resolution of microdialysis, the study illustrated by this schematic revealed a previously unknown, descending pathway by which adenosine receptors in prefrontal cortex modulate acetylcholine release in the pons. (Used with permission from Van Dort et al., 2009.) **B.** Ratio of ACh to GABA in the pontine reticular formation (PRF). The graph expresses levels of ACh and GABA as a percent of their levels measured during wakefulness. Use of the ACh/GABA ratio provides an innovative proportionality index of neurochemical excitation and inhibition. For example, the waking ratio of 1 changes only to 1.2 during NREM sleep but to 3.5 during REM sleep. The use of such a proportionality index facilitates comparison of changes in analytes that vary with species, brain region, biosensor technology, and application of a wide range of independent variables. (Used with permission from Vanini et al., 2011.)

opioids morphine and fentanyl (Nelson et al., 2009). These decreases are blocked by naloxone, indicating mediation by μ-opioid receptors. The finding that inhibiting the breakdown of adenosine in the pontine reticular formation blocks the opioid-induced decrease in adenosine levels (Nelson et al., 2009) suggests a possible strategy for treating sleep disruption caused by opioids (Moore & Kelz, 2009). See also opioids in Ch. 54.

CONCLUSIONS AND FUTURE DIRECTIONS

Neurochemical studies of sleep are a relatively recent addition to neuroscience (Hobson & Steriade, 1986). Pioneering studies began with a bioassay of frog muscle contraction used to measure sleep-dependent changes in acetylcholine levels collected from cerebrospinal fluid above the cortex (Jasper & Tessier, 1971). Since then rapid progress in neurochemical sampling and measurement techniques now make it possible to humanely measure femtomole levels of endogenous neurotransmitters from intact, behaving animals. Figure 57-2 helps explain the enthusiasm for neurochemical studies of sleep by illustrating that sleep-dependent changes in neurotransmission remain to be quantified in most brain regions.

Progress in analytic chemistry continues to develop instrumentation that will improve the spatial and temporal resolution of neurochemical measurement (Watson et al., 2006; Burmeister et al., 2008; Robinson et al., 2008). The sleep-related brain regions in Figure 57-2 are drawn to scale and make clear the difficulty of obtaining sleep-dependent measures from small brain regions such as the ventrolateral preoptic area located at the base of the brain. Ongoing studies demonstrate that, even with current limitations, the ability to obtain *in vivo* neurochemical measures simultaneously from more than one brain region (Fig. 57-4A) provides a unique opportunity for characterizing neurochemical interactions between multiple brain regions. Just as no single brain region regulates sleep, all of the available evidence emphasizes that sleep is regulated by multiple neurotransmitters. Figure 57-4B illustrates the additional opportunity to quantify multiple neurotransmitters within a given brain region. Continued advances in sleep neurochemistry will come from the deployment of sensor and sampling techniques that measure multiple analytes from multiple brain regions. The clinical importance of sleep neurochemistry is clear from the cross-cutting relevance of sleep for cognitive function, performance, metabolism and mental health.

INSOMNIA

Helen A. Baghdoyan, Ralph Lydic

Insomnia is the most prevalent sleep disorder, affecting approximately 10% of the general population (NIH Consensus Panel Report, 2005). A diagnosis of chronic insomnia is based on patient complaints of difficulty falling asleep, difficulty staying asleep, early morning awakenings, and/or nonrestorative sleep for more than one month. These disturbances in sleep occur despite adequate opportunity for sleep and are accompanied by distress and/or impaired daytime functioning (Roth et al., 2010). Insomnia is classified as primary if the sleep disturbance does not result from other illnesses (psychiatric or medical), other sleep disorders, or the effects of substance abuse (Hall-Porter et al., 2010). Primary insomnia increases the risk for developing depression (Yokoyama et al., 2010), can lead to impaired ability to perform complex attentional tasks (Hall-Porter et al., 2010), and is associated with decreased health-related quality of life (Kyle et al., 2010). Insomnia creates a substantial economic burden due to absenteeism from work and reduced productivity (Léger & Bayon, 2010). Comorbid insomnia is the term used to describe chronic sleep disturbances that result from other illnesses, side effects of medications used to treat those illnesses, or drug abuse (NIH Consensus Panel Report, 2005). Comorbid insomnia is by far the most prevalent type, and reciprocal interactions between insomnia and coexisting illnesses are now recognized (Glidewell et al., 2010).

Human sleep is a complex phenotype regulated by interactions between environmental factors and multiple genes (Drake et al., 2008). Studies of twins have provided clear evidence of a genetic contribution to insomnia (Dauvilliers et al., 2005). One striking example of genetically based insomnia is fatal familial insomnia, which is caused by a point mutation in the prion protein gene (Montagna et al., 2003) (see Ch. 50). This disease is always fatal and is characterized by a loss of slow-wave sleep due to degeneration of the thalamus.

Neurochemical mechanisms of primary insomnia are poorly understood. Current models of insomnia are focused on hyperarousal caused by an interaction between biological, cognitive and emotional factors (Hall-Porter et al., 2010). Functional neuroimaging during sleep has provided support for the hypothesis that in patients with insomnia key brain regions regulating wakefulness (see text of this chapter) do not deactivate during sleep, nor are these brain regions adequately activated during wakefulness (Nofzinger, 2008). These data emphasize the importance of preclinical studies aiming to characterize sleep-dependent neurochemical changes in the multiple brain regions that regulate sleep and wakefulness.

Pharmacological treatment of insomnia involves a wide range of agents, and the efficacy of these agents fits with what is known about the underlying neurochemistry of sleep. Drugs approved for the treatment of insomnia by the United States Food and Drug Administration include nine benzodiazepine receptor agonists (BzRAs), the melatonin MT1 and MT2 receptor agonist ramelteon, and most recently the tricyclic antidepressant doxepin (Roth et al., 2010). BzRAs, which are recommended as first-line pharmacologic treatment, have a binding site on the $GABA_A$ receptor complex and act as allosteric modulators to enhance inhibition mediated by endogenous GABA. BzRAs are thought to increase sleep by inhibiting wakefulness-promoting monoaminergic and cholinergic neurons. Although some BzRAs have a benzodiazepine structure (flurazepam, triazolam, temazepam, estazolam, quazepam) and others are non-benzodiazepines (eszopiclone, zaliplon, zolpidem, zolpidem extended-release), all of these drugs bind to $GABA_A$ receptors composed of α1-3,5β2,3γ2,3 subunits (see Ch. 18). Whereas the benzodiazepines have similar affinity for all four α subunits, the non-benzodiazepine zolpidem has greater affinity for α1 than for the other α subtypes and the non-benzodiazepine eszopiclone is thought to have relatively high affinity for the α1 and α3 subtypes (Hambrecht-Wiedbusch et al., 2010; Roth & Roehrs, 2010). How these binding affinities translate into differential responses in patients is not yet known.

A point mutation expressed as an altered β3 subunit of the $GABA_A$ receptor complex has been identified in humans (Buhr et al., 2002). Of relevance to insomnia is that functional analyses of human $GABA_A$ receptors containing the mutated β3 subunit showed that the mutation causes faster deactivation of the chloride ion channel (Buhr et al., 2002). Such a mutation could have the effect of decreasing GABAergic transmission. One patient with chronic insomnia was found to have this mutation, suggesting the intriguing possibility that reduced GABAergic inhibition may contribute to insomnia in humans (Buhr et al., 2002). Mice lacking the β3 subunit show altered sleep responses to the benzodiazepine midazolam (Wisor et al., 2002), and point mutations in the α1 subunit cause mice to be insensitive to the sleep-inducing effects of diazepam (Tobler et al., 2001). An exciting opportunity for sleep neurochemistry is identification of genetically modified neurotransmitter receptor systems that increase risk for insomnia.

References

Buhr, A., Bianchi, M. T., Baur, R., Courtet, P., Pignay, V., Boulenger, J. P., et al. (2002). Functional characterization of the new human $GABA_A$ receptor mutation β3(R192H). *Human Genetics*, *111*, 154–160.

Dauvilliers, Y., Maret, S., & Tafti, M. (2005). Genetics of normal and pathological sleep in humans. *Sleep Medicine Reviews*, *9*, 91–100.

Drake, C. L., Schofield, H., & Roth, T. (2008). Vulnerability to insomnia: The role of familial aggregation. *Sleep Medicine*, *9*, 297–302.

Glidewell, R. N., Moorcroft, W. H., & Lee-Chiong, T. (2010). Comorbid insomnias: Reciprocal relationships and medication management. *Sleep Medicine Clinical*, *5*, 627–646.

Hall-Porter, J. M., Curry, D. T., & Walsh, J. K. (2010). Pharmacologic treatment of primary insomnia. *Sleep Medicine Clinical*, *5*, 609–625.

Hambrecht-Wiedbusch, V. S., Gauthier, E. A., Baghdoyan, H. A., & Lydic, R. (2010). Benzodiazepine receptor agonists cause drug-specific and state–specific alterations in EEG power

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and acetylcholine release in rat pontine reticular formation. *Sleep*, *33*, 909–918.

Kyle, S. D., Morgan, K., & Espie, C. A. (2010). Insomnia and health-related quality of life. *Sleep Medicine Reviews*, *14*, 69–82.

Léger, D., & Bayon, V. (2010). Societal costs of insomnia. *Sleep Medicine Reviews*, *14*, 379–389.

Montagna, P., Gambetti, P., Cortelli, P., & Lugaresi, E. (2003). Familial and sporadic fatal insomnia. *Lancet Neurology*, *2*, 167–176.

NIH Consenses Panel Report (2005). National Institutes of Health state of the science conference statement: Manifestations and management of chronic insomnia in adults. *Sleep*, *28*, 1049–1057.

Nofzinger, E. A. (2008). Functional neuroimaging of sleep disorders. *Current Pharmaceutical Design*, *14*, 3417–3429.

Roth, T., & Roehrs, T. A. (2010). Pharmacotherapy for insomnia. *Sleep Medicine Clinical*, *5*, 529–539.

Tobler, I., Kopp, C., & Rudolph, U. (2001). Diazepam-induced changes in sleep: Role of the α1 $GABA_A$ receptor subtype. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 6464–6469.

Wisor, J. P., DeLorey, T. M., Homanics, G. E., & Edgar, D. M. (2002). Sleep states and sleep electroencephalographic spectral power in mice lacking the β3 subunit of the $GABA_A$ receptor. *Brain Research*, *955*, 221–228.

Yokoyama, E., Kaneita, Y., Saito, Y., Uchiyama, M., Matsuzaki, Y., Munezawa, T., et al. (2010). Association between depression and insomnia sybtypes: A longitudinal study on the elderly in Japan. *Sleep*, *33*, 1693–1702.

References

Alam, M. A., & Mallick, B. N. (2008). Glutamic acid stimulation of the perifornical–lateral hypothalamic area promotes arousal and inhibits non-REM/REM sleep. *Neuroscience Letters*, *439*, 281–286.

Basheer, R., Strecker, R. E., Thakkar, M. M., & McCarley, R. W. (2004). Adenosine and sleep–wake regulation. *Progress in Neurobiology*, *73*, 379–396.

Benedetti, F., & Smeraldi, E. (2009). Neuroimaging and genetics of antidepressant response to sleep deprivation: Implications for drug development. *Current Pharmaceutical Design*, *15*, 2637–2649.

Berridge, C. W. (2008). Noradrenergic modulation of arousal. *Brain Research Reviews*, *58*, 1–17.

Brevig, H. N., & Baghdoyan, H. A. (2010). Neurotransmitters and neuromodulators regulating sleep and wakefulness. In G. F. Koob, M. Le Moal & R. F. Thompson (Eds.), *Encyclopedia of behavioral neuroscience* (pp. 456–463). Oxford: Elsevier.

Brown, E. N., Lydic, R., & Schiff, N. D. (2010). General anesthesia, sleep, and coma. *The New England Journal of Medicine*, *363*, 2638–2650.

Burmeister, J. J., Pomerleau, F., Huettl, P., Gash, C. R., Werner, C. E., Bruno, J. P., et al. (2008). Ceramic-based multisite microelectrode arrays for simultaneous measures of choline and acetylcholine in CNS. *Biosensors & Bioelectronics*, *23*, 1382–1389.

Carter, L. P., Koek, W., & France, C. P. (2009). Behavioral analyses of GHB: Receptor mechanisms. *Pharmacology & Therapeutics*, *121*, 100–114.

Chase, M. H., Monoson, R., Watanabe, K., & Babb, M. I. (1976). Somatic reflex response-reversal of reticular origin. *Experimental Neurology*, *50*, 561–567.

Chaudhuri, K. R., & Logishetty, K. (2009). Dopamine receptor agonists and sleep disturbances in Parkinson's disease. *Parkinsonism & Related Disorders*, *15*(Suppl. 4), S101–S104.

Chhangani, B. S., Roehrs, T. A., Harris, E. J., Hyde, M., Drake, C., Hudgel, D. W., et al. (2009). Pain sensitivity in sleepy pain–free normals. *Sleep*, *32*, 1011–1017.

Cirelli, C., & Tononi, G. (2008). Is sleep essential? *PLoS Biology*, *6*, 3216.

Coleman, C. G., Baghdoyan, H. A., & Lydic, R. (2006). Dialysis delivery of an adenosine A_{2A} agonist into the pontine reticular formation of C57BL/6J mouse increases pontine acetylcholine release and sleep. *Journal of Neurochemistry*, *96*, 1750–1759.

Colten, H. R., & Altevogt, B. M. (Eds.). (2006). *Sleep disorders and sleep deprivation: An unmet public health problem*. Washington, D.C: National Academies Press.

Dash, M. B., Douglas, C. L., Vyazovshiy, V. V., Cirelli, C., & Tononi, G. (2009). Long-term homeostasis of extracellular glutamate in the rat cerebral cortex across sleep and waking states. *Journal of Neuroscience*, *29*, 620–629.

Datta, S. (2010). Cellular and chemical neuroscience of mammalian sleep. *Sleep Medicine*, *11*, 431–440.

Datta, S., & MacLean, R. R. (2007). Neurobiological mechanisms for the regulation of mammalian sleep–wake behavior: Reinterpretation of historical evidence and inclusion of contemporary cellular and molecular evidence. *Neuroscience and Biobehavioral Reviews*, *31*, 775–824.

de Lecea, L. (2010). A decade of hypocretins: Past, present and future of the neurobiology of arousal. *Acta Physiologica*, *198*, 203–208.

Dworak, M., McCarley, R. W., Kim, T., Kalinchuck, A. V., & Basheer, R. (2010). Sleep and brain energy levels: ATP changes during sleep. *Journal of Neuroscience*, *30*, 9007–9016.

Eisenach, J. C., Rauck, R. L., & Curry, R. (2003). Intrathecal, but not intravenous adenosine reduces allodynia in patients with neuropathic pain. *Pain*, *105*, 65–70.

Fava, M., McCall, W. V., Krystal, A., Wessell, T., Rubens, R., Caron, J., et al. (2006). Eszopiclone co-administered with fluoxetine in patients with insomnia coexisting with major depressive disorder. *Biological Psychiatry*, *59*, 1052–1060.

Ferini-Strambi, L., & Manconi, M. (2009). Treatment of restless legs syndrome. *Parkinsonism & Related Disorders*, *15*(Suppl. 4), S65–S70.

Fink, K. B., & Göthert, M. (2007). 5-HT receptor regulation of neurotransmitter release. *Pharmacological Reviews*, *59*, 360–417.

Flint, R. R., Chang, T., Lydic, R., & Baghdoyan, H. A. (2010). $GABA_A$ receptors in the pontine reticular formation of C57BL/6J mouse modulate neurochemical, electrographic, and behavioral phenotypes of wakefulness. *Journal of Neuroscience*, *30*, 12301–12309.

Friedman, E. B., Sun, Y., Moore, J. T., Hung, H.-T., Meng, Q. C., Perera, P., et al. (2010). A conserved behavioral state barrier impedes transitions between anesthetic-induced unconsciousness and wakefulness: Evidence for neural inertia. *PLoS One*, *5*, e11903.

Fronczek, R., Baumann, C. R., Lammers, G. J., Bassetti, C. L., & Overeem, S. (2009). Hypocretin/orexin disturbances in neurological disorders. *Sleep Medicine Reviews*, *13*, 9–22.

Halassa, M. M., Fellin, T., & Haydon, P. G. (2009a). Tripartite synapses: Roles for astrocytic purines in the control of synaptic physiology and behavior. *Neuropharmacology*, *57*, 343–346.

Halassa, M. M., Florian, C., Fellin, T., Munoz, J. R., Lee, S. -Y., Abel, T., et al. (2009b). Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. *Neuron*, *61*, 213–219.

Hambrecht-Wiedbusch, V. S., Gauthier, E. A., Baghdoyan, H. A., & Lydic, R. (2010). Benzodiazepine receptor agonists cause drug-specific and state-specific alterations in EEG power and acetylcholine release in rat pontine reticular formation. *Sleep*, *33*, 909–918.

Harris, R. E., Sundgren, P. C., Craig, A. D., Kirshenbaum, E., Sen, A., Napadow, V., et al. (2009). Elevated insular glutamate in fibromyalgia is associated with experimental pain. *Arthritis and Rheumatism*, *60*, 3146–3152.

Hass, H. L., Sergeeva, O. A., & Selbach, O. (2008). Histamine in the nervous system. *Physiological Reviews*, *88*, 1183–1241.

Hassani, O. K., Lee, M. G., Henny, P., & Jones, B. E. (2009). Discharge profiles of identified GABAergic in comparison to cholinergic and putative glutamatergic basal forebrain neurons across the sleep–wake cycle. *Journal of Neuroscience*, *29*, 11828–11840.

Henny, P., & Jones, B. E. (2006). Innervation of orexin/hypocretin neurons by GABAergic, glutamatergic, or cholinergic basal forebrain terminals evidenced by immunostaining for presynaptic vesicular transporter and postsynaptic scaffolding proteins. *Journal of Comparative Neurology*, *499*, 645–661.

Henny, P., & Jones, B. E. (2008). Projections from basal forebrain to prefrontal cortex comprise cholinergic, GABAergic and glutamateric inputs to pyramidal cells or interneurons. *The European Journal of Neuroscience*, *27*, 654–670.

Herring, W. J., Budd, K. S., Hutzelmann, J., Snyder, E., Snavely, D., & Liu, K., et al. (2010). Efficacy and tolerability of the dual orexin receptor antagonist MK-4305 in patients with primary insomnia: randomized, controlled, adaptive crossover polysomnography study. *Sleep*, *33* (Abstr Suppl), A199.

Hobson, J. A. (1999). *Consciousness*. New York: Scientific American Library.

Hobson, J. A., & Steriade, M. (1986). The neuronal basis of behavioral state control. In F. E. (1986). Bloom (Ed.), *Handbook of physiology—the nervous system* (Vol. 4, pp. 297–338). Bethesda, MD: American Physiological Society.

Hong, Z. -Y., Huang, Z. -L., Qu, W. -M., Eguchi, N., Urade, Y., & Hayaishi, O. (2005). An adenosine A_{2A} receptor agonist induces sleep by increasing GABA release in the tuberomammillary nucleus to inhibit histaminergic systems in rats. *Journal of Neurochemistry*, *92*, 1542–1549.

Huang, Z. -L., Urade, Y., & Hayaishi, O. (2007). Prostaglandins and adenosine in the regulation of sleep and wakefulness. *Current Opinion in Pharmacology*, *7*, 33–38.

Jasper, H. H., & Tessier, J. (1971). Acetylcholine liberation from cerebral cortex during paradoxical (REM) sleep. *Science*, *172*, 601–602.

John, J., Ramanathan, L., & Siegel, J. M. (2008). Rapid changes in glutamate levels in the posterior hypothalamus across sleep–wake states in freely behaving rats. American Journal of Physiology. Regulatory. *Integrative and Comparative Physiology*, *295*, R2041–R2049.

Jones, B. E. (2005). From waking to sleeping: Neuronal and chemical substrates. *Trends in Pharmacological Sciences*, *26*, 578–586.

Jouvet, M. (1969). Biogenic amines and the states of sleep. *Science*, *163*, 32–41.

Jouvet, M. (1999). Sleep and serotonin: An unfinished story. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *21*, 24S–27S.

Kaneko, T., Itoh, K., Shigemoto, R., & Mizuno, N. (1989). Glutaminase-like immunoreactivity in the lower brainstem and cerebellum of the adult rat. *Neuroscience*, *32*, 79–98.

Kelz, M. B., Sun, Y., Chen, J., Cheng Meng, Q., Moore, J. T., Veasey, S. C., et al. (2008). An essential role for orexins in emergence from general anesthesia. *Proceedings of the National Academy of Sciences of the United States of America*, 1309–1314.

Kilduff, T. S., & Peyron, C. (2000). The hypocretin/orexin ligand-receptor system: Implications for sleep and sleep disorders. *Trends in Neurosciences*, *23*, 359–365.

Kiyashchenko, L. I., Mileykovskiy, B. Y., Maidment, N., Lam, H. A., Wu, M. F., John, J., et al. (2002). Release of hypocretin (orexin) during waking and sleep states. *Journal of Neuroscience*, *22*, 5282–5286.

Kryger, M. H., Roth, T., & Dement, W. C. (Eds.), (2011). *Principles and practice of sleep medicine* (5th ed.). St. Louis: Elsevier.

Krystal, A. D., Durrence, H. H., Scharf, M., Jochelson, P., Rogowski, R., Ludington, E., et al. (2010). Efficacy and safety of doxepin 1 mg and 3 mg in a 12-week sleep laboratory and outpatient trial of elderly subjects with chronic primary insomnia. *Sleep*, *33*, 1553–1561.

Landolt, H.-P. (2008). Sleep homeostasis: A role for adenosine in humans? *Biochemical Pharmacology*, *75*, 2070–2079.

Landolt, H.-P., & Wehrle, R. (2009). Antagonism of serotonergic $5\text{-}HT_{2A/2C}$ receptors: Mutual improvement of sleep, cognition and mood?. *The European Journal of Neuroscience*, *29*, 1795–1809.

Lavigne, G., Sessle, B. J., Choinière, M., & Soja, P. J. (Eds.), (2007). *Sleep and pain*. Seattle: IASP.

Lee, M. G., Hassani, O. K., & Jones, B. E. (2005). Discharge of identified orexin/hypocretin neurons across the sleep–waking cycle. *Journal of Neuroscience*, *25*, 6716–6720.

Lenfant, C. (2003). 2003 National sleep disorders research plan. In <http://www.nhlbi.nih.gov/health/prof/sleep/res_plan/preface.html>: National Institutes of Health.

Li, Y., Gao, X.-B., Sakurai, T., & van den Pol, A. N. (2002). Hypocretin/orexin excites hypocretin neurons via a local glutamate neuron—a potential mechanism for orchestrating the hypothalamic arousal system. *Neuron*, *36*, 1169–1181.

Liang, C. L., & Marks, G. A. (2009). A novel GABAergic afferent input to the pontine reticular formation: The mesopontine GABAergic column. *Brain Research*, *1297*, 32–40.

Liu, Z.-W., & Gao, X.-B. (2007). Adenosine inhibits activity of hypocretin/orexin neurons by the A1 receptor in the lateral hypothalamus: A possible sleep-promoting effect. *Journal of Neurophysiology*, *97*, 837–848.

López-Rodríguez, F., Kohlmeier, K., Morales, F. R., & Chase, M. H. (1994). State dependency of the effects of microinjection of cholinergic drugs into the nucleus pontis oralis. *Brain Research*, *649*, 271–281.

Lydic, R., & Baghdoyan, H. A. (2002). Ketamine and MK-801 decrease acetylcholine release in the pontine reticular formation, slow breathing, and disrupt sleep. *Sleep*, *25*, 615–620.

Lydic, R., & Baghdoyan, H. A. (2005). Sleep, anesthesiology, and the neurobiology of arousal state control. *Anesthesiology*, *103*, 1268–1295.

Lydic, R., & Baghdoyan, H. A. (2007). Neurochemical mechanisms mediating opioid-induced REM sleep disruption. In G. Lavigne, B. J. Sessle, M. Choinière & P. J. Soja (Eds.), *Sleep and pain* (pp. 99–122). Seattle: IASP.

Lydic, R., & Baghdoyan, H. A. (2008). Acetylcholine modulates sleep and wakefulness: A synaptic perspective. In J. M. Monti, S. R. Pandi-Perumal & C. M. Sinton (Eds.), *Neurochemistry of sleep and wakefulness* (pp. 109–143). New York: Cambridge University Press.

Mamelak, M. (2009). Narcolepsy and depression and the neurobiology of gammahydroxybutyrate. *Progress in Neurobiology*, *89*, 193–219.

Manfridi, A., Brambilla, D., & Mancia, M. (1999). Stimulation of NMDA and AMPA receptors in the rat nucleus basalis of Meynert affects sleep. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *277*, R1488–R1492.

Marks, G. A., Shaffery, J. P., Speciale, S. G., & Birabil, C. G. (2003). Enhancement of rapid eye movement sleep in the rat by actions at A1 and A2a adenosine receptor subtypes with a differential sensitivity to atropine. *Neuroscience*, *116*, 913–920.

McKenna, J. T., Tartar, J. L., Ward, C. P., Thakkar, M. M., Cordeira, J. W., McCarley, R. W., et al. (2007). Sleep fragmentation elevates behavioral, electrographic and neurochemical measures of sleepiness. *Neuroscience*, *146*, 1462–1473.

Mileykovskiy, B. Y., Kiyashchenko, L. I., & Siegel, J. M. (2005). Behavioral correlates of activity in identified hypocretin/orexin neurons. *Neuron*, *46*, 787–798.

Monti, J. M., & Monti, D. (2007). The involvement of dopamine in the modulation of sleep and waking. *Sleep Medicine Reviews*, *11*, 113–133.

Moore, J. T., & Kelz, M. B. (2009). Opiates, sleep and pain. The adenosinergic link. *Anesthesiology*, *111*, 1175–1176.

Nelson, A. M., Battersby, A. S., Baghdoyan, H. A., & Lydic, R. (2009). Opioid induced decreases in rat brain adenosine levels are reversed by inhibiting adenosine deaminase. *Anesthesiology*, *111*, 1327–1333.

NIH Consensus Panel Report, (2005). National Institutes of Health state of the science conference statement: Manifestations and management of chronic insomnia in adults. *Sleep*, *28*, 1049–1057.

Nishino, S., Okuro, M., Kotorii, N., Anegawa, E., Ishimaru, Y., Matsumura, M., et al. (2010). Hypocretin/orexin and narcolepsy: New basic and clinical insights. *Acta Physiologica*, *198*, 209–222.

Nofzinger, E. A. (2005). Neuroimaging and sleep medicine. *Sleep Medicine Reviews*, *9*, 157–172.

Nofzinger, E. A. (2008). Functional neuroimaging of sleep disorders. *Current Pharmaceutical Design*, *14*, 3417–3429.

Oishi, Y., Huang, Z. -L., Fredholm, B. B., Urade, Y., & Hayaishi, O. (2008). Adenosine in the tuberomammillary nucleus inhibits the histaminergic system via A_1 receptors and promotes non-rapid eye movement sleep. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 19992–19997.

Pace-Schott, E. F., & Hobson, J. A. (2002). The neurobiology of sleep: Genetics, cellular physiology, and subcortical networks. *Nature Reviews Neuroscience*, *3*, 591–605.

Parmentier, R., Anaclet, C., Guhennec, C., Brousseau, E., Bricout, D., Giboulot, T., et al. (2007). The brain H_3-receptor as a novel therapeutic target for vigilance and sleep–wake disorders. *Biochemical Pharmacology*, *73*, 1157–1171.

Porkka-Heiskanen, T., Strecker, R. E., & McCarley, R. W. (2000). Brain site-specificity of extracellular adenosine concentration changes during sleep deprivation and spontaneous sleep: An *in vivo* microdialysis study. *Neuroscience*, *99*, 507–517.

Robinson, D. L., Hermans, A., Seipel, A. T., & Wightman, R. M. (2008). Monitoring rapid chemical communication in the brain. *Chemical Reviews*, *108*, 2554–2584.

Rodrigo-Angulo, M. L., Heredero, S., Rodríguez-Veiga, E., & Reinoso-Suárez, F. (2008). GABAergic and non-GABAergic thalamic, hypothalamic and basal forebrain projections to the ventral oral pontine reticular formation: Their implication in REM sleep modulation. *Brain Research*, *1210*, 116–125.

Roth, T., Lines, C., Vandormael, K., Ceesay, P., Anderson, D., & Snavely, D. (2010). Effect of gaboxadol on patient-reported measures of sleep and waking function in patients with primary insomnia: Results from two randomized, controlled, 3-month studies. *Journal of Clinical Sleep Medicine*, *6*, 30–40.

Roth, T., Rogowski, R., Hull, S., Schwartz, H., Koshorek, G., Corser, B., et al. (2007). Efficacy and safety of doxepin 1 mg, 3 mg, and 6 mg in adults with primary insomnia. *Sleep*, *30*, 1555–1561.

Sakurai, T. (2007). The neural circuit of orexin (hypocretin): Maintaining sleep and wakefulness. *Nature Reviews Neuroscience*, *8*, 171–181.

Sakurai, T., Mieda, M., & Tsujino, N. (2010). The orexin system: Roles in sleep/wake regulation. *Annals of the New York Academy of Sciences*, *1200*, 149–161.

Sander, K., Kottke, T., & Stark, H. (2008). Histamine H_3 receptor antagonists go to clinics. *Biological & Pharmaceutical Bulletin*, *31*, 2163–2181.

Saul, S. (2006). Record sales of sleeping pills are causing worries. *New York Times*

Scharf, M. T., Naidoo, N., Zimmerman, J. E., & Pack, A. I. (2008). The energy hypothesis of sleep revisited. *Progress in Neurobiology*, *86*, 264–280.

Sebastião, A. M., & Ribeiro, J. A. (2009). Adenosine receptors and the central nervous system. In C. N. Wilson & S. J. Mustafa (Eds.), *Adenosine receptors in health and disease* (pp. 471–534). Berlin: Springer.

Siegel, J. M. (2009). Sleep viewed as a state of adaptive inactivity. *Nature Reviews Neuroscience*, *10*, 747–753.

Stenberg, D. (2007). Neuroanatomy and neurochemistry of sleep. *Cellular and Molecular Life Sciences*, *64*, 1187–1204.

Stepanski, E. J. (2002). The effect of sleep fragmentation on daytime function. *Sleep*, *25*, 268–276.

Steriade, M., & McCarley, R. W. (2005). *Brain control of wakefulness and sleep* (2nd ed.). New York: Plenum.

Szymusiak, R., Gvilia, I., & McGinty, D. (2007). Hypothalamic control of sleep. *Sleep Medicine*, *8*, 291–301.

Takahashi, K., Lin, J. -S., & Sakai, K. (2006). Neuronal activity of histaminergic tuberomammillary neurons during wake–sleep states in the mouse. *Journal of Neuroscience*, *26*, 10292–10298.

Tanase, D., Baghdoyan, H. A., & Lydic, R. (2004). Dialysis delivery of an adenosine A_1 receptor agonist to the pontine reticular formation decreases acetylcholine release and increases anesthesia recovery time. *Anesthesiology*, *98*, 912–920.

Thakkar, M. M., Winston, S., & McCarley, R. W. (2002). Orexin neurons of the hypothalamus express adenosine A1 receptors. *Brain Research*, *944*, 190–194.

Thakkar, M. M., Engemann, S. C., Walsh, K. M., & Sahota, P. K. (2008). Adenosine and the homeostatic control of sleep: Effects of A1 receptor blockade in the perifornical lateral hypothalamus on sleep–wakefulness. *Neuroscience*, *153*, 875–880.

Torrealba, F., Yanagisawa, M., & Saper, C. B. (2003). Colocalization of orexin A and glutamate immunoreactivity in axon terminals in the tuberomammillary nucleus in rats. *Neuroscience*, *119*, 1033–1044.

Trenkwalder, C., & Paulus, W. (2010). Restless legs syndrome: Pathophysiology, clinical presentation and management. *Nature Reviews Neurology*, *6*, 337–346.

Tsujino, N., & Sakurai, T. (2009). Orexin/hypocretin: A neuropeptide at the interface of sleep, energy homeostasis, and reward system. *Pharmacological Reviews*, *61*, 162–176.

Ursin, R. (2002). Serotonin and sleep. *Sleep Medicine Reviews*, *6*, 57–69.

Van Dort, C. J., Baghdoyan, H. A., & Lydic, R. (2009). Adenosine A_1 and A_{2A} receptors in mouse prefrontal cortex modulate acetylcholine release and behavioral arousal. *Journal of Neuroscience*, *29*, 871–881.

Vanini, G., Baghdoyan, H. A., & Lydic, R. (2010). Relevance of sleep neurobiology for cognitive neuroscience and anesthesiology. In G. A. Mashour (Ed.), *Consciousness, awareness, and anesthesia* (pp. 1–23). Cambridge University Press.

Vanini, G., Watson, C. J., Lydic, R., & Baghdoyan, H. A. (2008). GABAergic neurotransmission in the pontine reticular formation modulates hypnosis, immobility, and breathing during isoflurane anesthesia. *Anesthesiology*, *109*, 978–988.

Vanini, G., Baracy, C. R., Lydic, R., Baghdoyan, H.A. (2010b). GABA levels in cat basal forebrain and cortex are greater during

non-rapid eye movement (NREM) sleep than during REM sleep and wakefulness. Society for Neuroscience Meeting Planner Online Program No.:798.1.

Vanini, G., Torterolo, P., Baghdoyan, H. A., & Lydic, R. (2011). The shared circuits of sleep and anesthesia. In G. A. Mashour & R. Lydic (Eds.), *The Neuroscientific Foundations of Anesthesiology* (pp. 33–44). New York: Oxford University Press.

Vanini, G., Wathen, B. L., Lydic, R., & Baghdoyan, H. A. (2011). Endogenous GABA levels in the pontine reticular formation are greater during wakefulness than during rapid eye movement sleep. *Journal of Neuroscience*, *31*, 2649–2656.

Wang, H. -L., & Morales, M. (2009). Pedunculopontine and laterodorsal tegmental nuclei contain distinct populations of cholinergic, glutamatergic and GABAergic neurons in the rat. *The European Journal of Neuroscience*, *29*, 340–358.

Watson, C. J., Venton, B. J., & Kennedy, R. T. (2006). *In vivo* measurements of neurotransmitters using microdialysis sampling. *Analytical Chemistry*, *78*, 1391–1399.

Watson, C. J., Baghdoyan, H. A., & Lydic, R. (2010). Neuropharmacology of sleep and wakefulness. *Sleep Medicine Clinical*, *5*, 513–528.

Wigren, H.-K., Schepens, M., Matto, V., Stenberg, D., & Porkka-Heiskanen, T. (2007). Glutamatergic stimulation of the basal forebrain elevates extracellular adenosine and increases the subsequent sleep. *Neuroscience*, *147*, 811–823.

Xi, M. C., & Chase, M. H. (2010). The injection of hypocretin-1 into the nucleus pontis oralis induces either active sleep or wakefulness depending on the behavioral state of the animal when it is administered. *Sleep*, *33*, 1236–1243.

Zhang, Y., Conklin, D. R., Li, X., & Eisenach, J. C. (2005). Intrathecal morphine reduces allodynia after peripheral nerve injury in rats via activation of a spinal A1 adenosine receptor. *Anesthesiology*, *102*, 416–420.