

THE NEUROBIOLOGY OF SLEEP: GENETICS, CELLULAR PHYSIOLOGY AND SUBCORTICAL NETWORKS

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To appreciate the neural underpinnings of sleep, it is important to view this universal mammalian behaviour at multiple levels of its biological organization. Molecularly, the circadian rhythm of sleep involves interlocking positive- and negative-feedback mechanisms of circadian genes and their protein products in cells of the suprachiasmatic nucleus that are entrained to ambient conditions by light. Circadian information is integrated with information on homeostatic sleep need in nuclei of the anterior hypothalamus. These nuclei interact with arousal systems in the posterior hypothalamus, basal forebrain and brainstem to control sleep onset. During sleep, an ultradian oscillator in the mesopontine junction controls the regular alternation of rapid eye movement (REM) and non-REM sleep. Sleep cycles are accompanied by neuromodulatory influences on forebrain structures that influence behaviour, consciousness and cognition.

CIRCADIAN RHYTHMS
Biological rhythms of physiology and behaviour that have a 24-h periodicity, which have evolved in response to the 24-h astronomical cycle to which all organisms are exposed.

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Sleep is a global state, the control mechanisms of which are manifested at every level of biological organization, from genes and intracellular mechanisms to networks of cell populations, and to all central neuronal systems at the organismic level, including those that control movement, arousal, autonomic functions, behaviour and cognition.

Recent genetic findings indicate that the molecular mechanisms that control **CIRCADIAN RHYTHMS**, which set the stage for sleep and are inseparable from sleep in a deep biological sense, are highly conserved phylogenetically^{1,2}. Molecular and behavioural conservation indicates that sleep conferred a selective advantage on ancestral mammals that might persist in modern populations. Prolonged sleep loss impairs temperature control, dietary metabolism and immune function, and leads ultimately to death³.

In the mammalian nervous system, genetic instructions are expressed at the progressively higher levels of gene transcription, protein synthesis and intracellular events, individual neuronal and neuronal-network dynamics, and ultimately behaviour, of which cognition is a specific covert form. In this review, we discuss the

genetic mechanisms, cellular neurophysiology and subcortical neuronal-population networks that are involved in sleep. In a forthcoming review⁴, we will discuss the cognitive neuroscience of sleep at the levels of subcortico-cortical neuronal-population networks, behaviour and cognition.

Many brain loci have specific functions in sleep at each of these organizational levels (FIG. 1a). The bottom tier of subcortical structures is addressed here. FIGURE 1b shows a schematic presentation of the molecular, cellular and subcortical networks. For a discussion of the peripheral physiology of sleep, sleep medicine and sleep in psychiatry, which are beyond the scope of this review, see REF. 5.

The circadian pacemaker

Starting with the first demonstration of a circadian gene in the fruitfly⁶, genetic approaches have begun to illuminate the intranuclear and cytoplasmic events that are associated with circadian rhythms and sleep. Most notable has been the elucidation of the genetic control of the mammalian circadian pacemaker^{2,7}, which can now explain the near-perfect 24-h rhythmicity of the

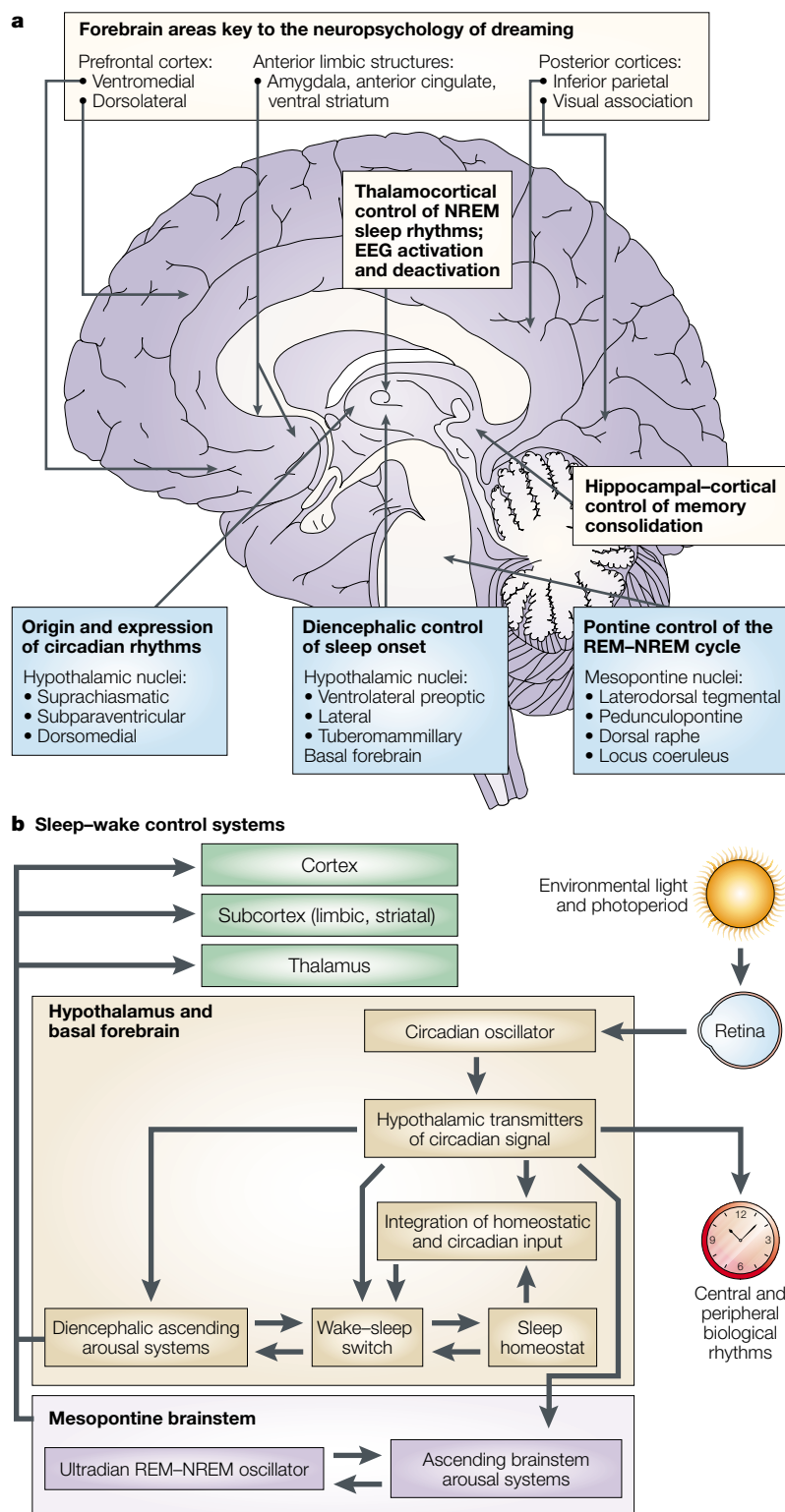


Figure 1 | Brain regions and regulatory circuits involved in sleep. a | Brain regions of current interest to the neurobiology of sleep. This review considers the bottom tier of subcortical regions (blue boxes), which control sleep-wake transitions and, within sleep, REM-NREM sleep alternation. The top tier includes areas that are key to the generation of the EEG rhythms of sleep, the subjective experience of sleep mentation or dreaming, and sleep's effects on cognition; these are considered in REF. 4. **b** | Schematic representation of the regulatory circuits that control sleep-wake and REM-NREM transitions, as well as their key inputs and outputs. Parts of this network are considered in more detail in the main text. EEG, electroencephalogram; NREM, non-REM; REM, rapid eye movement.

human circadian clock⁸. More details on the molecular circadian clock than can be provided here are available in REFS 1,2,7,9.

The genetically controlled molecular circadian clock is synchronously expressed collectively and individually¹⁰ by each of the ~20,000 cells of the mammalian SUPRACHIASMATIC NUCLEUS (SCN)², which is situated bilaterally in the hypothalamus, just above the optic chiasm. The SCN contains the 'master clock' mechanism, the entrainment of which by the daily light-dark cycle sets the 24-h rhythm for all other physiological rhythms in the organism. The SCN does this, in part, by controlling molecularly related peripheral cellular oscillators that exert local control over physiological rhythms at sites closer to the expression of these rhythms². Transplantation of the SCN of hamsters that have normal circadian rhythms into hamsters that have mutant rhythms restores normal periodicity in the implanted hamsters; mutant-to-normal transplantation has the opposite effect¹¹.

Mammalian circadian rhythms are maintained intracellularly by interlocking positive- and negative-feedback control of the transcription (and subsequent translation to protein) of three period genes (*Per1-3*), two cryptochrome genes (*Cry1,2*), and the *Clock* and *Bmal1* (brain and muscle ARNT-like 1) genes^{2,7} (FIG. 2a). The products of *Clock* and *Bmal1* exist as a heterodimer that is a key component of a transcription factor (abbreviated Clock:Bmal1) that promotes the transcription of *Per* and *Cry* genes by binding to their regulatory DNA sequences (E-box elements^{2,7}). The *Per* and *Cry* messenger RNAs are translocated to the cytoplasm for translation to proteins that form complexes that then re-enter the nucleus to exert feedback control on the Clock:Bmal1 transcription factor. Products of several other genes modulate this intracellular mechanism^{2,7}. For example, the product of the *tau* (*Csnk1e*) gene, casein kinase 1 ϵ (REF. 12), phosphorylates *Per* proteins, which affects their translocation between the cytoplasm and the nucleus¹³.

Briefly, molecular feedback control of the circadian clock in SCN neurons operates as follows (see REFS 2,7 for details). At the beginning of the organism's SUBJECTIVE DAY, CIRCADIAN TIME (CT) 0 (FIG. 2b), the transcription and translation of *Per* and *Cry* are accelerated by Clock:Bmal1 heterodimers that have accumulated over the previous subjective night (CT 12–24). The levels of the *Per* and *Cry* complexes peak at the beginning of the organism's subjective night (CT 12). Protein complexes that contain the products of the *Cry* gene exert negative feedback on the *Clock:Bmal1* promoter, thereby slowing the transcription of *Per* and *Cry* (FIG. 2a). At the same time, a protein complex that contains *Per2* exerts positive feedback by promoting the transcription of *Bmal1* (FIG. 2a). *Bmal1* begins to accumulate as raw material for new Clock:Bmal1 heterodimers during the subjective day, and its level peaks during the subjective night (FIG. 2b). Negative feedback on the Clock:Bmal1 promoter causes the levels of *Per* and *Cry* to reach a minimum during the subjective night (FIG. 2b). Simultaneously, positive feedback on *Bmal1* transcription raises the levels of Clock:Bmal1 heterodimers, which, when released from

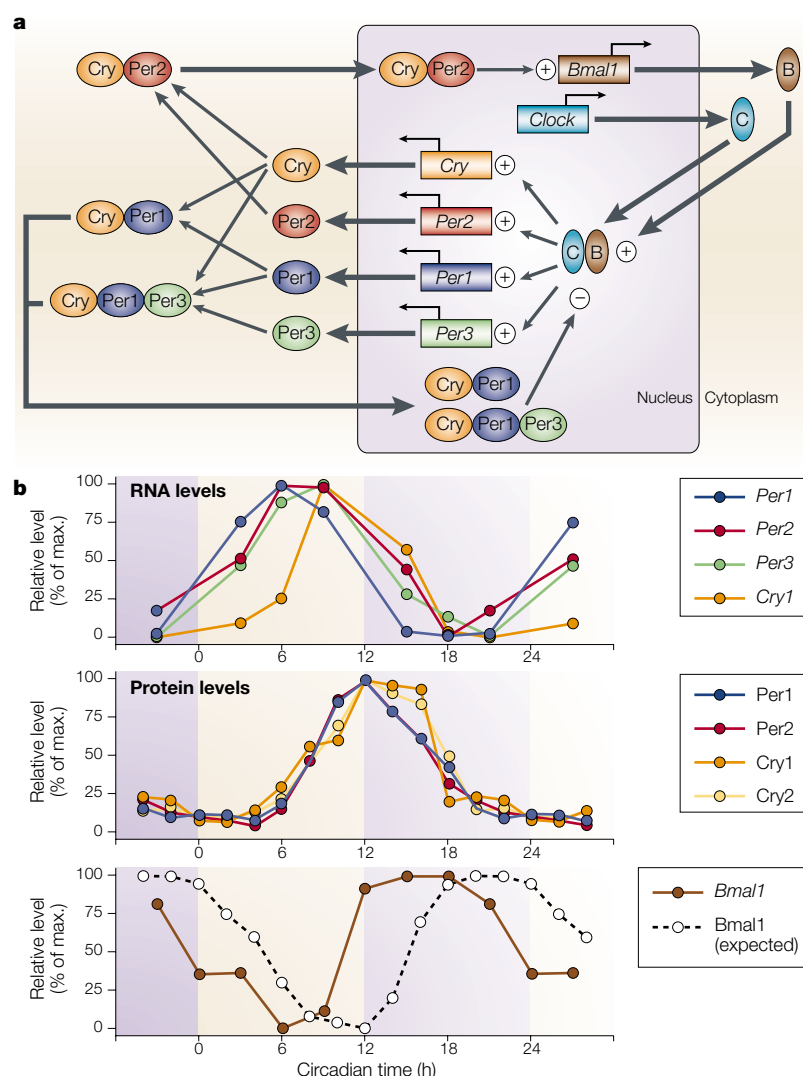


Figure 2 | **The molecular control of circadian rhythms.**

a | The molecular basis of the circadian clock expressed in a single cell of the suprachiasmatic nucleus of the anterior hypothalamus. *Clock*:*Bmal1* (labelled C and B) is a protein heterodimer that is a key component of a transcription factor that promotes the transcription of period (*Per*) and cryptochrome (*Cry*) genes. The RNA products of *Per*, *Cry*, *Bmal1* and *Clock* are translocated to the cytoplasm, where they are translated to proteins. *Per* and *Cry* proteins form complexes that are translocated back into the nucleus to exert feedback effects on *Clock*:*Bmal1*. Protein complexes that contain *Cry* exert negative feedback control on the *Clock*:*Bmal1* heterodimer, and slow the transcription of *Per* and *Cry*, whereas complexes that contain *Per2* proteins enhance the transcription of *Bmal1*. The promotion of *Per* and *Cry* gene expression by *Clock*:*Bmal1* causes the levels of *Per* and *Cry* to peak at the end of the subjective day, at which point the feedback inhibition of *Clock*:*Bmal1* (by complexes composed of *Per* and *Cry*) reverses this trend, causing their levels to fall to a minimum during the following subjective night (**b**). During the subjective day, however, protein complexes that contain *Per2* also enhance *Bmal1* transcription, causing the levels of *Bmal1* to peak during the subjective night (**b**). This favours the formation of new *Clock*:*Bmal1* heterodimers; these promote the transcription of *Per* and *Cry* when released from inhibition by minimal levels of *Per* and *Cry* as the next subjective day begins, thus restarting the cycle. Note that this is a highly simplified schematic, in which important feedback-related cofactors in protein complexes, as well as factors that favour the translocation of gene products within the cell², are omitted. Circled + or - indicates promotion and inhibition of gene transcription (or heterodimer formation), respectively. Light arrows represent gene transcription. Medium arrows represent gene promotion/inhibition and protein combination. Heavy arrows represent translocation of RNA transcripts and proteins between the nucleus and the cytoplasm. **b** | Variation over the circadian day in the levels of messenger RNA and protein products of the core circadian clock genes — *Per1*, *Per2*, *Cry1* and *Cry2*, and *Bmal1* mRNA and protein (dashed line). Bars indicate subjective night (purple) and subjective day (yellow). Modified, with permission, from REF. 2 © 2001 Annual Reviews.

SUPRACHIASMATIC NUCLEUS

The mammalian circadian pacemaker, or 'master clock', which consists of two tiny, bilaterally symmetrical nuclei in the anterior hypothalamus, located just above the optic chiasm (where the main fibre tracts, or optic nerves, from the two eyes meet). It is therefore ideally situated to receive photic input from the retina through the retinohypothalamic tract, which follows these nerves.

SUBJECTIVE DAY AND SUBJECTIVE NIGHT

The time during which an organism is normally active is referred to as the subjective day. The subjective night describes the period during which an organism is normally inactive and in which its sleep normally occurs. Therefore, a nocturnal animal's subjective day occurs during the astronomical night.

feedback inhibition, can again begin to promote the transcription of *Per* and *Cry* as the organism begins its new subjective day.

The combined action of positive- and negative-feedback loops creates a suite of molecular signals that reliably recur at precise times over 24-h cycles. These molecular signals can be read by cytoplasmic mechanisms in SCN cells and translated into reliably recurring cellular events, such as changes in membrane potential². Such signals, in turn, can be transmitted to connecting neurons and, ultimately, to those neural structures that control physiological processes with a circadian rhythmicity.

Many of the details of the genetic mechanisms of mammalian circadian rhythms have been elucidated by studies of mutations in these genes². For example, a mouse mutant of the *Clock* gene shows lengthening of the circadian period¹⁴. Cloning of these mammalian genes, beginning with *Clock*¹⁵, has allowed the precise molecular analysis of normal and mutant circadian genes². A recent study in humans has reported a heritable, familial trait for **advanced sleep-phase syndrome**

with autosomal-dominant transmission¹⁶. This finding might constitute the first step in identifying molecular components of the human circadian system that are analogous to those described in animals.

The precise timing of the appearance of these endogenously reliable signals relative to the astronomical day can be entrained to ambient light–dark cycles by light impinging on the retina. The reliable circadian output of SCN cells results not only from the endogenous cycling of transcriptional/translational signals described above, but also from temporally ordered sensitivity of these clock cells to input from the retina and other brain structures⁹. Such neurochemical feedback allows control of the SCN by neuronal responses it has itself previously elicited⁹.

Light-mediated entrainment of SCN cells is believed to result from glutamatergic stimulation of NMDA (*N*-methyl-D-aspartate) receptors through the retinohypothalamic tract (RHT)⁹. Administration of glutamate to SCN slices *in vitro* effects phase shifts of cell firing that presumably reflect the molecular mechanisms of light-induced entrainment^{9,17,18}. Photic and glutamatergic

CIRCADIAN TIME

A 24-h period divided into a 12-h activity phase and a 12-h rest phase. In diurnal animals, such as humans, circadian time (CT) 0 designates the start of the activity phase and CT 12 designates the beginning of the rest phase. In nocturnal animals, such as the rat, CT 12 is at the beginning of the activity phase and CT 0 is at the start of the rest phase.

TETRODOTOXIN

A potent marine neurotoxin that blocks voltage-gated sodium channels. Tetrodotoxin was originally isolated from the tetraodon pufferfish, and contains a positively charged guanidinium group and a pyrimidine ring.

stimulation of SCN cells in the early subjective night causes phase delay, whereas such stimulation late in the subjective night causes phase advance⁹.

The responses of SCN cell-membrane receptors to glutamate are modulated by neuromodulators that can shift the rhythms of SCN cell firing. For example, nitric oxide (NO) is an essential component of the photic resetting of circadian rhythms¹⁹. In most cases, the response depends on circadian phase (for a review, see REF. 9). For example, cholinergic mechanisms that involve the activation of muscarinic M1 receptors in the SCN are involved in resetting the circadian clock only during the subjective night²⁰. Similarly, SCN cells are most sensitive to melatonin feedback at subjective dusk and dawn²¹ (see below). By contrast, photic resetting of the circadian clock during the subjective day is believed to be mediated by pituitary adenylyl-cyclase-activating peptide (PACAP) and cyclic AMP^{9,22}.

Receptor activation in the SCN leads to membrane changes and second-messenger cascades, the pathways and sensitivity of which also depend on circadian phase. For example, photic or glutamate-induced phase delays that occur early in the subjective night are mediated by ryanodine receptors and elevated intracellular calcium^{9,23}, whereas late-night phase advance by the same signals results from a cyclic GMP pathway⁹. Similar temporal dependency occurs in the cAMP/protein kinase A (PKA) second-messenger pathway, with cAMP/PKA facilitating SCN state changes early in the night, but opposing such changes late in the night²⁴.

Second-messenger cascades lead ultimately to the intranuclear phosphorylation of cAMP-responsive-element-binding protein (CREB) and the subsequent downstream transcription of clock-related genes². The induction of *Per* might be the first step in resetting the SCN clock^{2,25}. Like membrane receptors and intracellular pathways, such intranuclear events also show specific sensitivity to circadian phase. For example, CREB phosphorylation occurs only during the night².

SCN neurons communicate circadian time to other brain structures primarily by action potentials that are mediated by sodium channels², and most SCN cells contain the inhibitory transmitter GABA (γ-aminobutyric acid)²⁶. However, the circadian clock continues to keep accurate time even when SCN cell firing is blocked by tetrodotoxin^{2,10}. SCN firing peaks at the middle of the circadian day, and circadian variations in SCN action potentials are believed to be produced by similar variations in SCN cell-membrane properties^{2,9}. Surface rhythms, such as variation in membrane potential or ion channel activity, are likely to be controlled by proteins such as preproressophysin (a precursor of arginine vasopressin, AVP) — products of 'clock-controlled genes' that are under the transcriptional control of core clock genes, such as *Clock* and *Bmal1* (REF. 2).

SCN neurons probably synchronize primarily by using GABA^{27,28}, although diffusible substances and cell-surface constituents might also have non-synaptic roles^{2,28}. Peripheral circadian oscillators that are

molecularly similar to those in the SCN also exist throughout the body^{2,29}. However, current evidence indicates that the maintenance of these oscillators depends on periodic input from the SCN².

Narcolepsy. The important role of genetics in sleep research is also exemplified by the discovery of the genetic basis of narcolepsy^{30,31}. These insights have led to the discovery of an excitatory wake-promoting neuromodulatory system that originates in the orexin (or hypocretin)-producing cells of the lateral hypothalamus. For further information on the orexin system, see REFS 32,33. The putative roles of orexin in modulating ascending arousal systems are described briefly later in this article.

Gene expression during sleep and wakefulness. Immediate early genes (IEGs), such as *c-fos*, are reliably transcribed and translated to protein products shortly after a neuron becomes active. Their products are probably involved in the subsequent transcription of other genes³⁴. Regional expression of these genes is highly state dependent, and most brain cells express *c-fos* and other IEGs at higher levels during waking^{34,35}.

Other genes that are activated selectively during sleep or waking might encode proteins that, unlike IEGs, are involved specifically in state-dependent processes³⁵. Extensive screening of the rat genome has been undertaken recently to identify genes that are upregulated selectively during sleep or waking³⁵. Using cortical cells of sleeping, waking and sleep-deprived rats, ~30–75% of the rat genome has been screened. State-dependent changes in regulation were found in less than 1% of the screened genome, and most of these genes were upregulated selectively during normal or sleep-deprived wakefulness.

The functions of the few rat genes that are upregulated selectively in sleep are unknown^{35–37}. Genes that are upregulated selectively during short (3 h) periods of wakefulness include IEGs and related transcription factors, as well as components of the mitochondrial genome, including the gene for subunit I of cytochrome c oxidase, an enzyme that is involved in oxidative metabolism. Such wake-related activation of the mitochondrial genome might facilitate a rapid response to the metabolic demands of initial, brief or unpredictable wakefulness, as the expression of these genes returns to baseline levels after sustained wakefulness³⁵.

Genes that are upregulated during sustained waking include some that are involved in glucose metabolism and responses to physiological stress^{35,37}. Most interesting to us are genes that encode elements of synaptic neurotransmission, such as presynaptic transporters and postsynaptic receptors. Upregulation of these genes could reflect a wake-related increase in demand that is associated with synaptic efficacy and neuroplastic processes. For example, the expression of aryl sulphotransferase, which is involved in the breakdown of the wake-related catecholamine neurotransmitters, is upregulated during extended sleep deprivation^{35,37}.

The expression of some wake-related genes is modulated by noradrenergic mechanisms: in rats with unilateral lesions of the **LOCUS COERULEUS**, the lesioned hemisphere fails to show the markedly increased expression during waking compared with sleep of both IEGs and plasticity-related genes, such as CREB, despite normal electroencephalographic (EEG) activity and behaviour^{35,38,39}. This finding links a known wake-related ascending arousal system with the expression of genes that are associated with a wake-related cognitive function — learning³⁵. Interestingly, this was not true for the serotonin (5-hydroxytryptamine, 5-HT) system — another wake-related ascending arousal system — as rats with lesions of the **DORSAL RAPHE NUCLEUS** showed normal gene expression³⁵. Although these new findings from molecular biology usher in a new era of sleep research, the results need to be replicated and evaluated more critically.

Intracellular events, sleep and neuroplasticity. The intracellular second-messenger systems and their downstream nuclear/genomic effects might represent an early stage of the neuroplastic changes that are initiated by waking experience, which are then consolidated or otherwise modified during sleep^{4,40}. The concept that neuroplastic changes are consolidated in sleep, especially during REM, is controversial. Some investigators point to animal studies that show increases in REM sleep after learning, learning decrements after REM sleep deprivation, and neuronal replay during sleep, and to specific correlations of sleep-stage percentages with procedural learning in humans⁴⁰. However, others argue that the effects of REM deprivation on learning might be epiphenomena of stress, and they offer more general homeostatic and ecological explanations for the adaptive value of sleep and its component stages⁴¹.

Notwithstanding this controversy, Graves *et al.*⁴² present compelling arguments that changes in the activity of the PKA second-messenger system and its third messenger, CREB, are associated with both state-dependent neuromodulatory changes in the hippocampus and performance of hippocampus-dependent learning tasks⁴⁰. Consolidation of hippocampus-based learning in rats is sensitive to disruption at specific times after training by both REM sleep deprivation and the intraventricular injection of inhibitors of PKA or the subcutaneous injection of inhibitors of protein synthesis. These manipulations could disrupt a PKA → CREB → gene transcription → protein synthesis pathway that is necessary for the consolidation of learning and is specifically facilitated by REM sleep. The hypothetical mechanism of this facilitation is REM-related enhanced cholinergic and diminished serotonergic modulation of adenylyl cyclase activity (linked to specific acetylcholine (ACh) and serotonin membrane receptors), which determines the activity of the PKA signalling pathway⁴². Notably, rats that are exposed to rich sensorimotor experiences during waking have elevated levels of the plasticity-associated IEG *zif-268* (also known as early growth response 1, *Egr1*) during subsequent sleep⁴³.

Cellular neurophysiology of REM and NREM

The original reciprocal-interaction model^{44,45} proposed that aminergic and cholinergic neurons of the mesopontine junction interact in a manner that results in the **ULTRADIAN** alternation of mammalian REM and NREM sleep (FIG. 3a,b). In this model, REM-on cells of the pontine reticular formation are cholinergically excited postsynaptically and/or cholinergically excitatory at their synaptic endings. Pontine REM-off cells are noradrenergically or serotonergically inhibitory. During waking, the pontine aminergic system is **TONICALLY** activated and inhibits the pontine cholinergic system. During NREM sleep, aminergic inhibition wanes and cholinergic excitation waxes. At REM sleep onset, aminergic inhibition is shut off, and cholinergic excitability peaks while other outputs are inhibited.

Recent evidence supports this model^{46,47}, but intermediate synaptic steps might intervene in the initiation and augmentation of REM at the level of both REM-on mesopontine neurons and REM-off pontine aminergic nuclei (FIG. 3b,c). Such synaptic details can be integrated with the reciprocal-interaction model without altering the basic effects of aminergic and cholinergic influences on the REM sleep cycle (FIG. 3a). For example, excitatory cholinergic–non-cholinergic interactions that involve ACh and glutamate enhance the firing of mesopontine REM-on cells^{48–50} (FIG. 3c). A role for mesopontine cholinergic REM-on cells in REM sleep generation was shown by their increased firing before the onset of REM sleep⁵¹.

Inhibition of locus coeruleus noradrenergic and dorsal raphe serotonergic neurons by GABA contributes to the release of mesopontine REM-on cells from aminergic suppression^{52–56}. As well as abundant evidence for noradrenergic, serotonergic and autoreceptor cholinergic suppression of mesopontine REM-on cells⁴⁶ (FIG. 3c), there is also evidence that intermediary non-cholinergic neurons might mediate the serotonergic inhibition of cholinergic REM-on cells⁵⁷. Other neurotransmitters, such as GABA and NO, modulate these interactions (FIG. 3c and see below). For further details, see REF. 46; for references to interactions depicted in FIG. 3c, see REF. 47.

The roles of brainstem neuromodulation in the REM–NREM cycle continue to be investigated; ongoing findings support cholinergic facilitation and aminergic inhibition of REM sleep^{57–59} (for reviews, see REFS 47, 60–62). Greater detail on the specific roles of aminergic and cholinergic systems can be found in REFS 50,61,63 (cholinergic systems), REF. 64 (noradrenergic systems) and REF. 65 (serotonergic systems).

Networks that generate the signs of REM sleep. A distributed neuronal network generates the characteristic signs of REM sleep (FIG. 4). The initiation of REM-sleep-related activity in these networks results from changes in the activity of ‘executive’ neuronal populations in the mesopontine junction, as identified in the reciprocal-interaction model (FIG. 3). The net result of REM-related activity in executive neuronal populations is the strong tonic and phasic activation of brainstem reticular and sensorimotor relay neurons in

LOCUS COERULEUS
A nucleus of the brainstem that is the main supplier of noradrenaline to the brain.

DORSAL RAPHE NUCLEUS
A nucleus of the brainstem that comprises a large cluster of serotonin-containing neurons. An important supplier of serotonin to the forebrain and to other brainstem nuclei.

ULTRADIAN RHYTHMS
Biological rhythms that have a periodicity of less than 24 h, such as the approximately 90-min REM–NREM cycle of the adult human.

TONIC
Physiological events that occur in a sustained manner, unlike phasic events, which occur only transiently with intervening periods of inactivity.

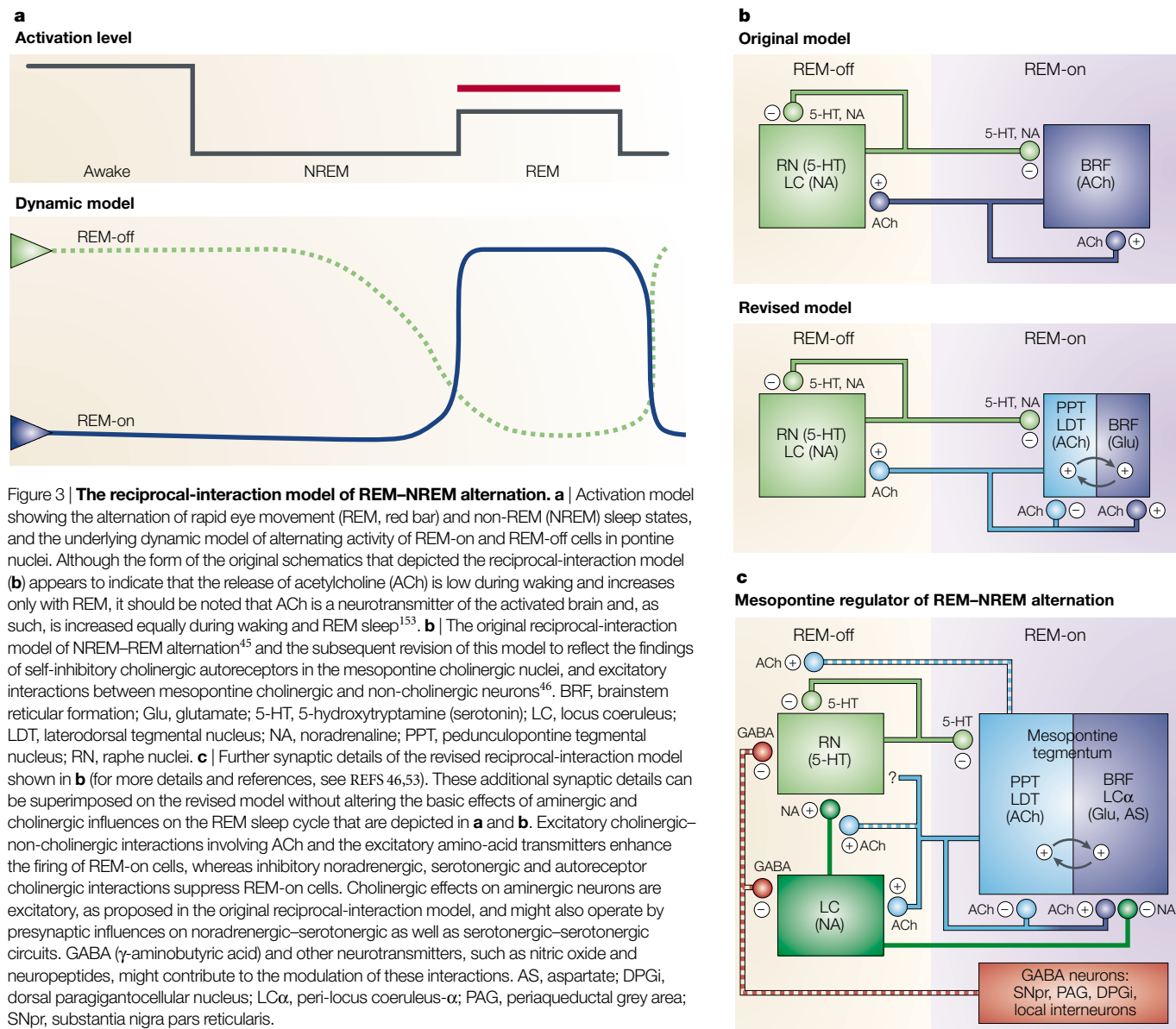


Figure 3 | The reciprocal-interaction model of REM–NREM alternation. a | Activation model showing the alternation of rapid eye movement (REM, red bar) and non-REM (NREM) sleep states, and the underlying dynamic model of alternating activity of REM-on and REM-off cells in pontine nuclei. Although the form of the original schematics that depicted the reciprocal-interaction model (**b**) appears to indicate that the release of acetylcholine (ACh) is low during waking and increases only with REM, it should be noted that ACh is a neurotransmitter of the activated brain and, as such, is increased equally during waking and REM sleep¹⁵³. **b** | The original reciprocal-interaction model of NREM–REM alternation⁴⁵ and the subsequent revision of this model to reflect the findings of self-inhibitory cholinergic autoreceptors in the mesopontine cholinergic nuclei, and excitatory interactions between mesopontine cholinergic and non-cholinergic neurons⁴⁶. BRF, brainstem reticular formation; Glu, glutamate; 5-HT, 5-hydroxytryptamine (serotonin); LC, locus coeruleus; LDT, laterodorsal tegmental nucleus; NA, noradrenaline; PPT, pedunculopontine tegmental nucleus; RN, raphe nuclei. **c** | Further synaptic details of the revised reciprocal-interaction model shown in **b** (for more details and references, see REFS 46,53). These additional synaptic details can be superimposed on the revised model without altering the basic effects of aminergic and cholinergic influences on the REM sleep cycle that are depicted in **a** and **b**. Excitatory cholinergic–non-cholinergic interactions involving ACh and the excitatory amino-acid transmitters enhance the firing of REM-on cells, whereas inhibitory noradrenergic, serotonergic and autoreceptor cholinergic interactions suppress REM-on cells. Cholinergic effects on aminergic neurons are excitatory, as proposed in the original reciprocal-interaction model, and might also operate by presynaptic influences on noradrenergic–serotonergic as well as serotonergic–serotonergic circuits. GABA (γ-aminobutyric acid) and other neurotransmitters, such as nitric oxide and neuropeptides, might contribute to the modulation of these interactions. AS, aspartate; DPGi, dorsal paragigantocellular nucleus; LCα, peri-locus coeruleus-α; PAG, periaqueductal grey area; SNpr, substantia nigra pars reticularis.

REM sleep⁶⁶. The characteristic signs of REM sleep are postulated to be further mediated as follows:

- EEG activation results from a net tonic increase in reticular, thalamocortical and cortical neuronal firing rates⁶⁷.
- Phasic potentials that are recorded sequentially in the pons, thalamic lateral geniculate body and occipital cortex of the cat, termed ponto-geniculo-occipital (PGO) waves, are the result of tonic disinhibition and phasic excitation of burst cells in the lateral pontomesencephalic tegmentum^{51,68}.
- REMs are the result of phasic firing by reticular and vestibular cells; the latter directly excite oculomotor neurons⁶⁶.
- The change in the hippocampal EEG from irregular rhythms to a regular THETA RHYTHM is influenced by the brainstem and mediated by the medial septal nucleus of the basal forebrain^{69,70}.

THETA RHYTHM
Rhythmic neural activity with a frequency of 4–8 Hz.

- Muscular atonia results from tonic postsynaptic inhibition of spinal anterior horn cells by the pontomedullary reticular formation⁷¹.

Other neurotransmitters and the REM–NREM cycle.

As well as cholinergic and aminergic neurons, other neurotransmitter systems modulate the REM–NREM oscillator and might interact with aminergic and cholinergic control^{47,60,62,72,73}.

Histamine is an amine transmitter of an arousal system that originates in neurons of the tuberomammillary nucleus (TMN) of the posterior hypothalamus (FIG. 5; see below), the firing of which, like that of serotonergic and noradrenergic neurons, is minimal during REM sleep^{74,75}. By contrast, firing of midbrain dopaminergic neurons of the substantia nigra and ventral tegmental area does not seem to vary in phase with the REM–NREM cycle^{76,77}, leading us to suppose that the

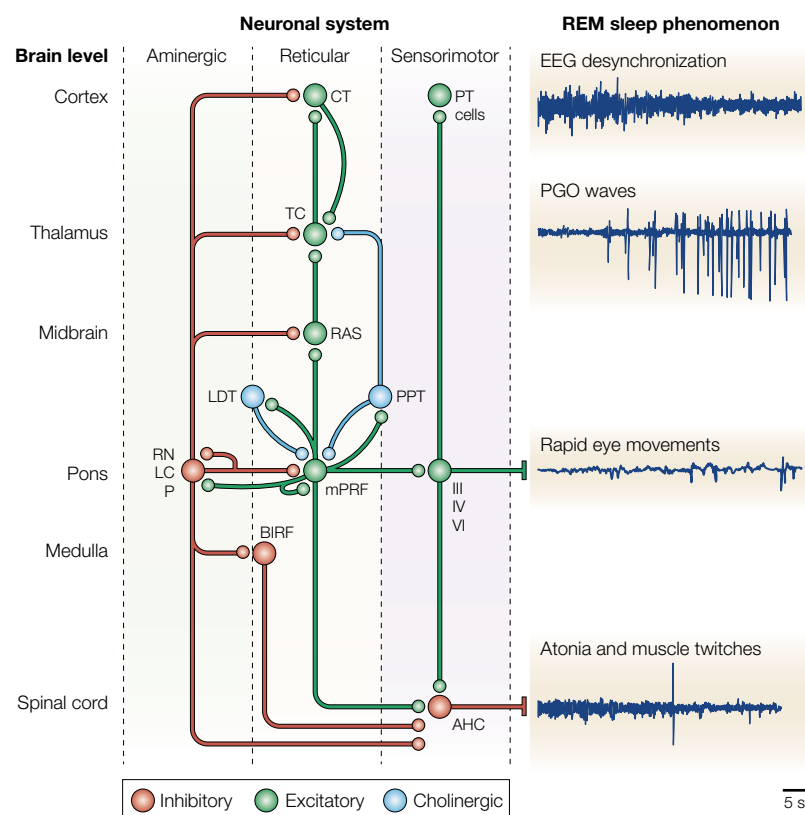


Figure 4 | Schematic representation of the process of REM sleep generation. The network is represented as comprising three neuronal systems (aminergic, reticular and sensorimotor) that mediate rapid eye movement (REM) sleep electroencephalographic (EEG) phenomena (right). The actual synaptic signs of many of the aminergic and reticular pathways remain to be shown and, in many cases (such as thalamus and cortex), the neuronal architecture is far more complex than is indicated here. III, oculomotor; IV, trochlear; VI, abducens; AHC, anterior horn cell; BIRF, bulbospinal inhibitory reticular formation (for example, gigantocellular tegmental field, parvocellular tegmental field, magnocellular tegmental field); CT, cortical; LC, locus coeruleus; LDT, laterodorsal tegmentum; mPRF, meso- and medio-pontine tegmentum (for example, gigantocellular tegmental field, parvocellular tegmental field, parvocellular tegmental field); P, peribrachial region; PGO, ponto-geniculo-occipital; PPT, pedunculopontine tegmentum; PT cell, pyramidal cell; RAS, midbrain reticular activating system; RN, raphe nuclei; TC, thalamocortical. Modified, with permission, from REF. 66 © 1986 The American Physiological Society.

effects of dopamine on normal sleep might be mediated by its interactions with other neurotransmitter systems. However, extrinsically augmented dopaminergic neurotransmission can influence both sleep–wake and REM–NREM cycles. For example, the enhancement of waking and prevention of sleep by dopamine reuptake inhibitors (the common psychostimulants) and dopamine receptor agonists are the basis for their use in the treatment of narcolepsy and somnolence associated with hypodopaminergic states such as **Parkinson's disease**⁷⁸. Moreover, some neurons in the periaqueductal grey area (PAG) are wake active⁷⁹.

The effects of dopaminergic drugs on REM sleep are more complex, possibly depending on the dose that is used and the receptor subtype that is targeted, and include both REM suppression⁸⁰ and REM enhancement⁸¹. Moreover, REM deprivation alters dopamine receptor function⁸². It has been suggested that a 'meso-thalamic' dopamine system might exist, in which axon

collaterals of dopaminergic neurons of the mesostriatal system synapse on motor and limbic-related thalamic nuclei, thus linking the mesostriatal dopaminergic system directly to thalamically mediated ascending arousal systems⁸³. The effects of endogenous dopamine on the sleep–wake and REM–NREM cycles await further study.

GABA and glutamate also influence the REM–NREM cycle. Inhibition by GABA both facilitates and inhibits REM sleep. For example, GABA inputs to the dorsal raphe nucleus and locus coeruleus from the PAG, substantia nigra pars reticulata, dorsal paraventricular nucleus and local interneurons could be the final synaptic step responsible for shutting down REM-off serotonergic and noradrenergic cells, thereby disinhibiting pontine cholinergic REM-on networks^{52,54–56} (for a review, see REF. 53), probably through both GABA_A and GABA_B receptors⁶⁹. But GABA also directly inhibits the mesopontine cholinergic neurons that generate PGO waves⁶⁸. Furthermore, there is evidence for a GABA-mediated 'switch' between waking and REM in the nucleus pontis oralis⁸⁴. Similarly, Boissard *et al.*⁸⁵ have suggested that the onset of REM in the rat is triggered by the release of neurons of the pontine dorsal subcoeruleus nucleus from tonic, wake-related, GABA-mediated inhibition by neurons of the PAG. Another inhibitory amino acid, glycine, regulates specific physiological manifestations of REM: medullary glycinergic cells inhibit somatic motor neurons during REM atonia⁸⁶.

Glutamate interacts with cholinergic and cholinceptive neurons to generate the exponential increase in mesopontine and pontine reticular activity that is associated with the onset of REM sleep⁵⁰. Moreover, glutamatergic cells of the pons excite glycinergic and GABA-containing neurons of the medulla, which, in turn, inhibit somatic motor neurons to produce REM sleep atonia⁷¹. Excitatory glutamatergic neurotransmission has a prominent role in the thalamocortical processes that underlie the characteristic oscillations of NREM sleep⁸⁷.

NO has been implicated widely in sleep-cycle modulation, and functions primarily as an intercellular messenger that can enhance capillary vasodilation and the synaptic release of neurotransmitters such as ACh⁸⁸. It is produced by cholinergic mesopontine neurons, and might help to maintain the cholinergically mediated REM sleep state in the pons and thalamus^{88,89}. The level of extracellular NO is elevated when the activity of mesopontine cholinergic neurons increases⁹⁰, and NO modulates the release of ACh in the basal forebrain⁹¹. The vascular effects of NO might interact with state-dependent alterations in cholinergic excitability and ascending cholinergic activation, which, in turn, could produce the REM-related changes in regional blood flow that are seen in neuroimaging studies⁹².

Finally, neuropeptides such as vasoactive intestinal polypeptide (VIP)⁹³, as well as numerous hormones⁹⁴, are increasingly thought to regulate REM–NREM cycles. Research on the REM–NREM cycle is now beginning to extend from the neurotransmitters and their receptors to

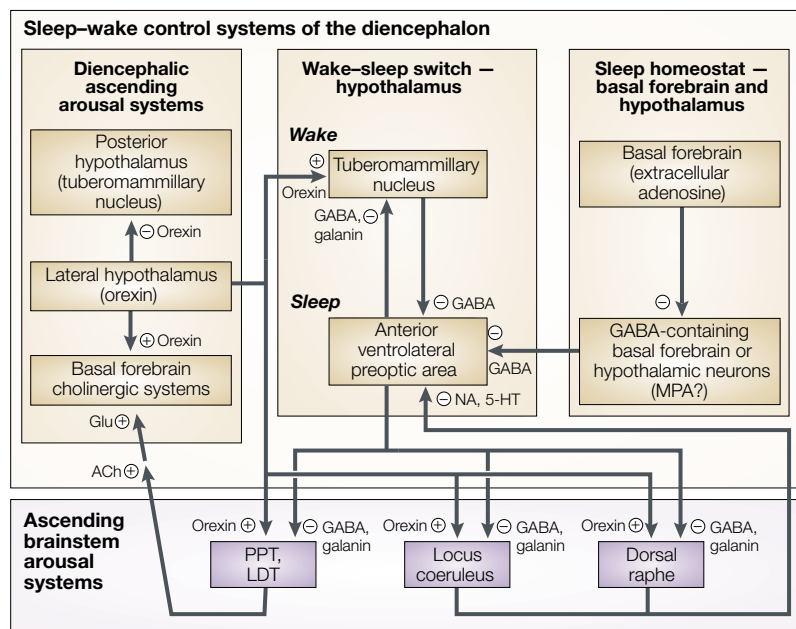


Figure 5 | **Sleep-wake control systems of the subthalamic diencephalon and their links to input from the circadian clock and to ascending arousal systems of the brainstem.**

Key interactions include sleep-promoting disinhibition of ventrolateral pre-optic area (VLPO) neurons by the adenosinergic inhibition of GABA (γ -aminobutyric acid)-containing basal forebrain neurons^{96,117}; VLPO GABA-mediated inhibition of diencephalic and brainstem ascending arousal systems^{95,99}; reciprocal inhibition of VLPO cells by noradrenergic and serotonergic input from ascending brainstem arousal systems, and by GABA-containing cells of the tuberomammillary nucleus that are co-localized with histaminergic neurons^{74,97}; wake-related orexinergic stabilization of these same ascending arousal systems⁷⁴; and brainstem cholinergic augmentation of wake-related basal forebrain cholinergic activity through a glutamatergic intermediary¹⁵⁴. ACh, acetylcholine; Glu, glutamate; 5-HT, 5-hydroxytryptamine (serotonin); LDT, laterodorsal tegmental nucleus; MPA, medial preoptic area; NA, noradrenaline; PPT, pedunculopontine tegmental nucleus.

the roles of intracellular second messengers⁶³ and the molecular biology of gene transcription^{35,42}.

Histamine–GABA interactions and NREM onset. Sherin *et al.* described a sleep-promoting region in the ventrolateral pre-optic area (VLPO) of the anterior hypothalamus⁹⁵. Further investigations revealed histaminergic and GABA-mediated interactions between sleep-promoting anterior regions and wake-promoting posterior regions of the hypothalamus^{74,96}. Neurons in the VLPO produce GABA and the inhibitory neuropeptide **galanin**^{74,95–99}. The activity of these neurons is proportional to the amount of time spent in sleep⁹⁵. They project to the ascending aminergic arousal systems of the brainstem (locus coeruleus and dorsal raphe nucleus) and to the hypothalamus (TMN)^{98,99}, and *in vitro* studies have shown that VLPO cells can use GABA to inhibit the histaminergic TMN¹⁰⁰.

The VLPO consists of two subregions, which are termed ‘tightly clustered’ and ‘diffuse’ because of their distribution pattern^{74,101}. Neurons of the tightly clustered subregion project to the TMN, and a lesion study indicates that they might selectively promote NREM sleep¹⁰¹. By contrast, VLPO cells in the diffuse subregion project to the brainstem aminergic nuclei (locus coeruleus and dorsal raphe nucleus), and might promote REM sleep¹⁰¹.

The histaminergic arousal system originates in the TMN and innervates the entire forebrain as well as brainstem regions that are involved in behavioural-state control^{74,75,95,98,102,103}. The histaminergic neurons of the hypothalamus fire in relation to behavioural state with a pattern identical to that of the noradrenergic and serotonergic neurons of the lower brainstem: they change their output in the same distinctive REM-off pattern^{74,75}. So, this cell group can be thought of as the hypothalamic component of an aminergic wake-state-enhancement system that involves serotonin, noradrenaline and histamine. These systems are all modulated by orexin⁷⁴ (FIG. 5; see below).

The anterior sleep-promoting and posterior arousal-promoting regions of the hypothalamus are thought to be mutually inhibitory⁷⁴ (FIG. 5). The wake-promoting neuromodulators noradrenaline, serotonin and ACh inhibit VLPO neurons⁹⁷. Although histamine has not been shown to inhibit VLPO neurons⁹⁷, histaminergic neurons are co-localized in the TMN with GABA neurons, which might reciprocally inhibit the GABA-containing VLPO neurons that project to the histaminergic TMN cells⁷⁴ (FIG. 5). In support of these ideas, serotonergic dorsal raphe, noradrenergic locus coeruleus and histaminergic TMN neurons project to the VLPO (REF. 104). Saper *et al.*⁷⁴ propose that this arrangement forms the dynamic basis for a pattern of bistability analogous to a ‘flip-flop’ electrical circuit. Either sleep or the waking state is self-reinforcing when its component neurons are sufficiently active; transitional states arise when either wake or sleep neuronal activity wanes, but they are transient because the system tends to revert to one of the two stable configurations⁷⁴.

The orexin system of the lateral hypothalamus. The orexin system — an important system for the control of behavioural state — was identified by investigators seeking the genetic basis of narcolepsy^{32,33}. In the rat, orexin induces a dose-dependent increase in wakefulness when it is injected intracerebroventricularly¹⁰⁵ or perfused by microdialysis into the basal forebrain¹⁰⁶. The densest projection of orexinergic cells in the rat is to the locus coeruleus¹⁰⁷ (FIG. 5), the noradrenergic output of which favours the cortical arousal of waking, but opposes REM-associated arousal^{46,108}. In humans, orexinergic neurons in the perifornical, lateral and medial hypothalamus project heavily to the locus coeruleus¹⁰⁹. Interestingly, human narcoleptics show a large reduction in the number of orexinergic neurons in the lateral hypothalamus^{110,111} and a deficiency of orexin in the cerebrospinal fluid¹¹².

On the basis of the bistable hypothalamic sleep–wake switch model, Saper *et al.*⁷⁴ have proposed a specific functional role for orexin in the normal sleep–wake cycle that might explain the dysregulated state transitions that occur in its absence in narcolepsy. They propose that the orexinergic drive on key nuclei in the wake-promoting, aminergic half of the bistable sleep–wake switch stabilizes the wake state and prevents untimely transitions from waking to sleep. In narcolepsy, orexinergic deficiencies make abnormal wake–sleep switches more likely⁷⁴. Orexin might indirectly inhibit sleep-promoting neurons of the

VLPO by exciting co-localized inhibitory wake-on neurons in the preoptic hypothalamus¹¹³. Clearly, this important modulator of primary sleep–wake and REM–NREM mechanisms will continue to be a focus of intense study.

It has been proposed that orexin promotes waking by its excitatory influence on aminergic arousal systems in the locus coeruleus, dorsal raphe, TMN, ventral tegmental area, and cholinergic ascending arousal systems in the pons (the pedunculopontine tegmental nucleus, or PPT, and laterodorsal tegmental nucleus, or LDT) and basal forebrain^{32,114}. Notably, orexin activates G proteins in locus coeruleus, dorsal raphe and pontine reticular neurons¹¹⁵, and excites cholinergic neurons of the basal forebrain¹¹⁴. During NREM, GABA-mediated inhibition of orexinergic and aminergic nuclei by VLPO neurons causes globally decreased arousal³². During REM sleep, inhibition of aminergic nuclei by brainstem GABA neurons (in the PAG, for example) blocks their excitation by orexin, but allows orexinergic excitation of brainstem cholinergic systems; this complements the disinhibition of these cholinergic cells that results from decreased aminergic inhibition³². Kilduff and Peyron propose that this release of REM-on mesopontine cells from aminergic inhibition contributes to sudden attacks of REM sleep (cataplexy) in narcolepsy³².

The homeostatic process of sleep–wake control. Adenosine is a putative endogenous SOMNOGEN; its accumulation in the brain during prolonged wakefulness might constitute the physiological basis of homeostatic sleep need^{116,117}. This is termed ‘process S’, and it interacts with circadian factors (‘process C’) in Borbely’s two-process model of sleep propensity¹¹⁸. For example, both Shiromani *et al.*⁹⁶ and Strecker *et al.*¹¹⁷ have proposed that, during prolonged wakefulness, accumulating adenosine inhibits specific anterior hypothalamic and basal forebrain GABA-containing neurons that have been inhibiting the sleep-active VLPO neurons during waking (FIG. 5). Disinhibited sleep-active GABA neurons of the VLPO and adjacent structures then inhibit the wake-active histaminergic neurons of the TMN, as well as those of the pontine aminergic (locus coeruleus, dorsal raphe nucleus) and cholinergic (LDT/PPT) ascending arousal systems, thereby initiating NREM sleep⁹⁶.

Microdialysis in the cat has shown that the basal forebrain is the site of adenosine accumulation in response to sleep deprivation, and is where the behavioural-state effects of experimentally altered adenosine levels are expressed^{117,119}. In addition, the demonstration that binding of adenosine to A1 receptors in the basal forebrain increases the binding of DNA by the transcription factor NF- κ B (nuclear factor κ B) might extend our understanding of the physiological basis of sleep debt into the cell nucleus¹²⁰. Although adenosine continues to generate the most interest, certain cytokine neuropeptides¹²¹ and the fatty acid oleamide¹²² have also been nominated as endogenous somnogens.

According to the two-process model of sleep regulation¹¹⁸, the homeostatic increase in sleep pressure with duration of wakefulness must be integrated with circadian propensity to initiate sleep. Structures responsible

for the integration of these processes seem to reside in the diencephalon; candidate structures include the medial preoptic area (MPA) and the anterior paraventricular thalamic nucleus¹²³ (FIG. 6). Another is the dorso-medial hypothalamic nucleus (DMH), which receives dense projections from the SCN and, together with the MPA, projects to the VLPO¹⁰⁴. Finally, the SCN projects monosynaptically to the VLPO¹²⁴, although SCN afferents to this region are sparser than those from the DMH and MPA¹⁰⁴. It seems likely that the integration of circadian and homeostatic influences occurs largely in anterior hypothalamic regions near to where each influence is individually regulated.

Thalamic maintenance of NREM sleep. Once NREM sleep has been initiated by the combined influence of homeostatic pressure and circadian propensity on structures in the hypothalamus, prominent oscillatory rhythms can be detected in EEG recordings⁸⁷. These oscillations include the DELTA and SPINDLE RHYTHMS that are described in BOX 1, as well as a recently described slow (<1 Hz) oscillation that is maintained by alternating hyperpolarization and depolarization of intracortical neurons, which, in turn, influences the timing of delta and spindle rhythms⁸⁷. These thalamocortical rhythms are described in detail in REFS 4,84.

Sleep–wake control and circadian entrainment

Connection between photoreceptors and the SCN. Glutamatergic retinal ganglion cells (RGCs) project to the SCN along the RHT. This is the pathway by which photic input entrains the SCN’s circadian clock^{2,9}. Signals from RGCs also reach the SCN along two indirect pathways, one through the lateral geniculate nucleus of the thalamus and the other through the dorsal raphe nucleus of the brainstem; however, these are believed to mediate non-photoc signals².

In the past year, the neuroanatomical and neurochemical basis of the direct pathway by which light input to the retina entrains the circadian pacemaker in the SCN has been elucidated^{125–127}. Melanopsin has been identified as the circadian photopigment in the RGCs of the RHT^{126–129}. RGCs that are photosensitive (depolarized by light even when isolated from rods and cones¹²⁵) also express melanopsin¹²⁷, and light effects on melanopsin are probably the first step in the entrainment of the SCN circadian clock¹²⁶. So, the RGCs might be the primary photoreceptors for the system by which light entrains the circadian clock¹²⁵.

SCN, hypothalamus and brainstem connections. Long after the discovery and elaboration of specific circadian and sleep-cycle control mechanisms, the connections between these oscillators have begun to emerge. The initial steps in the entrainment of biological rhythms have recently been linked to hypothalamic structures near the SCN, including the paraventricular nucleus (PVH), the subparaventricular zone (SPZ; the hypothalamic area that receives the greatest number of projections from the SCN) and the DMH (which receives projections from the SPZ)^{108,130,131} (FIG. 6).

SOMNOGEN

An agent that promotes sleep. Endogenous somnogens accumulate during prolonged waking, tending to produce sleep despite opposing pressures of the circadian cycle. Putative somnogens include adenosine, cytokines, hormones, melatonin, oleamide and prostaglandins

DELTA RHYTHM

Rhythmic neural activity with a frequency of 1–4 Hz that is characteristic of stage III and IV NREM sleep (also known as slow-wave sleep).

SPINDLE RHYTHM

Phasic episodes of 12–14-Hz neural activity that are characteristic of stage II NREM sleep, having a waxing and waning, spindle-like morphology.

ELECTROCULOGRAPHY

The polysomnographic measurement of eye movement by electrodes mounted adjacent to each eye, which detect the electrical dipole produced by the retina.

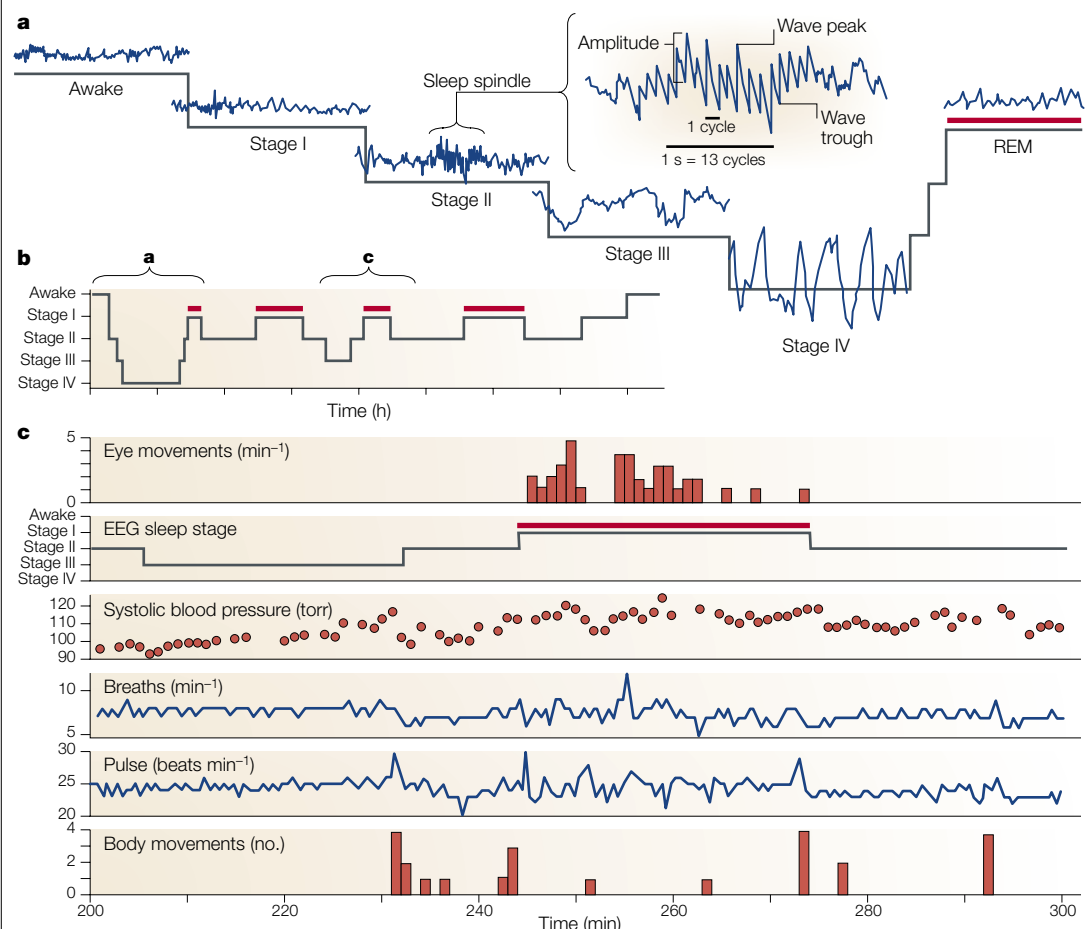
Box 1 | Sleep-cycle basics

In mammals, there are two types of sleep — rapid eye movement (REM) and non-REM (NREM). They are defined in terms of electrophysiological signs that are detected with a combination of electroencephalography (EEG), ELECTROCULOGRAPHY (EOG) and electromyography (EMG), the measurement of which in humans is collectively termed polysomnography¹⁵⁰. REM sleep (also known as paradoxical, active or ‘desynchronized’ sleep) is characterized by the following: wake-like and ‘activated’ (high-frequency, low-amplitude, or ‘desynchronized’) activity in the EEG; singlets and clusters of REMs in the EOG; and very low muscle tone (atonia) in the EMG¹⁵¹. Note that the term ‘desynchronized’ for the activated states of waking and REM has been rendered obsolete by the discovery of highly synchronized gamma-frequency (30–80 Hz) activity in these states⁴.

NREM sleep is divided into four stages, corresponding to increasing depth of sleep, as indicated by progressive dominance of the EEG by high-voltage, low-frequency (‘synchronized’) wave activity. Such low-frequency waves dominate the deepest stages of NREM (stages III and IV, also termed slow-wave sleep). Stage II NREM is characterized by distinctive sleep spindle and K-complex waveforms, as well as a slow (<1 Hz) oscillation, which influences their timing. Panel a shows the characteristic waveforms of the different sleep stages; panel c shows changes in peripheral physiology associated with these stages¹⁵².

NREM and REM sleep alternate in each of the four or five cycles that occur in each night of adult human sleep. Early in the night, NREM sleep is deeper and occupies a disproportionately large amount of time, especially in the first cycle, when the REM epoch might be short or aborted. Later in the night, NREM sleep is shallow, and more of each cycle is devoted to REM (red bars). Panel b illustrates these changes over the course of a night’s sleep. Panel a depicts, in detail, features of an early-night sleep cycle in which NREM reaches its greatest depth at stage III and IV (delta) sleep, whereas panel c depicts a late-night cycle in which NREM descends only to stage III. The constant period length of the NREM–REM cycle indicates that it is timed by a reliable oscillator (for a discussion of ultradian alternation of REM and NREM sleep, see the main text), the amplitude of which varies according to extrinsic factors.

The cyclic organization of sleep varies within and between species. The period length of each REM–NREM epoch increases with brain size across species, and the depth and proportion of the NREM phase in each cycle increases with brain maturation within species. NREM sleep complexity is a function of brain systems, such as the thalamocortical circuitry, that reach their maximum development in mature humans only to decline in post-mature age. It can therefore be concluded that the differentiation of sleep is a function of brain differentiation, a rule that indicates both mechanistic and functional links between sleep and other brain functions.



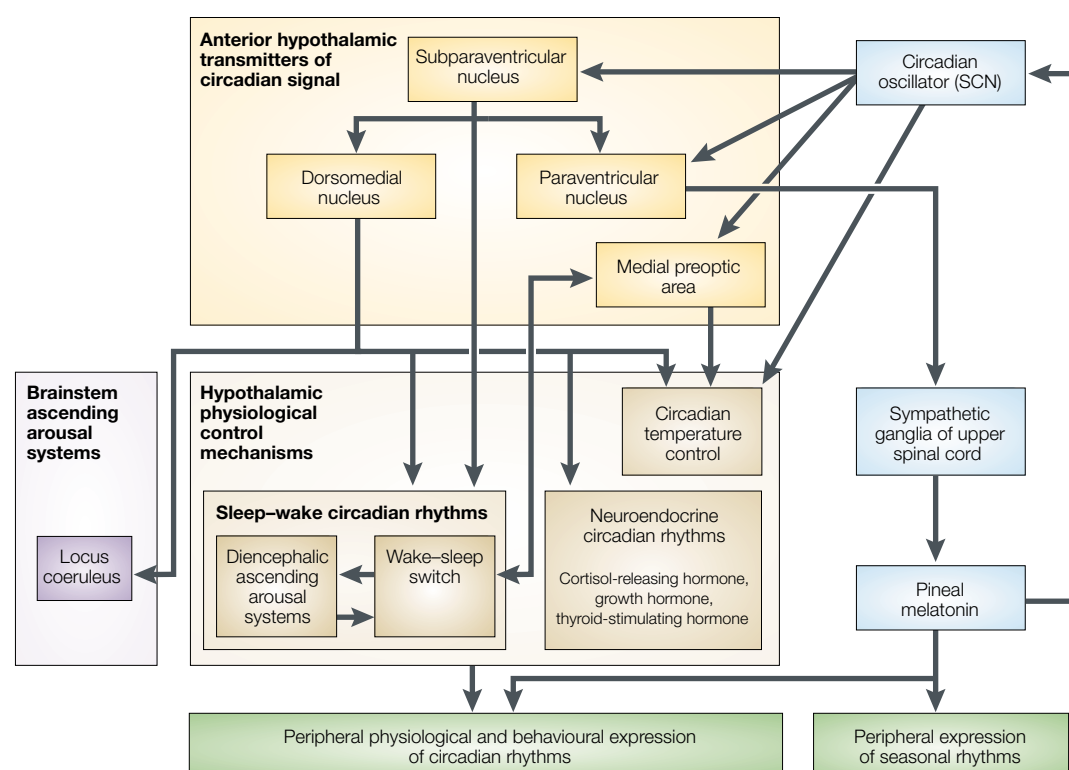


Figure 6 | Transmission of circadian information from the circadian oscillator to hypothalamic systems that control circadian rhythms. Hypothalamic systems control central and peripheral circadian rhythms, including the sleep–wake cycle. Key interactions include the multisynaptic transmission of circadian information from the suprachiasmatic nucleus (SCN) to physiological control systems through adjacent nuclei of the anterior hypothalamus^{104,130,131}; multisynaptic transmission of circadian information from the SCN to the pineal gland and feedback to the SCN by the pineal hormone melatonin¹⁴¹; direct pathways from the SCN to sleep- and arousal-promoting nuclei, the functional significance of which awaits elucidation¹⁰⁸; and a putative integration of homeostatic and circadian information in the medial preoptic area¹²³.

Excitotoxic lesions of the SPZ cause damping of the circadian rhythms of sleep, locomotor activity and body temperature¹³¹. Such lesions reveal regional differentiation of the SPZ, with dorsal lesions having more effect on body-temperature rhythms and ventral lesions having more effect on circadian rhythms of sleep and activity¹³¹. The SPZ projects to the VLPO¹³¹, which is involved extensively in NREM sleep regulation (see above). Another major projection target of SPZ neurons is the DMH, which also projects to the VLPO^{104,131}. As with lesions of the SPZ, excitotoxic lesions of the DMH decrease the amplitude of the circadian sleep and body-temperature rhythms of rats, indicating that these nuclei might link SCN circadian output to the behavioural and physiological expression of circadian rhythms^{130,131}. Whereas the SCN innervates the VLPO only weakly, the DMH innervates the VLPO more strongly, supporting the idea that the DMH is an intermediary structure between the SCN and the VLPO¹⁰⁴.

Trans-synaptic retrograde tracing has revealed indirect projections from the SCN through the DMH, PVH and VLPO nuclei of the hypothalamus to the noradrenergic locus coeruleus¹⁰⁸. As circadian periodicity in locus coeruleus activity is eliminated by lesions of the DMH, it seems that the SCN → DMH → locus coeruleus pathway is a physiologically functional circuit¹⁰⁸. The DMH contains orexinergic neurons¹⁰⁸, and anterograde tracing

has revealed projections from the SCN of the rat to orexinergic cells in the lateral hypothalamus¹³² (FIG. 3). Orexin increases both locus coeruleus activity^{107,133} and behavioural arousal¹³³. Aston-Jones *et al.*¹⁰⁸ suggest that orexin might help to transmit circadian information to ascending arousal systems, as the densest projection of orexinergic neurons is to the locus coeruleus¹⁰⁹.

Circadian information might be transmitted from the SCN to circuits that subserve stage transitions within sleep, as well as sleep–wake transitions. Wurts and Edgar¹³⁴ have used REM-deprived, SCN- and sham-lesioned rats to show that circadian inputs from the SCN gate the rats' propensity to enter REM during their normal sleep phase, but not in the normal active phase, whereas REM sleep homeostatic processes determine the duration of REM bouts in both states. Such complex interactions between circadian and homeostatic influences of sleep stage must be mediated through hypothalamic structures that link the SCN to sleep control switches. In this regard, it is notable that separate subpopulations of VLPO neurons might promote NREM and REM sleep⁷⁴.

Output of the SCN to other structures. The output of the SCN to other neural structures proceeds initially through adjacent hypothalamic structures; it is modulated by these structures¹³¹ and forms the basis of the

circadian rhythmicity of a remarkable array of physiological processes^{135,136} (FIG. 6). These include not only the sleep, temperature and activity rhythms, but also circadian neuroendocrine rhythms, such as those of cortisol, thyroid-hormone- and **parathyroid-hormone-stimulating hormones**, **growth hormone**, **prolactin** and, notably, melatonin¹³⁶ (see below). The SCN controls rhythmic processes such as sleep, activity, temperature and feeding through its projections to adjacent regions of the anterior hypothalamus, such as the SPZ and the DMH¹³¹. The MPA is proposed to be particularly important for the circadian control of sleep–temperature interactions¹³⁷.

Melatonin is produced by the pineal gland, which receives its circadian signal from the SCN through a multi-synaptic pathway that involves the PVH and sympathetic ganglia of the upper spinal cord¹³⁸. It reliably regulates important behaviours such as seasonal reproduction on the basis of environmental photoperiod¹³⁹. Melatonin can, in turn, influence the circadian clock by feedback effects on melatonin receptors¹⁴⁰. It might affect the circadian rhythms of the SCN through a protein kinase C second-messenger system²¹ and, like photic stimuli, its effect might involve the phosphorylation of CREB¹⁴¹. The ability of melatonin to reset the circadian clock is especially prominent at subjective dusk and dawn²¹.

The SCN also projects to non-hypothalamic brain regions, such as the basal forebrain and amygdala¹⁴². The precise areas in which SCN efferents terminate are determined to some extent by anatomical and neurochemical differentiation of the SCN itself into a core, which receives visual afferents, and a shell, which receives non-visual afferents¹⁴². Apart from the pineal, other structures that are involved in the physiological expression of circadian rhythms also feed back on the SCN¹⁴³ and include additional sources of neuromodulatory influences on the pacemaker.

Higher forebrain structures

The significance to forebrain functioning of the neuromodulatory outputs of the brainstem and diencephalic structures that regulate behavioural state (FIG. 1a,b) will be covered in more detail in a forthcoming review⁴. Like rodents and cats, primates (including humans) have ascending cholinergic, serotonergic, noradrenergic, dopaminergic, histaminergic and orexinergic activating systems with receptive fields in widespread cortical and subcortical areas^{109,144–147}. In addition to the metabolic and bioregulatory roles that have been proposed for state-dependent neuromodulatory changes, their associated cognitive effects have great potential significance for cognitive neuroscience, psychiatry and behavioural neurology.

State-dependent changes in cholinergic modulation of the hippocampus are involved in memory formation, consolidation and retrieval. Hasselmo¹⁴⁸ suggests that, in waking, high levels of cholinergic input suppress feedback circuits within the hippocampus and between the hippocampus and the entorhinal cortex. However, ACh does not suppress feedforward circuits that convey

information from the cortex to the hippocampus, where it is temporarily stored as the neural basis of episodic memory. Therefore, ACh might facilitate memory encoding by preventing interference of incoming information by previous hippocampal experience-related synaptic modifications. By contrast, during quiet waking and slow-wave sleep, low levels of ACh in the hippocampus allow such feedback to occur, permitting new hippocampal synaptic modifications to be strengthened within the hippocampus and conveyed back to the neocortex.

Many structures in the cortex and limbic forebrain are reciprocally connected to the brainstem and diencephalic structures that are involved in sleep–wake and REM–NREM cycles, and might influence behavioural state in a top-down manner. For example, input from the central nucleus of the amygdala to the mesopontine junction might influence the timing of REM onset¹⁴⁹.

Conclusions

Over the past decade, a remarkable explosion of new findings has allowed us to construct a much more complete picture of the genetic mechanisms, cellular neurophysiology and subcortical networks that underlie the neurobiology of sleep. This explosion has been made possible by technological advances in molecular biology and biotechnology (such as gene cloning and DNA sequencing), as well as in cellular neurophysiology (such as sophisticated combinations of microdialysis, unit recording, axonal tracers and immunohistochemistry).

We now know that an interlocking positive–negative-feedback mechanism that controls gene transcription in individual cells of the SCN of the hypothalamus is the molecular basis of circadian rhythmicity in mammals. This endogenous periodicity can be entrained to the ambient photoperiod by photons that impinge on the circadian photopigment, melanopsin, in RGCs, which convey this information to the SCN monosynaptically through the RHT. Circadian rhythmicity emerges from SCN cells by action potentials that impinge on adjacent nuclei of the anterior hypothalamus, including the PVH, SPZ and DMH; in turn, these nuclei convey circadian rhythmicity to structures that control rhythmic physiological processes, such as sleep, temperature and endocrine output. Feedback to the SCN circadian oscillator can occur through melatonin from the pineal gland, which reliably secretes this sleep-related hormone in response to polysynaptically conveyed signals from the SCN.

A key hypothalamic structure that receives circadian output from the SCN through the SPZ and the DMH is the VLPO, which promotes NREM sleep. The VLPO might initiate sleep onset through reciprocal inhibition of cholinergic, noradrenergic and serotonergic arousal systems in the brainstem, as well as histaminergic systems of the posterior hypothalamus and cholinergic systems of the basal forebrain, all of which are modulated by the orexinergic arousal system of the lateral hypothalamus. These arousal systems conspire to promote the activated brain states of waking, whereas the cholinergic system acts alone to promote the activated state of REM sleep.

The VLPO is triggered to initiate sleep onset by both circadian input from the anterior hypothalamus and sleep–wake homeostatic information from endogenous chemical signals, such as adenosine, that accumulate in proportion to time awake. Once sleep is initiated, an ultradian oscillator in the mesopontine junction controls the regular alternation of NREM and REM sleep. The executive control of this oscillator involves a reciprocal interaction between aminergic REM-on and cholinergic REM-off cell groups, whose influence on one another is mediated by interposed excitatory, inhibitory and autoregulatory circuits that involve GABA and glutamate. Circadian influences on

this ultradian oscillator are now also being sought in brainstem–hypothalamic connectivity.

Both the sleep–wake and REM–NREM oscillators give rise to regularly recurring changes in neuromodulation of the forebrain structures that mediate behaviour, consciousness and cognitive processes such as memory consolidation. The burgeoning literature detailing molecular-biological, cellular and neuro-modulatory mechanisms indicates that sleep research has entered a new era. Because it provides so many opportunities for interdisciplinary integration, the study of sleep has become a mainstream scientific enterprise.

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