Sleeping with the hypothalamus: emerging therapeutic targets for sleep disorders

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Delineating the basic mechanisms that regulate sleep will likely result in the development of better treatments for sleep disorders. The hypothalamus is now recognized as a key center for sleep regulation, with hypothalamic neurotransmitter systems providing the framework for therapeutic advances. An increased awareness of the close interaction between sleep and homeostatic systems is also emerging. Progress has occurred in the understanding of narcolepsy—molecular techniques have identified the lateral hypothalamic hypocretin (orexin) neuropeptide system as key to the disorder. Other sleep disorders are now being tackled in the same way and are likely to yield to efforts combining basic and clinical research. Here we highlight the role of the hypothalamus in sleep physiology and discuss neurotransmitter systems, such as adenosine, dopamine, GABA, histamine and hypocretin, that may have therapeutic applications for sleep disorders.

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Sleep disorders are increasingly prevalent and are associated with significant morbidity[1] (**Table 1**). In the United States alone, the number of sleep disorder centers and accredited sleep physicians has more than doubled since 1996. Research into the neurobiology of sleep has recently accelerated as a result of the rapid development of sleep medicine, but current treatments still target symptoms rather than the underlying pathophysiology of sleep disorders (**Table 1**). Thus there is a critical need to apply basic research to these common clinical problems, as recently recognized in a comprehensive research plan from the U.S. National Institutes of Health (http://www.nhlbisupport.com/sleep/research/research-a.htm).

The hypothalamus has been rediscovered as a key regulator of sleep and wakefulness, shifting focus away from brainstem and thalamocortical systems. This role for the hypothalamus was first recognized with the encephalitis lethargica epidemic (1918–1926), in which affected individuals showed major sleep abnormalities and Parkinsonism. It was suggested that posterior hypothalamic and midbrain junction lesions resulted in sleepiness, whereas anterior hypothalamic inflammation resulted in insomnia and chorea (with striatal involvement)[2]. These findings were subsequently overshadowed by animal studies where profound (often lethal) physiological abnormalities occurred after hypothalamic lesions, thus masking sleep effects[3]. The discovery of the brainstem ascending reticular activating system[4] further shifted focus away from the hypothalamus.

Sleep control systems in the hypothalamus and their interaction with the circadian pacemaker in the suprachiasmatic nuclei (SCN) have been identified. Furthermore, the interactions between sleep and other hypothalamic functions, such as the regulation of food intake, metabolism, hormone release and temperature, are increasingly coming to light. Sleep deprivation alters hormone release, increases body temperature, stimulates appetite and activates the sympathetic nervous system[5,6]. Sleep control systems within the hypothalamus are therefore closely integrated with homeostatic systems. These systems need to be studied together during pharmacological manipulations.

Here, we first review how the monoaminergic and cholinergic systems form the basis of most models of sleep regulation. The rest of this review focuses on recent progress in the field of sleep neurobiology, with emphasis on the hypothalamus and promising neurotransmitters for therapeutic application in sleep disorders medicine. Syndromes such as obstructive sleep apnea and restless leg syndrome (RLS) will only be briefly addressed.

Monoaminergic and cholinergic control of sleep

Sleep oscillates between rapid eye movement (REM) sleep, light sleep and deep slow-wave sleep (SWS); the latter two are collectively called non-REM (NREM) sleep. Sleep-state control (for example, transitions from NREM to REM) is attributed to reciprocal monoaminergic–cholinergic interactions in the brainstem[7], whereas the electrophysiological expression of sleep versus wakefulness is attributed to the synchronization and desynchronization of thalamocortical circuits[7,8] (**Fig. 1**). In this model, serotoninergic (raphe nuclei, RN), adrenergic (locus coeruleus, LC) and histaminergic (tuberomammillary nucleus, TMN) activity is high during wakefulness, decreases during NREM stages, and becomes almost silent during REM sleep[6]. In contrast, brainstem cholinergic activity (laterodorsal tegmental area, LDT, and pedunculopontine nuclei, PPT) is high during wakefulness and REM sleep[7].

High monoaminergic and cholinergic tones are responsible for electroencephalogram (EEG) desynchronization during wakefulness and REM sleep, respectively. Cholinergic projections to the thalamus are critical to EEG activation in this model, complementing cholinergic projections from the basal forebrain area (BFA) to the cortex that are especially active while one is

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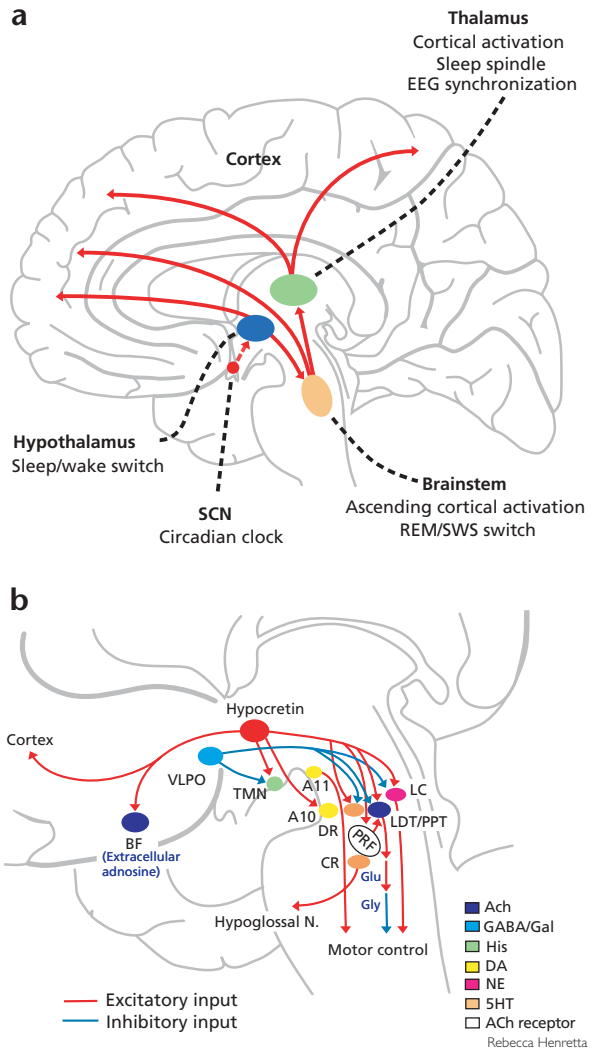

Fig. 1. Hypothalamic and brainstem sleep/wake regulation systems, in relation to common sleep disorders and their pharmacological treatment. (**a**) Distinct roles of the brainstem, thalamus, hypothalamus and cortex for vigilance control. (**b**) Recent discoveries of the sleep-promoting GABAergic/galanininergic (Gal) neurons in the ventrolateral preoptic area (VLPO) and the wake-promoting hypocretin/orexin neurons in the lateral hypothalamus focus attention on the hypothalamus. Destruction of these systems induces insomnia and narcolepsy, respectively, mirroring the pioneering clinical observations during the encephalitis lethargica epidemic. Although no direct interaction between the two systems has been reported, both VLPO and hypocretin systems innervate the main components of the ascending arousal system, such as adrenergic locus coeruleus (LC), serotoninergic dorsal raphe (DR) and histaminergic tuberomammillary nucleus (TMN). The VLPO system inhibits, and the hypocretin system activates, these systems. Thus, the hypothalamus may serve as a center for the 'sleep switch' under the influence of the circadian clock. In addition to these ascending arousal systems, pharmacological evidence suggests the involvement of dopamine (DA), especially in the ventral tegmental area (A10), for the control of alertness. The DA system, including descending A11 projections, may also be particularly important for sleep-related motor control in conditions such as cataplexy and PLM. Recent anatomical and pharmacological evidence suggests that serotonin (5HT) is important for the motor control of the hypoglossal nucleus, and dysregulation of this system may be involved in upper airway resistance in obstructive sleep apnea. Histamine (His) is a wake-promoting amine, but it has a variety of peripheral actions. Histaminergic compounds are not widely used in sleep medicine, except the CNS-permeable H1 antagonists for a small number of insomnia patients. Benzodiazepines (BZs) act on the $GABA_A$/BZ-Cl^- macromolecular complex, and may act on any part of the arousal system shown here. BF, basal forebrain cholinergic nuclei; LDT/ PPT, laterodorsal tegmental nuclei/pedunculopontine tegmental nuclei; CR, caudal raphe; PRF, pontine reticular formation; ACh, acetylcholine; PLM, periodic leg movements during sleep; NE, norepinephrine; GLY, glycine; GLU, glutamate.

awake. The synchronization of thalamocortical circuits results in the expression of sleep spindles or slow-wave activity during NREM sleep[8]. It may also be essential to blocking sensory input during sleep[7].

Dopaminergic mechanisms in sleep regulation

Amphetamine-like stimulants, the most potent wake-promoting agents, increase wakefulness by blocking dopamine reuptake and/or by stimulating dopamine release[9]. Dopamine transporter knockout mice have sleep abnormalities and are hyperactive[9], but animals with midbrain dopaminergic lesions have impaired wakefulness only in response to behavioral stimulation. Contrary to other monoaminergic cell groups, dopaminergic neurons do not change their activity greatly throughout the sleep cycle[10]. In addition, dopaminergic receptor agents have differential effects on sleep depending on their affinity for dopaminergic receptor subtypes and for presynaptic versus postsynaptic receptors.

Despite these complexities, there is growing evidence (some based on reports of sleep disturbances in dopaminergic disorders such as Parkinson's disease[11]) that emphasizes dopamine's role in sleep regulation. There may be a greater functional and pharmacological heterogeneity of dopaminergic cell groups than is generally acknowledged. Thus, sleep regulation by dopamine may be as complex as that mediated by cholinergic systems, where sleep state firing patterns are variable between and within cell groups. For example, sleep can be controlled via D2 or D3 presynaptic receptor modulation of the ventral tegmental area (VTA), but not substantia nigra (SN), activity[9].

Dopaminergic neurons of the VTA, but not SN, are excited by hypocretins (discussed below), and there is greater hypocretin innervation of the VTA than SN (K. Eriksson, pers. comm.). Recent work indicates the existence of sleep-state dependent dopaminergic neurons in the ventral periaqueductal gray (PAG)[12], a rostral extension of the VTA that is contiguous with caudal diencephalic dopaminergic groups. Dopaminergic neurons of the VTA and PAG may thus be more important in regulating wakefulness than other groups (such as SN) that are more relevant to motor control. Descending dopaminergic neurons (A11) from the telencephalon to the spinal cord may contribute to abnormal movements during sleep (such as PLM or RLS, **Table 1**). Dopaminergic cell groups may coordinate sleep with respect to motivated behavior and locomotor activity.

GABAergic mechanisms as therapeutic targets

The importance of inhibitory GABAergic mechanisms in sleep regulation is well established. The widespread nature of GABAergic transmission in the brain makes it difficult to delineate specific sleep circuits, but the importance of GABAergic input to thalamocortical circuits has been emphasized[7,8]. Most hypnotics (such as benzodiazepine, BZ, **Table 1**) increase GABAergic transmission by acting on the $GABA_A$/BZ-Cl^- ligand-gated ion channel. This receptor is a pentameric protein that surrounds the transmembrane chloride channel. The implicated subunit genes have been cloned (7 subunits, 18 isoforms), and the corresponding proteins have been studied[13]. The functional significance of these

Table 1. Most common sleep disorders and their treatments.

Sleep disorder	Prevalence	Common symptoms and pathophysiology	Commonly used treatments	Future treatments
Insomnia	9–15%	Difficulty initiating and maintaining sleep; difficulty initiating sleep in adolescence is often a circadian phase maladjustment called 'delayed sleep phase syndrome'; can be associated with sleep-disordered breathing and other sleep disorders	Benzodiazepine (BZ) and BZ receptor–acting hypnotics (e.g. zolpidem, zopiclone, zaleplon), sedative antidepressants and antihistaminergic drugs (e.g. trazodone), melatonin, behavioral treatments	GABAergic and BZ compounds with improved neural specificity (e.g. VLPO, TMN); hypocretin/orexin antagonists; non-GABAergic hypnotics such as histaminergic H1 receptor antagonists and sedative antidepressants with shorter half lives; melatonin receptor agonists
Sleep-disordered breathing (obstructive sleep apnea)	10–20%	Snoring and breathing pauses causing sleep disruption and most often excessive daytime sleepiness; exacerbated by alcohol and sedatives Associated with airway anatomical abnormalities and/or central control of ventilation; multi-system disorder associated with obesity, hypertension, diabetes mellitus, hyperlipidemia	Continuous positive airway therapy (CPAP) or bilevel positive airway pressure (BiPAP) therapy; dental appliances; surgery of the upper airway	Improved ventilatory devices; 5HT3 and 5HT2A/C antagonists; adenosine A1 receptor agonists
Restless legs syndrome (RLS)	2–5%	Parasthesias followed by akathisia (urge to move limbs, usually legs); often associated with periodic leg movements (PLM), brief, repetitive muscular jerks of the legs during sleep and wakefulness; pathophysiology possibly due to abnormalities in dopaminergic system and/or brain iron metabolism	Dopamine agonists; opiates; gabapentin	Dopamine (D2/3) agonists with improved neural specificity (e.g. A11); hypocretin/orexin compounds; factors influencing central iron metabolism
REM sleep behavior disorder (RBD)	0.5%	Increased motor activity during REM sleep; multiple causes, often drug induced; frequently predates Parkinsonism	Clonazepam	GABAergic and BZ compounds with increased neural specificity
Narcolepsy	0.02–0.06%	Excessive daytime sleepiness, cataplexy (loss of muscle tone in response to emotions), short REM sleep latency; disturbed nocturnal sleep; often associated with PLM and RBD; HLA association and probable autoimmune etiology causing hypocretin deficiency	Amphetamine-like stimulants, Modafinil (sleepiness); REM suppressant antidepressants (cataplexy); Gamma-hydroxybutyric acid (GHB, all symptoms)	Hypocretin/orexin agonists or transplantation of hypocretin-producing cells (all symptoms); dopamine uptake inhibitors or histamine H3 receptor antagonists (sleepiness); GHB receptor or $GABA_B$ receptor agonists (all symptoms)

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receptor subtypes remains unclear, but it may be possible to dissociate hypnotic activity from unwanted effects. As $GABA_A$ alpha-1 subunit knockouts are not viable, animals have been produced with a point mutation in the alpha-1 subunit (H101R), resulting in subunits that are insensitive to BZs. It has thus been possible to dissociate the sedative from SWS-reducing effects of BZs and zolpidem[14,15]. Partial BZ agonists, which lack side effects such as ataxia or amnesia, are being explored as potentially important hypnotics.

Interest is growing in older sedative anti-epileptics, some with GABAergic effects; these are gabapentin (also used in PLM), pregabalin and tigabin. This interest was rekindled with the approval of gammahydroxybutyric acid (GHB/sodium oxybate) for the treatment of narcolepsy. GHB is a GABA metabolite, but whether its primary action is GABAergic is uncertain[16]. Primary GHB receptor and $GABA_B$ mechanisms have been suggested, with secondary effects on dopamine neurotransmission. GHB has a short half-life and, unlike BZ-like drugs, dramatically increases SWS (and REM sleep in some studies). Narcoleptic patients are frequently insomniac and GHB consolidates sleep and improves daytime symptoms such as cataplexy. Unfortunately, because of its positive effects on mood, libido and SWS-related growth hormone release, GHB is abused recreationally and by athletes. The existence of a SWS-enhancing compound suggests that more effective and restorative hypnotics are possible.

The lateral/posterior hypothalamus

The importance of histamine in sleep regulation is evidenced by the hypnotic effects of histamine receptor H1 antagonists. Posterior hypothalamic lesions, by inactivating histaminergic TMN neurons, produce long-term hypersomnia in animals. Electrophysiological studies and *in vivo* microdialysis of histamine release indicate higher histaminergic activity during wakefulness versus sleep and during the active versus inactive periods[17]. Histidine decarboxylase knockout mice have wake fragmentation, increased REM sleep, slower EEG activity while awake, and an inability to maintain wakefulness in novel environments[18]. Overall, 24-hour sleep and wake amounts were normal under undisturbed conditions. Similar results have been reported using locomotor measurements in H1-receptor knockouts. Notably, excessive muscle activity reminiscent of REM behavior disorder has been observed in H3-receptor knockouts[19]. These results indicate that histamine is a major wake-promoting neurotransmitter whose role in the pathophysiology of sleep disorders requires further exploration.

The discovery of hypocretins (also called orexins) and their involvement in narcolepsy have been recently reviewed[20,21]. Human narcolepsy is caused by deficient hypocretin neurotransmission in the lateral hypothalamus. Preprohypocretin gene knockout mice and a mouse model with ataxin-3 driven hypocretin cell loss have abnormal wake–REM transitions, behavior arrests reminiscent of cataplexy, and increased sleep during the active period.

The hypocretin system encompasses two peptides (hypocretin-1 [orexin A] and 2 [orexin B]) that are encoded by the same precursor and two receptors (hcrtr1 and 2). Genetic canine narcolepsy is caused by mutations in the hcrtr2 gene, suggesting a primary role for this receptor in sleep disturbances. Hypocretin neurons have widespread projections, with dense excitatory projections to all monoaminergic and cholinergic cell groups. Within monoaminergic projections, hcrtr2 is densely located in the TMN where it is excitatory[20–22]. The adrenergic LC also receives

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dense excitatory projections (via hcrtr1). Other monoaminergic groups (RN, VTA and SN) have both receptor subtypes. Notably, hcrtr2 is highly expressed in the diagonal band where wake-promoting BFA cholinergic neurons are located[20–22].

In rats, intracerebroventricular hypocretin-1 increases wakefulness and body temperature and stimulates the hypothalamo-pituitary-adrenal axis and sympathetic nervous system[20–22]. Hypocretin release is higher in the active period[23]. The pattern of hypocretin release throughout the sleep cycle is uncertain, however, with a single study indicating slightly lower release during NREM sleep compared with wake or REM sleep states, and a positive correlation with activity[24]. Hypocretin neurotransmission is also activated by sleep deprivation[23], suggesting that hypocretin tone is subject to circadian, homeostatic and ultradian regulation.

Wake-promoting actions of hypocretins are attenuated when histaminergic transmission is impaired[25]. It has been suggested that hypocretin neurons drive monoaminergic activity to control sleep/wake states, with a quantitatively important effect via histamine (hcrtr2)[20,21]. This picture is oversimplified, as histaminergic neurons (and perhaps other monoaminergic systems) also project to hypocretin neurons, with probable excitatory effects. Monoaminergic and hypocretinergic systems are therefore more likely to work in concert than in succession. Substantial differences between these systems are apparent not only in the phenotype of the knockouts, but also in various aspects of sleep regulation and general physiology. For example, hypocretinergic neurons may be more sensitive to metabolic cues such as glucose, leptin and ghrelin[20,21]. Also, hypocretinergic, but not histaminergic, neurons are activated by locomotion during wakefulness. This indicates a coordinated activity of these two systems during wakefulness, with partial specialization. Hypocretins may, for example, be more important in resisting sleep after sleep-deprivation or during periods of intense activity. Increased hypocretin activity may also mediate some of the effects of sleep deprivation, including deleterious metabolic and autonomic effects (via sympathetic and hypothalamo-pituitary–adrenal axis activation) and antidepressant action (via monoaminergic stimulation)[21].

The preoptic hypothalamus

The importance of the preoptic hypothalamus in the generation of SWS has long been recognized. Electrophysiological recordings have identified SWS-active neurons in this area where lesions produce insomnia in animals and humans. Interest in the preoptic area was renewed by the observation that a subgroup of GABAergic cells in the ventrolateral preoptic area (VLPO) was activated in correlation with the amount of SWS[26]. These cells also contain galanin and project to all monoaminergic systems (especially the TMN), inhibiting activity during NREM sleep. Projections from the VLPO may be an important substrate for the hypnotic action of GABAergic compounds (see above). An extended area has also been identified as REM-active, with projections onto brainstem serotoninergic and adrenergic nuclei[26]. A second cluster of SWS-active neurons also exists in the median preoptic area[27]. Genetically based lesions of these systems are awaited. These systems are not known to contain any specific neurotransmitter that could be easily manipulated.

Circadian and homeostatic regulation of sleep

The regulation of sleep is classically viewed as the dual interaction of circadian (SCN-based) and homeostatic processes[28]. The propensity to sleep or be awake at any given time is a consequence of a sleep debt (process S) and its interaction with signals from the SCN circadian clock (process C). In a variation of this model—the opponent process of sleep regulation[29]—a wake-promoting signal is generated by the SCN in the latter part of the active period (in the evening for most humans). This wake-promoting signal opposes the sleep debt that increases from sleep onset, ensuring an even degree of alertness throughout the day[29].

Whereas little is known about the nature of the sleep homeostat, a detailed model of SCN cells that are genetically controlled to provide circadian rhythmicity to the rest of the organism has been established[7,30]. Eight mammalian genes (for example, *Per1-3*, *Cry1,2*, *Clock*, *Arntl* and *Cnk1e*) create a translation–transcription feedback loop that provides a roughly 24-hour rhythmicity. As the influence of circadian rhythms on health become increasingly apparent, there is a growing need to manipulate circadian regulatory factors. Most of the factors discovered to date, however, are likely to be difficult pharmacological targets.

An exciting area of research focuses on how the signal leaves the circadian clock to regulate sleep. There may be logical relays in the hypothalamus, but the pathways are probably indirect. Neurons in the SCN shell project to the preoptic area, but only weakly to the posterior hypothalamus. Lesion studies indicate that a projection from the SCN to the subventricular zone (SVZ, a region that also receives direct retinal input) is essential to entrain locomotor activity rhythms. The fact that the SCN is day-active in both nocturnal and diurnal animals[7,30] suggests the existence of multisynaptic connections.

Experiments in which the SCN was isolated show that there are diffusible factors that entrain the rest of the organism. Two potentially important molecules are TGF-α and prokineticin-2 (refs. 31 and 32, respectively). Both are released during the rest period and decrease locomotor activity when injected intracerebroventricularly. Expression is high in the SCN and peaks during the inactive period in rodents. TGF-α action on locomotor rhythms is mediated via the EGF receptor within the SVZ. Notably, TGF-α is also expressed in a subset of retinal ganglion cells, and EGF receptor knockouts are insensitive to the masking effects of light (decreased locomotor activity induced by light regardless of circadian time). EGF receptor–positive ganglionic cells may provide direct input from the retina to the hypothalamus and mediate other effects of light, such as masking[31].

The downstream effects of prokineticin are less clear. Prokineticin-2 receptors are located in selected hypothalamic nuclei but not in the SVZ. Additional studies with sleep recording, including studies on prokineticin-2 knockouts, will further elucidate any physiological significance. Other unknown factors are likely to be involved in transmitting SCN information to regulate sleep, especially factors that increase wakefulness and activity.

Processes C and S have generally been viewed as independent, yet recent results suggest a complex interaction. Mouse and *Drosophila clock* gene mutants show abnormal rest/sleep rebound after rest/sleep deprivation[33,34]. Strikingly, cycle 01 *Drosophila* mutants died early after rest deprivation, an effect probably related to their inability to induce the expression of protective heat shock protein genes[33]. Similarly, various key neurotransmitter systems, such as wake-promoting hypocretins, appear to be regulated by both the circadian clock and the homeostat. Hypocretin release is maximal at the end of the active period in both diurnal monkeys (J. M. Zeitzer *et al., Soc. Res. Biol. Rhythms*, **8**, 18, 2002) and nocturnal rodents[23]. We propose that this increase is essential to maintain alertness in the evening in the face of a mounting sleep debt. This increase may be partially driven by the circadian clock, as predicted by the opponent model of sleep

regulation, but may also result from a direct effect of sleep pressure on hypocretin activity. This dual regulation may help to consolidate wakefulness across the entire active period, even when its duration varies because of external survival factors.

Adenosine has been proposed as a mediator of sleep homeostasis and a link between metabolism and sleep control[35]. Caffeine, an adenosine A1 receptor antagonist, is widely used to induce wakefulness, although its efficacy is low compared to dopaminergic stimulants. Adenosine levels are higher in the BFA during wakefulness than during sleep, and adenosine accumulates with prolonged wakefulness[35]. Increased levels are believed to inhibit wake-active cholinergic BFA neurons, providing a substrate for increased sleepiness. Elevated extracellular adenosine may also have long-term effects on various transcription factors in cholinergic neurons. However, preliminary studies in A1 receptor knockout mice have shown limited sleep/wake alterations.

Clinical opportunities

Despite the complexity of sleep-regulating systems, current treatments for sleep disorders act via a limited number of pathways (**Table 1**). Most hypnotics target GABAergic activity globally in the brain. Other commonly used hypnotics that were not designed to treat insomnia (sedative antidepressants and antihistamines) have long half-lives and peripheral side effects. Hypnotics that more selectively target VLPO projections sites (for example, GABAergic/BZ receptor subtypes within the TMN) are awaited. More selective manipulations of the adenosine/cholinergic BFA pathway may also have clinical applications.

Most sedatives are suspected to exacerbate disordered breathing during sleep. Studies are needed to understand these effects and to design countermeasures. Promising therapeutic pathways include serotoninergic projections to the hypoglossal nerve[36] and adenosinergic pathways regulating muscle tone and breathing during sleep. Similarly, there is a need to understand the pathophysiology of RLS and PLM to design new treatments.

Current treatments for hypersomnia typically enhance dopaminergic transmission. Treatments that increase the activity of other wake-promoting pathways such as hypocretin/orexin and histamine (for example, H3 antagonists) could be designed. Promising interventions include manipulation of hypocretin (hcrtr2) transmission for the treatment of narcolepsy (agonists) and insomnia (antagonists).

Hypocretin receptors were originally orphan G protein–coupled receptors (GPCRs) that were not suspected to be involved in sleep regulation. The systematic study of sleep when characterizing other GPCR/ligand systems, especially in animal knockouts, may provide further insight into sleep regulation and treatment of sleep disorders. Other potential therapeutic targets are components of the circadian clock and its output systems (for example, melatonin and casein kinase 1-epsilon)[30]. The rapid growth of basic and clinical sleep research promises to lead to new, more targeted, pharmacotherapy for sleep disorders.

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