

Hypothalamic regulation of sleep and circadian rhythms

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A series of findings over the past decade has begun to identify the brain circuitry and neurotransmitters that regulate our daily cycles of sleep and wakefulness. The latter depends on a network of cell groups that activate the thalamus and the cerebral cortex. A key switch in the hypothalamus shuts off this arousal system during sleep. Other hypothalamic neurons stabilize the switch, and their absence results in inappropriate switching of behavioural states, such as occurs in narcolepsy. These findings explain how various drugs affect sleep and wakefulness, and provide the basis for a wide range of environmental influences to shape wake–sleep cycles into the optimal pattern for survival.

In 1916, Baron Constantin von Economo, a Viennese neurologist, began to see patients with a new type of encephalitis that specifically attacked regions of the brain that regulate sleep and wakefulness¹. This disorder, which was eventually called encephalitis lethargica or von Economo's sleeping sickness, swept through Europe and North America during the second decade of the twentieth century; by the end of the following decade it had apparently disappeared, as only sporadic and unconvincing reports have appeared since. Although the virus that caused it was never identified, von Economo was able to identify the areas of the brain in which lesions caused specific alterations of wake–sleep regulation (Fig. 1). Only during the past decade has the accuracy of his observations come to be appreciated, as key components of the wake–sleep-regulatory system were found to reside at the sites that von Economo first identified.

In this review, we cover recent advances in understanding the brain circuitry that regulates sleep and produces wakefulness, including cell groups in the brainstem, hypothalamus and basal forebrain (BF) that are crucial for arousing the cerebral cortex and thalamus. These neurons are inhibited during sleep by a system of γ -aminobutyric acid (GABA)-containing neurons, in which the ventrolateral preoptic nucleus (VLPO) seems to have a key role. Mutual inhibition between the arousal- and sleep-producing circuitry results in switching properties that define discrete wake and sleep states, with sharp transitions between them. We examine how this switch is stabilized by orexin neurons in the lateral hypothalamus (LHA), and why loss of these neurons results in narcolepsy. We also explore how basic drives affect this system, including the homeostatic need to sleep that accumulates as a function of prolonged wakefulness, the circadian drive that moulds the daily cycles of sleep–wakefulness, and allostatic influences that adapt sleep and wakefulness to external behavioural events. Interactions with this circuitry might explain the effects of a range of drugs on sleep and wakefulness.

The ascending arousal system promotes wakefulness

The majority of patients with von Economo's encephalitis lethargica slept excessively. Many slept for 20 or more hours per day, arising only briefly to eat and drink. Their cognitive function was reasonably intact, but they would soon return to sleep — a cycle that lasted for

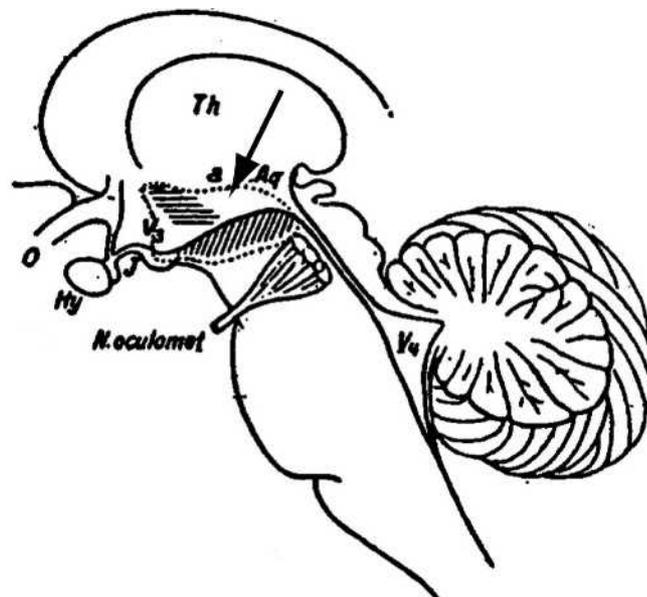


Figure 1 | A drawing of the human brainstem, taken from von Economo's original work. It illustrates the site of the lesion (diagonal hatching) at the junction of the brainstem and forebrain that caused prolonged sleepiness, and the site of the lesion (horizontal hatching) in the anterior hypothalamus that caused prolonged insomnia. The arrow points to a region between the two, including the posterior lateral hypothalamus. Von Economo suggested that narcolepsy was caused by lesions at this site.

many weeks before recovery¹. Von Economo found that these patients invariably had lesions at the junction of the midbrain and the diencephalon. He therefore proposed that there was an ascending arousal system originating in the brainstem that kept the forebrain awake (Fig. 1). During the years after the Second World War, investigators, such as Moruzzi and Magoun and their many colleagues, showed that this influence was mediated by an ascending arousal pathway that begins in the rostral pons and runs through the midbrain reticular for-

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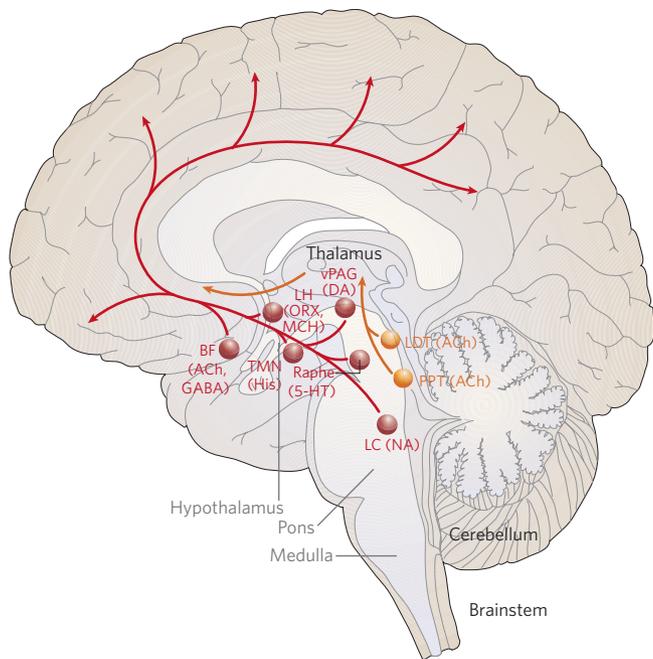


Figure 2 | A schematic drawing showing some key components of the ascending arousal system. A major input to the relay and reticular nuclei of the thalamus (yellow pathway) originates from cholinergic (ACh) cell groups in the upper pons, the pedunculopontine (PPT) and laterodorsal tegmental nuclei (LDT). These inputs facilitate thalamocortical transmission. A second pathway (red) activates the cerebral cortex to facilitate the processing of inputs from the thalamus. This arises from neurons in the monoaminergic cell groups, including the tuberomammillary nucleus (TMN) containing histamine (His), the A10 cell group containing dopamine (DA), the dorsal and median raphe nuclei containing serotonin (5-HT), and the locus coeruleus (LC) containing noradrenaline (NA). This pathway also receives contributions from peptidergic neurons in the lateral hypothalamus (LHA) containing orexin (ORX) or melanin-concentrating hormone (MCH), and from basal forebrain (BF) neurons that contain γ -aminobutyric acid (GABA) or ACh. Note that all of these ascending pathways traverse the region at the junction of the brainstem and forebrain where von Economo noted that lesions caused profound sleepiness.

mation²; hence, the concept of the 'ascending reticular activating system' achieved wide currency^{2,3}.

Studies in the 1970s and 1980s clarified the nature of this pathway (Fig. 2). A key finding was that the ascending arousal system largely originates from a series of well-defined cell groups with identified neurotransmitters⁴. This pathway has two major branches. The first branch is an ascending pathway to the thalamus that activates the thalamic relay neurons that are crucial for transmission of information to the cerebral cortex. The major source of upper brainstem input to the thalamic-relay nuclei, as well as to the reticular nucleus of the thalamus, is a pair of acetylcholine-producing cell groups: the pedunculopontine and laterodorsal tegmental nuclei (PPT/LDT)⁵. The neurons in the PPT/LDT fire most rapidly during wakefulness and rapid eye movement (REM) sleep, which is the stage accompanied by cortical activation, loss of muscle tone in the body and active dreams⁶. These cells are much less active during non-REM (NREM) sleep, when cortical activity is slow. Their input to the reticular nucleus is crucial, as it sits between the thalamic-relay nuclei and the cerebral cortex, acting as a gating mechanism that can block transmission between the thalamus and cerebral cortex, which is important for wakefulness⁷. Other inputs to the thalamic midline and intralaminar nuclei originate more broadly in the upper brainstem, including the reticular formation, the PPT/LDT, the monoaminergic systems (see below) and the parabrachial nucleus⁸. The intralaminar and midline nuclei are also believed to have a role in cortical arousal.

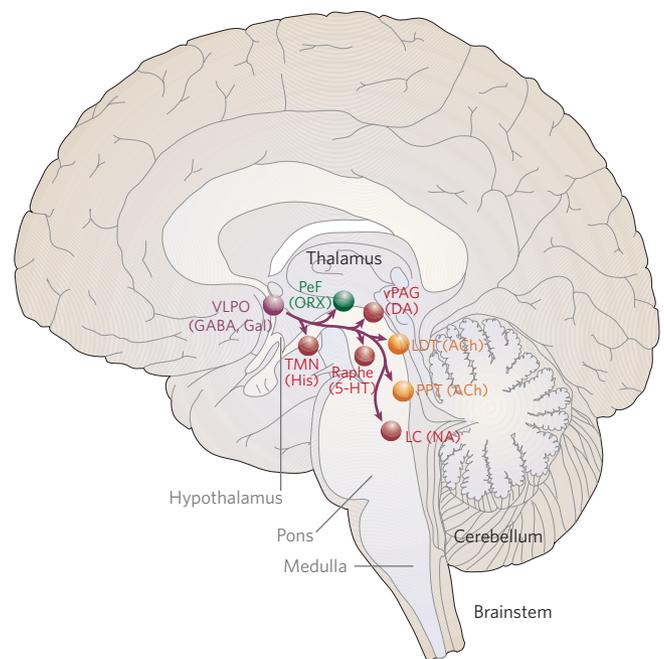


Figure 3 | A schematic drawing to show the key projections of the ventrolateral preoptic nucleus (VLPO) to the main components of the ascending arousal system. It includes the monoaminergic cell groups (red) such as the tuberomammillary nucleus (TMN), the A10 cell group, the raphe cell groups and the locus coeruleus (LC). It also innervates neurons in the lateral hypothalamus (LHA; green), including the perifornical (PeF) orexin (ORX) neurons, and interneurons in the cholinergic (ACh) cell groups (yellow), the pedunculopontine (PPT) and laterodorsal tegmental nuclei (LDT). Note that the VLPO neurons lie within the region outlined by von Economo for the anterior hypothalamic lesion that caused insomnia. 5-HT, serotonin; GABA, γ -aminobutyric acid; gal, galanin; NA, noradrenaline; His, histamine.

The second branch of the ascending arousal system bypasses the thalamus, instead activating neurons in the lateral hypothalamic area and BF, and throughout the cerebral cortex^{4,9,10}. This pathway originates from monoaminergic neurons in the upper brainstem and caudal hypothalamus, including the noradrenergic locus coeruleus (LC), serotonergic dorsal (DR) and median raphe nuclei, dopaminergic ventral periaqueductal grey matter and histaminergic tuberomammillary neurons. The input to the cerebral cortex is augmented by lateral hypothalamic peptidergic neurons (containing melanin-concentrating hormone (MCH) or orexin/hypocretin), and BF neurons (containing acetylcholine or GABA). Lesions along this pathway, particularly in the LHA and rostral midbrain, produce the most profound and long-lasting forms of sleepiness or even coma^{11,12}. Neurons in each of the monoaminergic nuclei that contribute to this pathway have the property of firing fastest during wakefulness, slowing down during NREM sleep and stopping altogether during REM sleep¹³⁻¹⁵. Orexin neurons in the LHA are, similarly, most active during wakefulness¹⁶⁻¹⁸, whereas MCH neurons are active during REM sleep¹⁹. Many BF neurons, including most cholinergic neurons, are active during both wake and REM sleep²⁰.

Given the anatomy and functions of these systems, it is not surprising that von Economo found that lesions at the junction of the mid-brain and forebrain, which block both ascending pathways, produce profound and long-lasting impairment of arousal.

The VLPO promotes sleep

Von Economo's second major observation was an opposite response in a small percentage of the victims of encephalitis lethargica¹. Rather than being sleepy, they became insomniac and slept for only a few hours each day. Typically, they were extremely tired, but found it diffi-

cult to fall asleep, could sleep for only a short time, and then awoke and were unable to fall back asleep. These patients had lesions involving the basal ganglia and adjacent anterior hypothalamus. Later experiments in animals identified a hypothalamic site involving the lateral preoptic area where lesions caused similar insomnia^{21,22}.

During the 1980s and 1990s, investigators began to examine the inputs to the monoaminergic cell groups that might be responsible for their remarkable, stereotyped and coordinated changes in firing patterns associated with sleep. One such cell group, the VLPO, was found to send outputs to all of the major cell groups in the hypothalamus and brainstem that participate in arousal²³ (Fig. 3). The VLPO neurons are primarily active during sleep, and contain the inhibitory neurotransmitters, galanin and GABA^{24–26}. These neurons form a dense cluster, as well as a more diffuse extended part of the nucleus^{24,25}.

These observations suggested that damage to the VLPO might have caused the insomnia in von Economo's patients. Experiments showed that cell-specific lesions of the VLPO in animals reduced both NREM and REM sleep by more than 50% (refs 27, 28). Lesions of the VLPO cluster primarily reduced NREM sleep, whereas lesions of the extended VLPO mainly disrupted REM sleep. The extended VLPO neurons provide the main output from the VLPO to the LC and DR, which are thought to be important in gating REM sleep^{27,29}. By contrast, the VLPO cluster more heavily innervates the histaminergic neurons, which are closely linked to transitions between arousal and NREM sleep^{27,30,31}.

The VLPO also receives afferents from each of the major monoaminergic systems³². Both noradrenaline and serotonin inhibit VLPO neurons³³. The latter do not have histamine receptors, but the tuberomammillary neurons also contain GABA³⁴, which is inhibitory to VLPO neurons³⁵, as well as several other potentially inhibitory peptides, such as galanin and endomorphin^{36,37}. Therefore, the VLPO can be inhibited by the very arousal systems that it inhibits during sleep (Fig. 4).

The flip-flop switch

A circuit containing mutually inhibitory elements sets up a self-reinforcing loop, where activity in one of the competing sides shuts down inhibitory inputs from the other side, and therefore disinhibits its own action. Such a circuit is called a 'flip-flop switch' by electrical engineers, who design them to produce discrete states with sharp transitions⁴. Flip-flop circuits tend to avoid transitional states, because when either side begins to overcome the other, the switch 'flips' into the alternative state. This flip-flop circuit model might explain why wake-sleep transitions are often relatively abrupt (one 'falls' asleep and suddenly awakes), and both humans and animals spend only a small part of each day (typically < 1–2%) in transitional states (Fig. 4). There are obvious adaptive advantages to a wake-sleep system that makes rapid and complete transitions, as it would be dangerous for animals to have impaired alertness while engaging in waking behaviours, and inefficient for them to spend their sleep periods half-awake.

However, flip-flop switches can also make unwanted transitions with little warning. When a small perturbation gives one side a sudden advantage, it can turn off the alternative state relatively abruptly (such as falling asleep during a momentary lapse of attention while driving). Interestingly, mathematical models show that when either side of a flip-flop neural circuit is weakened, homeostatic forces cause the switch to ride closer to its transition point during both states³⁸. As a result, there is an increase in transitions, both during the wake and the sleep periods, regardless of which side is weakened. This is certainly seen in animals with VLPO lesions, which fall asleep about twice as often as normal animals, wake up much more often during their sleep cycle and, on the whole, only sleep for about one-quarter as long per bout²⁸ — in other words, they wake up and are unable to fall back asleep during the sleep cycle, but also are chronically tired, falling asleep briefly and fitfully during the wake cycle, much like von Economo's patients. Interestingly, elderly people, who have similar problems although perhaps on a lesser scale³⁹, have been found to have

loss of neurons in the human equivalent of the VLPO²⁴ with ageing^{40–42}.

Orexin/hypocretin neurons and state stability

In the years that followed the epidemic of encephalitis lethargica, several well-known clinicians, such as Wilson in London and Spiller in Philadelphia, recorded a rash of cases of narcolepsy, a mysterious illness in which the patients had attacks of irresistible sleepiness, as well as episodes of cataplexy during which emotional situations would cause them to lose muscle tone and collapse to the floor⁴³. Wilson believed that at least some of the cases were post-encephalitic, and von Economo proposed that the responsible pathology involved the posterior hypothalamus¹.

In 1998, two groups of investigators simultaneously discovered a pair of closely related neuropeptides, named orexins by one group and hypocretins by the other^{44–46}. These peptides are produced exclusively by a cluster of neurons in the posterior half of the LHA. One year later, two groups of investigators again simultaneously found that a lack of orexins or their type 2 receptor can cause symptoms of narcolepsy in experimental animals^{46,47}. The following year, it was reported that humans who have narcolepsy with cataplexy have few orexin neurons

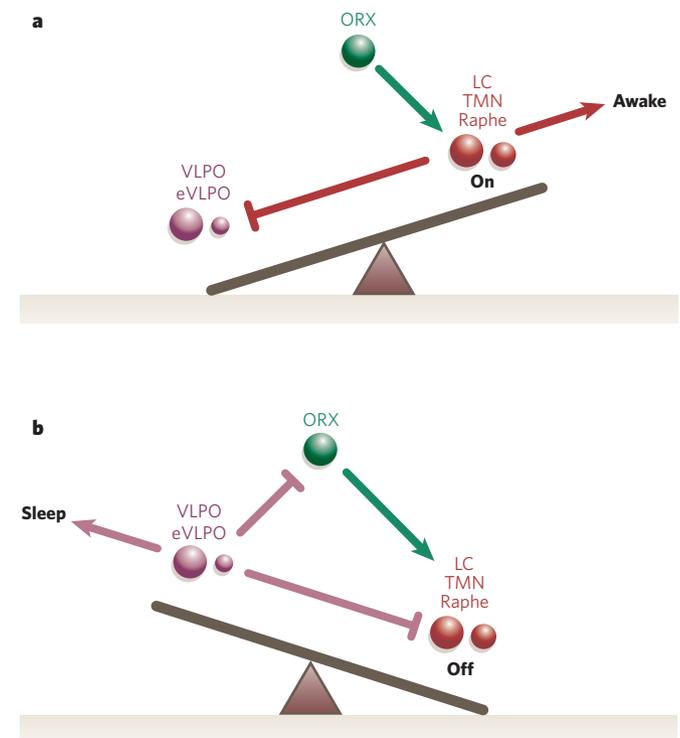


Figure 4 | A schematic diagram of the flip-flop switch model. During wakefulness (a), the monoaminergic nuclei (red) inhibit the ventrolateral preoptic nucleus (VLPO; purple), thereby relieving the inhibition of the monoaminergic cells, and that of the orexin (ORX) neurons (green), and the cholinergic pedunculopontine (PPT) and laterodorsal tegmental nuclei (LDT; yellow). Because the VLPO neurons do not have orexin receptors, the orexin neurons serve primarily to reinforce the monoaminergic tone, rather than directly inhibiting the VLPO on their own. During sleep (b), the firing of the VLPO neurons inhibits the monoaminergic cell groups, thereby relieving their own inhibition. This also allows it to inhibit the orexin neurons, further preventing monoaminergic activation that might interrupt sleep. The direct mutual inhibition between the VLPO and the monoaminergic cell groups forms a classic flip-flop switch, which produces sharp transitions in state, but is relatively unstable. The addition of the orexin neurons stabilizes the switch. 5-HT, serotonin; ACh, cholinergic; eVLPO, extended ventrolateral preoptic nucleus; GABA, γ -aminobutyric acid; gal, galanin; LC, locus coeruleus; NA, noradrenaline; PeF, perifornical; REM, rapid eye movement; TMN, tuberomammillary nucleus.

in the LHA and low orexin levels in the cerebrospinal fluid^{48–50}. So, within a remarkably short period of time, the molecular basis of this mysterious illness was identified.

Interestingly, few people with narcolepsy have mutations in either the orexin ligand or receptor genes^{49,51}. In most narcoleptics, the disease begins in the second or third decade of life, and the loss of orexin neurons is remarkably specific (producing no injury to the adjacent neurons that produce MCH)^{48,49}. The cause is believed to be autoimmune, although convincing evidence for this hypothesis is still lacking, and it might be a neurodegenerative condition⁵¹. Other patients with lesions of the posterior LHA due to a range of causes, such as those first reported by von Economo and Wilson, also acquired narcolepsy.

The orexin neurons are mainly active during wakefulness and especially during motor activity when animals actively explore their environment^{16–18}. They have ascending projections to the cerebral cortex, as well as descending projections to all the monoaminergic and cholinergic cell groups of the arousal systems^{47,52}. There are mutual projections between the VLPO neurons and the orexin neurons^{32,53,54}, but the former do not express orexin receptors⁵⁵. Hence, the orexin neurons reinforce the arousal systems, but probably do not directly inhibit the VLPO (Fig. 4). This asymmetric relationship could help stabilize the flip-flop switch, like a 'finger' on the switch that might prevent unwanted transitions into sleep. The increase in homeostatic sleep drive due to consolidated wakefulness might, in turn, help produce consolidated sleep. Narcoleptic people and animals lack this influence and behave as if their sleep flip-flop switch has been destabilized^{51,56}. They do not sleep more than normal individuals, but easily doze off during the day and wake more often from sleep at night⁵³, as the flip-flop model would predict.

Homeostatic regulation of sleep

Although the purpose of sleep remains unknown, it clearly has a restorative effect on the brain. Like other homeostatic systems, such as that governing body temperature, which seek to recover to a set point when perturbed, sleep deprivation is followed by extra recovery sleep that is proportional to the sleep loss. An influential model of sleep regulation, proposed by Borbely and colleagues, includes both homeostatic and circadian drives for sleep^{57,58}. The homeostatic influence is believed to be due to some structure or substance that accumulates 'need to sleep' during prolonged wakefulness, and discharges this

homeostatic need during sleep. NREM and REM sleep probably have separate homeostatic mechanisms, and, after a period of sleep deprivation, NREM sleep is typically repleted first.

The mechanisms for this homeostatic determinant remain unclear. The VLPO neurons, for example, do not accumulate a need to sleep, as they remain at a low level of activity after a prolonged period of sleep deprivation, until the animal actually falls asleep^{23,27}. At that point, VLPO neurons fire about twice as fast as they do during normal sleep²⁷, implying that they are under the influence of, but distinct from, the homeostatic factors that reflect sleep need.

Adenosine has been proposed as a homeostatic accumulator of the need to sleep^{6,59,60}. During prolonged wakefulness, the energy-producing systems in the brain run down, with exhaustion of brain glyco-gen reserves and depletion of ATP levels^{61,62}. During prolonged wakefulness, as ATP is degraded to ADP, AMP and eventually adenosine, extracellular adenosine levels rise in some parts of the brain, including the BF⁶³. Injection of adenosine or an adenosine A1 receptor agonist into the BF of cats⁶, or an adenosine A2a receptor agonist near the VLPO in rats, causes sleep; the latter also results in expression of Fos (a marker of neuronal activity) in VLPO neurons⁶⁴. In addition, adenosine might disinhibit the VLPO via presynaptic A1 receptors by reducing inhibitory GABAergic inputs³⁵. Hence, at least one mechanism for homeostatic sleep drive might be an accumulation of a sleep-promoting substance that enhances the activity of sleep-promoting cells and reduces the activity of wake-promoting neurons. In this way, adenosine, and perhaps other somnogens, can allow the activation of the VLPO that is necessary to trigger a sleep episode.

Circadian regulation of sleep

Borbely also proposed a circadian influence on sleep and wakefulness (process C) that is distinct from the homeostatic drive (process S) for sleep^{57,58}. Careful studies of humans in a forced desynchrony protocol, where they experience a 28-hour 'day' without any cues as to external time, have confirmed a strong 24-hour circadian rhythm in sleep drive⁶⁵. Recent studies in experimental animals have clarified how this drive is maintained.

The suprachiasmatic nucleus (SCN) serves as the brain's 'master clock'. Neurons in the SCN fire in a 24-hour cycle that is driven by a transcriptional-translational loop, which persists even when the neurons are dissociated in cell culture^{66,67}. Loss of the SCN abolishes the circadian rhythms of a range of behaviours and physiological

Box 1 | Drugs that affect sleep and wakefulness

Many of the drugs that are used to regulate sleep and wakefulness are now thought to act directly on the wake and sleep systems that have been outlined here.

Wake-promoting drugs, such as amphetamines, methylphenidate (Ritalin) and modafinil (Provigil), all act on the dopamine (DA)-reuptake transporter. Their potency is roughly proportional to their ability to block DA reuptake, and mice in which the DA-transporter gene has been deleted fail to respond to this entire class of medications⁹⁴. Modafinil might also interfere with noradrenaline reuptake, and this dual mode of action might explain why it can work without inducing other dopaminergic effects, such as addiction⁹⁵.

Some sleep-promoting drugs interfere with the actions of the ascending arousal system. For example, antihistamines that cross the blood-brain barrier, such as diphenhydramine (Benadryl), cause drowsiness by blocking the arousing influence of the central histaminergic system. This side effect accounts for the popularity of antihistamines that do not cross the blood-brain barrier and, hence, do not cause drowsiness. Centrally acting DA antagonists also cause drowsiness⁹⁶. These drugs are used primarily to treat psychotic disorders, and this side effect is often a limiting factor in drug acceptability. Paradoxically, D2-agonist drugs can also cause drowsiness at low dosages⁹⁶. The D2-presynaptic autoreceptor inhibits the firing of dopaminergic neurons, and therefore results in reduced dopaminergic activity. Similarly, dexmedetomidine, an α -2 adrenergic agonist, reduces firing of noradrenergic neurons, and is thought to induce loss of wakefulness by disinhibiting the ventrolateral preoptic nucleus

(VLPO), which is ordinarily under noradrenergic inhibition during wakefulness⁹⁷ (Fig. 4).

The largest class of sleep-promoting drugs consists of those that enhance the activity of γ -aminobutyric acid-A (GABA-A) receptors, including barbiturates, benzodiazepines, chloral hydrate, ethanol and most gaseous anaesthetics⁹⁸. Few of these drugs are direct agonists (that is, few compete with GABA for binding); rather, most bind to other components of the GABA-A receptor and enhance the action of GABA at the receptor. At low dosages, these drugs might act on the targets of the VLPO, which also uses GABA as a neurotransmitter to silence the arousal system. At higher dosages, they suppress firing in much of the central nervous system. Benzodiazepines, such as diazepam (Valium), act on GABA-A receptors that contain a γ -subunit and subunits of the α -1, α -2, α -3 or α -5 class⁹⁸. Newer 'non-benzodiazepines', such as zolpidem (Ambien) or eszopiclone (Lunesta), also bind at α - γ sites but do not have the classic benzodiazepine structure and are more selective for the α 1-containing receptors, which are important in sedation but not reduction of anxiety⁹⁸. Gaboxadol binds to GABA-A receptors that contain the α -4 and δ (but not γ) subunits. These receptors, which are at highest concentration in the thalamus, limbic system and cerebral cortex, are not accessible to benzodiazepines, and hence represent a distinct drug target⁹⁹. This might reduce the arousal drive from the medial temporal and prefrontal cortex that is seen during insomnia, as gaboxadol can activate Fos expression by neurons in the VLPO, and increases the amount of slow-wave sleep¹⁰⁰.

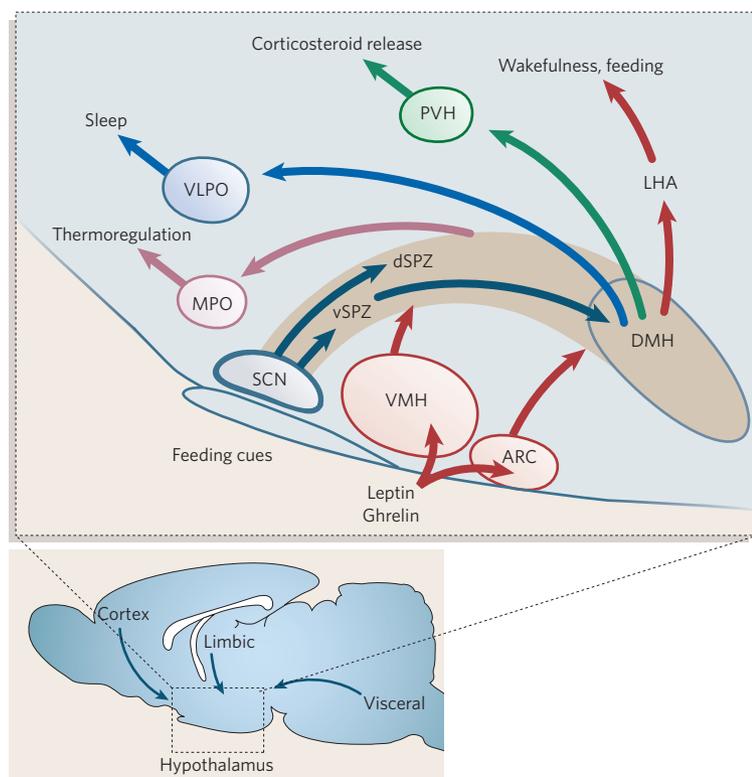


Figure 5 | A schematic diagram to illustrate the three-stage integrator for circadian rhythms. The suprachiasmatic nucleus (SCN) serves as a biological clock, but has few outputs to sleep-regulatory systems. Most of its output goes into the region in light brown, which includes the ventral (vSPZ) and dorsal (dSPZ) subparaventricular zone, and the dorsomedial nucleus of the hypothalamus (DMH). Neurons in the vSPZ relay information necessary for organizing daily cycles of wake-sleep, whereas dSPZ neurons are crucial for rhythms of body temperature. Outputs from the SPZ are integrated in the DMH with other inputs, and DMH neurons drive circadian cycles of sleep, activity, feeding and corticosteroid secretion. Cycles of body temperature are maintained by dSPZ projections back to the medial preoptic area (MPO), whereas the DMH is the origin of projections to the VLPO for sleep cycles, to the corticotropin-releasing hormone (CRH) neurons of the paraventricular nucleus (PVH) for corticosteroid cycles, and to the lateral hypothalamic (LHA) orexin and melanin-concentrating hormone neurons for wakefulness and feeding cycles. The integrative steps in the SPZ and DMH allow circadian rhythms to adapt to environmental stimuli, such as food availability (for example, leptin and ghrelin action via the ventromedial (VMH) and arcuate (ARC) nuclei), as well as visceral sensory inputs, cognitive influences from the prefrontal cortex and emotional inputs from the limbic system (inset).

processes, including sleep, if the animals are not given other external timing cues⁶⁸. Under normal circumstances, the SCN is reset on a daily basis by light inputs from the retina during the day, and by melatonin secretion from the pineal gland during the dark cycle^{69,70}. The light signal is received from a specialized set of retinal ganglion cells that contain the photopigment melanopsin⁷¹. These timing signals keep the clock in synchrony with the external day-night cycle.

The link between the SCN and the sleep system has been the subject of much recent investigation (Fig. 5). The SCN has relatively modest projections to the VLPO or the orexin neurons^{32,53,54,72}. However, the bulk of its output is directed toward the adjacent subparaventricular zone (SPZ) and the dorsomedial nucleus of the hypothalamus (DMH). The SPZ contains a ventral part, just above the SCN, and a dorsal part, just below the paraventricular nucleus⁷³. Cell-specific lesions of the ventral SPZ disrupt the circadian rhythms of sleep and wakefulness, as well as locomotor activity, but have minimal effects on body-temperature rhythms. Conversely, lesions of the dorsal SPZ severely impair circadian rhythms of body temperature, but not wake-sleep or locomotor activity⁷³. Therefore, direct projections from the SCN to sleep or thermoregulatory regions are not sufficiently strong to maintain the circadian rhythms of these functions, and the relay neurons in the SPZ are required.

The SPZ also has relatively limited projections to the VLPO, orexin neurons and other components of the wake-sleep-regulatory system^{32,53,54,74}. However, a major target is the DMH^{73,74}. This region receives inputs from many more neurons in the SPZ than the SCN, so the SPZ is in a position to amplify the output of the SCN. Cell-specific lesions of the DMH also profoundly diminish circadian rhythms of sleep and wakefulness, as well as locomotor activity, corticosteroid secretion and feeding⁷⁵. Interestingly, animals with DMH lesions sleep about one hour more each day and have much less locomotor activity, implying that the output of the DMH is mainly activating. This theme is also reflected in the corticosteroid levels, which, in animals with DMH lesions, remain at the lowest basal levels throughout the day. Body temperature retains a normal circadian variation, but is about 0.5 °C lower than in control animals⁷⁵.

The DMH is one of the largest sources of input to the VLPO and orexin neurons^{32,54,75,76}, and is crucial for conveying SCN influence to

the wake-sleep-regulatory system^{75,77}. The DMH projection to the VLPO comes largely from GABA-containing neurons (that is, those that promote wakefulness by inhibiting sleep), and the projection to the LHA originates from neurons containing glutamate and thyrotropin-releasing hormone (which should presumably be excitatory and promote wakefulness)⁷⁵. The DMH has relatively few direct outputs to the brainstem components of the ascending arousal system, but the orexin neurons have extensive projections to these targets^{47,52,76}. Examination of Fos patterns in the DMH show that it contains many more active neurons during wakefulness than during sleep⁷⁸.

Why might the brain need such a complex three-stage pathway for circadian control of sleep and other behaviours? The answer might lie in the observation that, in a wide range of both nocturnal and diurnal animals, the SCN is always active during the light cycle and the VLPO is always active during the sleep cycle^{23,24}. Therefore, in animals that are nocturnal versus diurnal, there must be an intervening set of circuitry that allows the circadian cycle to be set at opposite phases, despite an identical clock input and sleep-control system. In fact, the circadian rhythms in many animals are not entirely fixed. For example, bats are often considered to be the quintessential nocturnal animal. This is true for bats in Finland during the summer, when there are insects flying around at night for them to eat and predatory birds flying around during the day to discourage them. However, during the cooler weather of the spring and autumn, few insects fly at night and predatory birds have migrated to other climes. At these times of year, the activity cycles of bats shift so that they can take advantage of the food source that is available during the day, and they shift their activity pattern entirely to the daylight hours^{78,79}.

Similarly, it is possible to invert many of the circadian rhythms of laboratory rats by restricting their access to food to the daylight hours. For example, if animals are only permitted to eat during the latter half of the light period, they awaken, become active and have an increase in body temperature a few hours before the food is due to be presented⁸⁰. This behaviour persists even if the food is withheld completely for 2 days; hence, this circadian pattern is not dependent on external cues. The ability to alter the wake-sleep, activity, feeding, body temperature and corticosteroid rhythms of these animals correlates with a shift in activation of the DMH (but not the SCN, which

stays locked to the original light–dark cycle) to the new wake cycle, and lesions of the DMH prevent these behavioural shifts⁷⁸ (J. J. Gooley and C. B. Saper, unpublished observations). So, the DMH seems to integrate clock information from the SCN and SPZ with feeding, temperature, social and other cues, providing animals with the flexibility to adapt their behavioural and physiological cycles to the environment, thereby maximizing their chances of survival (Fig. 5).

Allostatic regulation of sleep

The rapid expansion of our knowledge about the substrates of the regulation of sleep–wakefulness and circadian rhythms highlights the lack of knowledge about how these systems are controlled in a complex and ever-changing environment. Although we are beginning to understand the homeostatic drive for sleep, it is clear that sleep patterns and the circadian cycle can be modified by external events, such as availability of food or external temperatures. Similarly, homeostatic and circadian drives for sleep can be overcome for brief periods when external events demand an emergency response.

McEwen and Stellar, in 1993, proposed the term ‘allostatic’ drive⁸¹ for situations where “rather than maintaining constancy, the physiologic systems within the body fluctuate to meet demands from external forces.” We know remarkably little about how these external forces overcome the homeostatic and circadian systems or reset them (Fig. 5). There are clearly cues from visceral sensory systems and feeding regulatory systems to the arousal systems^{54,82–85}. Visceral inputs relayed through the nucleus of the solitary tract, such as gastric stretch, have a synchronizing sleep-inducing influence⁸⁶, whereas absence of sufficient food causes an arousing influence⁸⁷. However, we know little about the mechanisms by which cognitive and emotional systems can act on the sleep or circadian control systems. Recent studies have emphasized the inputs to the SCN⁸², VLPO³² and orexin neurons^{53,54} from corticolimbic sites, such as the infralimbic cortex, ventral subiculum, lateral septum and bed nucleus of the stria terminalis, as possible routes by which this influence might be expressed.

Positron-emission tomography studies during sleep in humans with insomnia also show increased activity in corticolimbic sites, including the medial prefrontal cortex and medial temporal lobe, compared with sleeping subjects without insomnia⁸⁸. These inputs might maintain a hyperaroused state, which can be essential in an emergency situation, such as when a doctor must stay awake to care for a sick patient overnight. However, when the arousal systems override the homeostatic and circadian regulation of sleep during periods of behavioural stress or depression, the result might be unwanted and debilitating insomnia.

Conclusions

Understanding the interactions of these allostatic influences with the sleep and circadian systems is a major challenge for the next decade. However, learning to control our wake–sleep systems holds the promise of improved health and cognitive performance (Box 1). Individuals who lose even small amounts of sleep on a daily basis show progressive impairment of cognitive performance and elevation of C-reactive protein, which predicts cardiovascular risk^{89–91}. Because older individuals sleep about half an hour less per day, it is possible that at least some of their cognitive decline and increase in cardiovascular disease might be explained by sleep restriction⁹². Similarly, sleep loss might impair performance among adolescents who arise early for school, shift workers, overnight long-haul truckers and even medical personnel working in hospitals⁹³. The public-health implications of sleep loss indicate that there is a great deal at stake in working out the mechanisms that regulate our daily cycles of sleep and wakefulness. ■

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