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Neuroanatomy and neurochemistry of sleep

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Abstract. Sleep is regulated by homeostatic and circadian factors, and the regulation of sleep of mammals shares many molecular properties with the rest state of submammalian species. Several brain structures take part in waking: the basal forebrain, posterior and lateral hypothalamus, and nuclei in the tegmentum and pons. Active sleep mechanisms are located to the preoptic/anterior hypothalamic area. In addition to acetylcholine and monoamines, glutamate and hypocretin/orexin are important waking factors. Gamma-aminobutyric acid and several peptide factors, including cytokines, growth hormone-releasing hormone and prolactin, are related to sleep promotion. Adenosine is an important homeostatic sleep factor acting in basal forebrain and preoptic areas through A1 and A2A receptors. Prolonged waking activates inducible nitric oxide synthase in the basal forebrain, which through energy depletion causes adenosine release and recovery sleep. Numerous genes have been found differentially displayed in waking compared with sleep, and they relate to neural transmission, synaptic plasticity, energy metabolism and stress protection. The genetic background of a few sleep disorders has been solved.

Keywords. Sleep factor, sleep homeostasis, adenosine, nitric oxide, cytokines, genetics.

Introduction

Definition of sleep

Sleep is a reversible, physiological state with reduced motility and reduced responsiveness to sensory stimuli. It is subdivided into REM sleep (rapid eye movement sleep, paradoxical sleep, active sleep, D-sleep, REMS), and NREM sleep (non-REM sleep, NREMS). REMS is characterized by muscle atonia, activation of several brain areas, including the cortex, and phasically occurring eye movements, muscle twitches and changes in pulse rate, blood pressure and respiration. NREMS is characterized by body rest. In birds and mammals, slow (delta) waves emerge during NREMS, and phases when they dominate the electroencephalogram (EEG) are called slow-wave sleep (SWS). In poikilothermal animals, including evertebrates, cerebral electrical activity differs from the bird-mammal pattern. Sleep may be characterized by high-voltage spikes as in reptiles. In many species, no EEG can be recorded (e.g., fruit flies, small fishes), and it is therefore difficult to determine corresponding vigilance states. Even in the most primitive animals studied, rest-activity rhythms can be observed, and they appear homeostatically regulated like mammalian sleep/wake patterns.

Two-process model of sleep regulation

Human and animal sleep depends on two major factors: a circadian regulator, defining the diurnal rhythm, and a homeostatic regulator defining the relationship between wake time and sleep time [1]. The circadian regulation is the subject of an accompanying article in this volume [2]. A third factor, determining the dissipation of sleepiness after a bout of sleep (sleep inertia) may be added if one considers the effect of napping on performance during shift work [3–5].

Homeostatic regulation means that sleep is prolonged and often more profound after extended waking (recovery sleep). The search for regulatory factors has focused on brain chemicals that accumulate during prolonged wakefulness and decline during recovery sleep, or the opposite (decline during wake and restoration during sleep) (reviews in [6, 7]. Recently, experimental evidence has raised the pos-

sibility that the response to instrumentally prolonged wakefulness is regulated differently from the spontaneous sleep/wake cycle [8, 9].

Rest-activity homeostasis

Simple animal models are useful to study cellular and molecular mechanisms of sleep [10]. Evidence for circadian and homeostatic regulation of a sleep-like state has been found in insects [11, 12], notably fruit flies [13, 14], and in fish [15]. Prolonged rest deprivation by instrumental manipulations (shaking, lights, sounds) induces compensatory recovery rest when the disturbance is ended. In larval zebrafish, arousal thresholds were higher during the nocturnal rest period, and rest deprivation was followed by excess recovery rest [16]. Many of the molecular correlates of sleep-wake regulation are replicated in the rest/activity cycle of fruit flies, indicating that the regulatory mechanisms are analogous [13, 17].

CNS nuclei and structures involved in sleep and alertness

Nonmammalian

The insect CNS lacks analogous anatomical structures to those of mammals, but amine neurotransmitter and neuropeptide systems involved in mammalian sleep regulation are well developed (see [10, 18, 19]). In reptiles, the electrographic criteria of the rest state differ considerably from that of birds and mammals, mostly due to the more primitive telencephalon (see [20]). However, in freely moving box turtles brainstem neurons responded to sensory stimuli and increased their firing rate during motor activity, indicating homology to the 'reticular formation' of mammals [21].

Fish and mammalian brains have several homologies. For instance, the aminergic projection systems are well developed in zebrafish [22], as also the orexinergic system, which is connected to the aminergic and cholinergic system as in mammals [23]. Sleep structure in birds is quite similar to that in mammals [20].

Mammals

Evidence for posterior hypothalamic involvement in waking and anterior hypothalamic involvement in sleep mechanisms came from studies of patients with encephalitis in the influenza pandemic of 1917–1919. Patients with sleep-like coma had neuronal loss in the posterior hypothalamus and rostral mesencephalic area, whereas patients with insomnia had lesions in the anterior hypothalamic area [24, 25]. Lesions in the rostral hypothalamus produced sleeplessness in rats, while waking capacity was impaired by lesions in the lateral hypothalamus, indicating the presence of an active sleeping center [26] in the area we call the basal forebrain today. Active induction of sleep was suggested by the induction of sleep-like EEG by electrical stimulation of the basal forebrain [27]. Further studies have strengthened the notion of active sleep induction by neurons in the hypothalamic preoptic area [28].

Cats transected in the midbrain (*cerveau isolé*) had constant sleep EEG [29], indicating that preventing sensory input impaired waking. The finding that electrical stimulation of a large mesencephalic region induced waking in cats, whereas its lesion caused sleep, led to the concept of an 'ascending reticular activating system' (ARAS), activated by sensory input [30].

Lesion studies showed that REMS was generated in the pons (review in [31]), and specific lesions could result in REMS without muscle atonia, when the animals acted out their dream behavior (see [32]), a model of the REM Behavior Disorder of humans.

Since these classical studies, several wake-promoting areas have been characterized in the posterior and lateral hypothalamus, in the tegmentum and pons, while sleep-promoting neuron groups were found to be concentrated in the hypothalamic preoptic area (see [33], Fig. 1).

Basal forebrain and anterior hypothalamic neuronal groups involved in waking and sleep

The basal forebrain (BF) implied in wake/sleep regulation is a heterogeneous region adjacent to preoptic and supraoptic levels of the hypothalamus. It includes the cholinergic cell groups Ch1–4 as defined by Mesulam [34], but also glutamatergic and GABAergic neurons. The cholinergic neurons are located in several nuclei, from rostral to caudal: the medial septum, the vertical and horizontal diagonal bands of Broca, the magnocellular preoptic area, the substantia innominata and the nucleus basalis of Meynert [35]. These neurons receive input from brainstem arousal systems, and they project widely to the cortex and limbic system without thalamic relay (review in [36, 37]. When recorded in head-restrained rats, neurons that were immunohistochemically identified as cholinergic cells discharged in bursts during waking and REMS, and fell silent during NREMS [38]. However, of BF neurons projecting to the cortex more are GABAergic than cholinergic in the rat [39]. Of those projecting to the wake-regulating lateral hypothalamus, half can be considered GABAergic, about 25% glutamatergic and only 10% cholinergic [40]. In addition to the majority of wake-active and wake+REMS-active neurons, some REMS-active and a few NREMS-active neurons were found in head-restrained rats, and neurons could also vary their

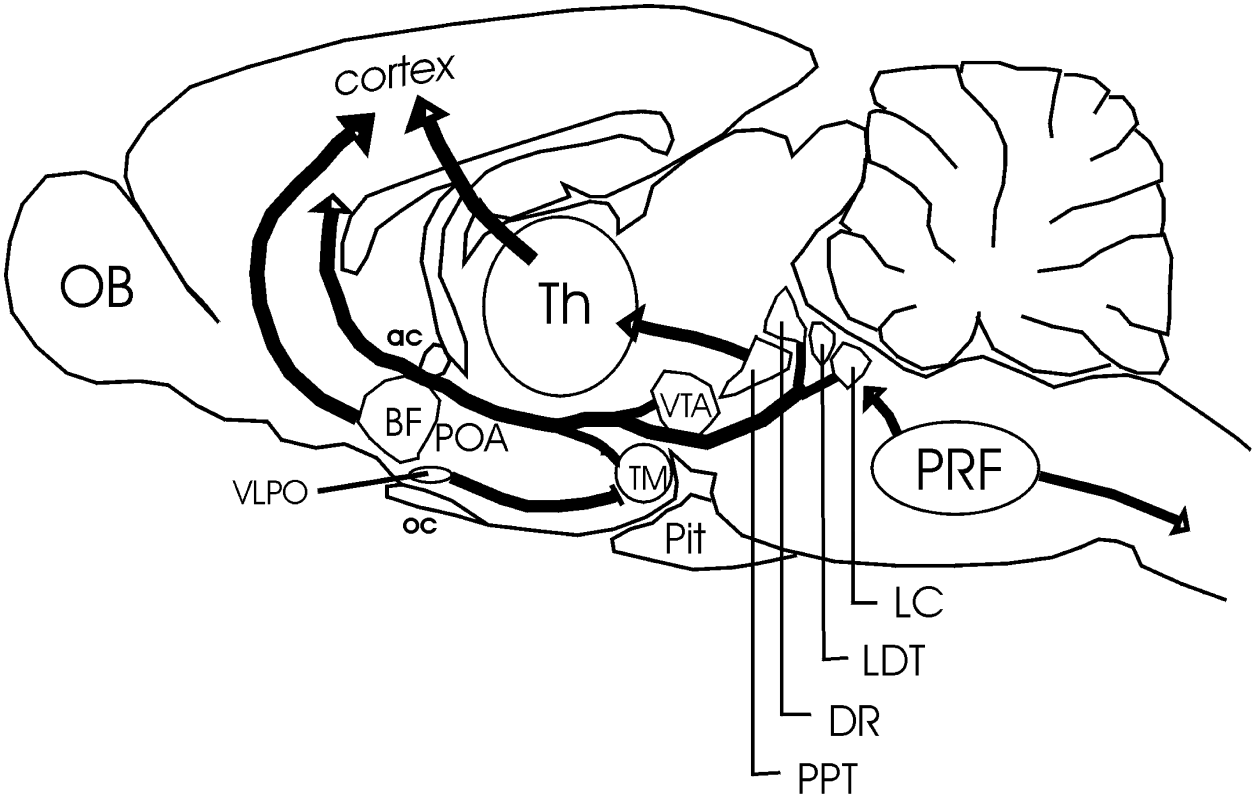

Figure 1. Sleep and wake-related structures in the rat brain. The structures may be more medial or lateral, but are projected onto the same sagittal section. OB = olfactory bulb, Th = thalamus, ac = anterior commissure, oc = optic chiasm, Pit = pituitary gland, BF = basal forebrain, VLPO = ventrolateral preoptic nucleus, POA = preoptic area, TM = tuberomamillary nucleus, PPT = pedunculopontine nucleus, DR = dorsal raphe, LDT = laterodorsal tegmental nucleus, LC = locus coeruleus, PRF = pontine reticular formation. The most important projections are schematically shown.

firing patterns [40]. Recording in freely moving rats showed similar distribution of the state dependency of neurons, and almost half of the neurons were inhibited during 3 h of sleep deprivation (SD), most of these being of wake-active type [41]. Possibly, extended waking leads to inhibition of the wake-promoting BF neurons.

Neurons which express Fos during sleep, and which colocalize GABA and galanin, have been found in the ventrolateral preoptic nucleus (VLPO) [42]. These neurons project to waking centers like the tuberomamillary nucleus (TM) in the posterior hypothalamus, the locus coeruleus and the raphe nuclei [42, 43].

Posterior and lateral hypothalamus

The TM in the posterior hypothalamus contains the only histaminergic neurons of the brain [44, 45]. They receive afferent inputs from the cholinergic BF, from the hypothalamus and from the VLPO. They project diffusely to all brain regions. Neurons in the TM fire in a robust, clocklike rhythm in waking, more slowly in NREMS and are virtually silent in REMS, and as these neurons are also inhibited by histamine auto-receptor (H_3) agonists, they are presumably histaminergic [46].

Neurons scattered in the lateral and mediolateral hypothalamus contain a peptide known as either hypocretin (Hcrt) [47] or orexin (OX) [48]. The peptide is coded by one gene, but expressed as two forms: Hcrt1/OX-A and Hcrt2/OX-B. Hcrt/OX neurons project diffusely in the brain, innervating major waking-promoting systems (see [49, 50, 33]. Inputs are from hypothalamic regions, brainstem, preoptic area, BF and amygdala [51, 52]. Identified orexinergic neurons in head-restrained rats discharged most intensely in aroused waking, and least in REMS [53]. Their intrinsic membrane properties maintain a depolarized state allowing tonic activity [54].

Tegmentopontine nuclei

The tegmentopontine cholinergic nuclei are the laterodorsal tegmental nucleus (LDT, Ch6) and the pedunculopontine nucleus (PPT, Ch5) [34, 55]. LDT is situated medially, between the raphe and the locus coeruleus, whereas PPT is more lateral. The neurons receive input from ascending sensory fibers as well as from neighboring nuclei. Major efferent projections

are to the thalamus, hypothalamus, BF, pontine reticular formation, locus coeruleus and raphe nuclei [55, 56, 57]. Some GABAergic neurons project from the LDT-PPT to the posterior lateral hypothalamus [58]. The discharge activity of most Ch5 and Ch6 neurons is high during wakefulness and REMS, and correlates with EEG signs of cortical activation [59, 60].

Cell groups in the pons and medulla named A1–A7 [61] contain noradrenaline, and the most important for sleep-wake regulation is the locus coeruleus (LC) or A6 (see [62, 33]). LC and A4 project to the thalamus, neocortex, hippocampus and cerebellum, whereas the hypothalamus receives noradrenergic innervation from A1, A2, A5 and A7 [63]. LC neurons receive input which is partly glutamatergic and GABAergic from adjacent areas in the pons. LC neurons fire most intensely in waking, less in NREMS and least in REMS [64], and changes in their firing frequency precede the change in vigilance state [65].

Serotonergic neurons are found in the raphe nuclei in the brainstem midline. Rostral nuclei (B4–B9) project to the forebrain, while caudal nuclei (B1–B3) project to the spinal cord [61]. The dorsal raphe (DR) nucleus (B7) is important for sleep-wake regulation. Like the LC, it receives input from adjacent tegmentopontine areas, and projects widely to the forebrain and limbic system. DR neurons also fire maximally in waking, less in NREMS and are virtually silent in REMS [66]. In cats with brainstem lesions preventing muscle atonia during REMS, DR discharge was not inhibited during REMS, indicating that some waking activity might be secondary to movement [67]. During waking, serotonergic neurons are activated during certain behaviors, like grooming and rhythmic movement, and might serve to limit cortical activation by inhibitory influences on other arousal-promoting neurons [33].

Dopaminergic neurons are found in the substantia nigra (A9), the ventral tegmental area (VTA, A10) and hypothalamic cell groups (A12–A14) [61]. A9 projects to the striatum, and is involved in waking motor activity. A10 projects to the prefrontal, orbital and cingulate cortices, as well as to the amygdala, the nucleus accumbens and other limbic areas ([68], see also [33]). A12–A14 regulate neuroendocrine functions. The activity of presumably dopaminergic neurons in A9 and A10 is not significantly modulated by the sleep-wake cycle [69, 70], but they increase their firing in relation to positively rewarding stimuli (see [33].

Thalamus

The thalamus is the gate for sensory input to the cortex, and so is of paramount importance for vigilance. The excitability of thalamocortical (TC) projection neurons is modulated by innervation from the thalamic reticular nuclear complex (RE), the neurons of which are in turn modulated by input from the LDT/PPT, LC and raphe (see [37, 71]). The thalamocortical network is quite complicated. TC neurons are glutamatergic and convey information to the cortex. RE neurons are GABAergic and inhibit TC neurons. They also influence each other through reciprocal connections. RE neurons are excited primarily by glutamatergic output from the cortex, but also modulated by the aminergic and cholinergic tegmentopontine nuclei. During NREMS, TC neurons are inhibited and incoming sensory messages are blocked from reaching the cortex. Three major types of thalamocortical oscillation occur during NREMS: sleep spindles, delta activity and slow oscillations. Activity bursts can be generated in the isolated RE nucleus when its neurons are hyperpolarized by G-protein-coupled potassium channel activation. *In situ*, these bursts cause rhythmic inhibition of TC neurons, which in turn fire in bursts onto cortical neurons to yield sleep spindle activity of 12–14 Hz frequency. Delta waves (at 1–4 Hz), on the other hand, rely on an intrinsic property of TC neurons to burst rhythmically when hyperpolarized to more than −65 to −70 mV. Cortical neurons also have intrinsic capability of delta frequency burst firing. The slow oscillation (<1 Hz) depends mostly on cortical neurons, and is then conveyed to thalamic RE and TC neurons through glutamatergic output.

Considerable evidence shows that sleep facilitates memory consolidation (see [72]). In fact, cortical responsiveness is maintained during NREMS although sensory input is shut out, and plastic changes in neuronal networks have been found to be enabled during sleep [73].

Imaging findings

Increasingly refined brain-imaging techniques provide information on the location of activity in various behaviors, including sleep-wake states in healthy humans and in sleep disorders (reviewed in [74, 75]), and the findings suggest 'where to look' for specific cellular and molecular changes. Areas deactivated during SWS include the prefrontal, anterior cingulate and temporal cortex, pons and mesencephalon, thalamus, basal ganglia and basal forebrain/hypothalamus [74]. During REMS, activation occurs in the pons, thalamus, amygdala, hippocampus, anterior cingulate cortex and occipital cortex, while the prefrontal cortex remains inactive [74].

Notable decrease of regional metabolic rate, measured by position emission tomography (PET), has been seen after SD in the frontoparietal cortex and in the thalamus, consistently with the known decline in performance in tests depending on prefrontal function [76, 75]. A novel approach to measure metabolic events in the human brain is proton spectroscopy (^{1}H-MRS). Brain lactate is measurably increased by cognitive stimulation, and this increase decreased after SD, indicating malfunction of energy metabolism [77, 78]. Functional brain imaging has also been applied to several sleep disorders (see [75]), but changes may often be secondary to the impaired sleep and not indicative of the location of primary disorder.

Neurochemistry of sleep and wake

Acetylcholine

Observations following local microinjections of acetylcholine into the brain of cats showed that acetylcholine could have opposite effects on sleep and waking in neighboring loci, and led to a theory of two antagonizing cholinergic sleep-regulatory systems: a rostral waking system, and a caudal sleep system [79, 80]. The muscarinic antagonist atropine given systemically to animals caused slow EEG waves while the animals remained awake – a first indication that drugs could cause 'dissociation' between behavior and electrical activity [81]. Acetylcholine release in the cortex was increased during waking [82]. Injecting acetylcholine or muscarinic agonists like carbachol into the pons could induce a REMS-like state with muscle atonia [83], reviewed in [84]. The source of cholinergic innervation was found to be the LDT-PPT [85], and electrical stimulation of the PPT increased acetylcholine release in the cholinoceptive pontine area [86]. The receptors were presumed to be muscarinic autoreceptors on LDT-PPT terminals, probably of the abundant M_2 subtype. *In vivo* microdialysis with various muscarinic receptor antagonist drugs supported this notion [87]. However, other evidence also implicated postsynaptic M_2 receptors. M_2 receptors are also involved in REMS generation in the mouse [88].

REMS-NREMS oscillation depends on the interplay between the cholinergic LDT-PPT and the pontine aminergic nuclei (the noradrenergic LC and the serotonergic DR). The reciprocal discharge rate in pontine RF neurons, the target for cholinergic input, and LC neurons led to a model for control of REMS [89]. Analysis of DR neuronal firing also showed inverse correlation with REMS, indicating a role in the control of REMS [90, 91]. REMS is thus dependent on the balance between cholinergic REM-on and aminergic REM-off activity, with clinical relevance to mood disorders (see [37]). These findings suggested that narcolepsy could result from an imbalance between cholinergic and aminergic brainstem mechanisms [92].

Cholinergic neurons in the BF are important in cortical activation during waking and REMS (see [36,33]). Their discharge activity correlates with gamma and theta EEG frequencies which are typical for waking and REMS, and negatively with delta frequencies. Their stimulation by injection of glutamatergic agonists AMPA and NMDA or noradrenaline induces fast cortical activity, whereas serotonin decreases gamma frequencies. They are also stimulated by histamine and hypocretin/orexin. They can be inhibited by GABA, and by acetylcholine, presumably through muscarinic autoreceptors, while they are excited by nicotinic receptors, which probably are autoreceptors on cholinergic projections from the LDT/PPT.

In vivo microdialysis shows that release of acetylcholine in the cortex is increased during waking and REMS [93], and even higher during REMS than during waking [94]. Acetylcholine probably exerts its effects in the cortex through both nicotinic and muscarinic receptors [95, 33]. Blocking nicotine receptors diminishes cortical activation. Blocking muscarinic receptors with atropine or scopolamine causes slow EEG activity while animals remain active, indicating that the muscarinic projection is more necessary for fast EEG activity than for the waking state as a whole. Degeneration of the cholinergic BF projection to the cortex may be responsible for the EEG slowing seen in patients with Alzheimer's disease even during waking [96].

Noradrenaline

Noradrenaline (NA) has a multitude of effects in the brain, including modulation of vigilance (review in [62]). Systemic administration of amphetamine, which enhances catecholamine release and prevents reuptake, produced arousal in animals, and after depletion of catecholamine synthesis with AMPT (α-methyl-paratyrosine), amphetamine lost this effect [97]. The catecholamine precursor L-DOPA also produced arousal in cats [98], and restored REMS after depletion of catecholamine stores with reserpine (see [99]). If SD in humans was combined with depletion of catecholamines, severe cognitive impairment followed, indicating the increased need for catecholamines to maintain waking and performance during prolonged waking [100].

NA acts postsynaptically on neurons through α1, α2 and β1-receptors. α2-receptors are also found as presynaptic inhibitory autoreceptors. The α2-agonist

clonidine inhibits LC activity [101]. In moderate doses it causes sedation, and in large doses also inhibition of NREMS and REMS [102]. α2-agonists have found clinical application as anesthetics and analgesics, especially in veterinary medicine [103]. α2-antagonists promote waking and counteract the effect of clonidine [102]. NA may promote waking also by inhibiting sleep-promoting GABAergic BF neurons through α2 receptors [104].

Activation of the LC causes EEG arousal [105], and alters sensory and orienting responses in a way to optimize their processing (reviewed in [62]). This includes decrease in spontaneous neuronal activity, while responses to both excitatory and inhibitory synaptic input are enhanced. Changes in LC neuronal activity precede changes in behavioral state [65], and are increased by novel sensory stimuli and by various stressors. Stressors activate the neurons in the paraventricular hypothalamic nucleus, which release CRH at terminals in the LC, increasing LC activity [106]. Mice lacking NA due to inactivation of the dopamine-β-hydroxylase gene fall asleep more rapidly than controls in stressful conditions [107].

NA has an inhibitory role in REMS regulation [89, 37, 72]. The α1/α2-antagonist phentolamine as well as the α1-antagonist prazosin increased REMS in cat [102, 108], raising the suspicion that blocking α1 receptors might aggravate narcoleptic attacks, as has subsequently been shown [109].

Serotonin

Pharmacological and lesion studies first implicated serotonin (5-hydroxytryptamine, 5-HT) as a promoter of sleep. Systemic or icv (intracerebroventiruclar) administration of 5-HT precursors 5-hydroxytryptophan (5-HTP) or L-tryptophan induced sleep in cats [110]. In these experiments, blocking of 5-HT synthesis with parachlorophenylalanine (PCPA) caused long-lasting insomnia, proportional to the reduction in 5-HT. 5-HTP promptly restored 5-HT and sleep. Neurotoxic or electrolytic lesion of the 5-HT-containing raphe nuclei also caused insomnia. However, electrical stimulation of the DR of cats during waking elicited arousal with orienting response, and intense stimulation caused rapid movements and suppressed food intake even in hungry cats [111]. Stimulation during REMS suppressed the PGO (ponto-geniculo-occipital) waves associated with REMS. DR stimulation never induced sleep or slow EEG activity. Local cooling of the DR induced sleep, not wakefulness [112]. Spontaneous neuronal activity was highest during waking, less in NREMS and inhibited in REMS [66]. In certain waking behaviors, like grooming, some DR neurons were maximally active [113]. Taken together, experimental evidence indicated that 5-HT projections from DR served to modulate forebrain activity during waking, but not directly induce sleep. However, 5-HT was proposed to produce a delayed increase in sleep through hypothalamic neuropeptides that are influenced by 5-HT release [114, 115].

Of 5-HT receptors [37, 116], 5-HT_1 and 5-HT_2 types are the ones studied most in relation to vigilance. 5-HT_2 receptors (A, B and C subtypes) activate phospholipase C (PLC), and can be considered excitatory. 5-HT_{2A}Rs are found in the cortex and basal ganglia, and mediate certain behavioral syndromes. 5-HT_{2A}R antagonists have been used in the treatment of schizophrenia. 5-HT_{2B}Rs are found e.g. in hypothalamus and medial amygdala. 5-HT_{2C}Rs (originally named 5-HT_{1C}Rs) are found in the choroid plexus, cortex, limbic areas, basal ganglia and DR. The nonselective 5-HT_{2A}/5-HT_{2C} antagonist ritanserin increases NREMS and has been used as a hypnotic [117, 118]. On the contrary, a specific 5-HT_{2B} antagonist was found to decrease sleep in rats [119] and mice [120], indicating opposite roles for 5-HT_{2A}Rs and 5-HT_{2B}Rs. While 5-HT_{2A} antagonists increased NREMS in wild-type mice, 5-HT_{2A} knockout mice had less NREMS during baseline and no EEG delta rebound after SD [120]. A 5-HT_{2B} antagonist had less effect in 5-HT_{2A} knockouts than in wild-type mice, indicating compensation of 5-HT_{2A}R absence in the knockouts by reduced influence of the opposing 5-HT_{2B}Rs. This illustrates one limitation of constitutional knockout design.

5-HT_1 Rs inhibit adenylyl cyclase and cause hyperpolarization by decreasing K^+ conductance [37, 116]. 5-HT_{1A}Rs mediate postsynaptic inhibition especially in the limbic system, and are inhibitory autoreceptors in the DR nucleus. 5-HT_{1B}Rs are inhibitory autoreceptors in cortex and basal ganglia. REMS is increased in 5-HT_{1A} as well as 5-HT_{1B} knockout mice, and corresponding antagonists increase REMS in wild-type mice, indicating that these receptors have tonic inhibitory control over REMS [121]. 5-HT_{1A} agonist microinjected into the DR reduces local 5-HT concentrations and increases REMS [37, 122, 123]. The uptake inhibitor citalopram, which strongly inhibits REMS [124], failed to exhibit this effect in 5-HT_{1A} knockout mice [125]. 5-HT uptake blockers like fluoxetine are widely used as antidepressants, and it has been pointed out that blocking the 5-HT_{1A} autoreceptors would enhance the synaptic 5-HT levels and the clinical effect, without disturbing sleep.

Histamine

Early H_1 receptor-blocking antihistamines penetrated the blood-brain barrier and induced sedation. H_1Rs are coupled through a G_q protein to PLC, and in most

cases mediate postsynaptic excitation (see [45]). H_2Rs, coupled through G_s to adenylyl cyclase and PKA, mediate more long-lasting potentiation of excitation. H_3-autoreceptors provide inhibitory feedback in the TM nucleus, but H_3Rs are also found on other nerve terminals, controlling the release of various other transmitters. Systemic administration of H_1 agonists or H_3 antagonists promotes wakefulness, while H_1 antagonists, H_3 agonists and synthesis inhibitors promote sleep (review in [126]). The rate of synthesis is limited by the enzyme histidine decarboxylase (HDC), and HDC knockout mice show less arousal and shorter sleep latency in novel environments, compared with wild-type mice [127].

Dopamine

The nigrostriatal dopamine (DA) pathway mediates activation of motor activity, including exploration, which may promote waking and inhibit sleep, although the discharge of nigral neurons is not dependent on vigilance states [70]. The mesocortical and mesolimbic dopaminergic projections from the VTA may influence cortical and limbic structures to modify wakefulness in relation to behavior. Mice lacking the dopamine transporter gene and thus having increased synaptic concentrations of dopamine had threefold waking amounts, were hyperactive and were hypersensitive with caffeine compared to wild-type mice [128]. The effect of dopamine on sleep-wakefulness may often be secondary to influences on motor activity and emotions.

DA exerts its effects in the brain mainly through D_1 and D_2 receptors, which can be divided into subgroups [129]. D_1Rs and D_2Rs have a multitude of cellular actions, and may act in synergy or opposition (e.g. D_1 Rs activate, and D_2Rs inhibit adenylyl cyclase). In addition, they can influence each other. Autoreceptors resemble the D_2 type. In animals, responses to systemic administration of dopamine agonists and antagonists are dose-dependent. For instance, a D_2 agonist at doses blocking autoreceptors promoted NREMS, whereas large doses acting postsynaptically inhibited REMS [130].

Clinical applications for DA-related drugs comprise the restless legs syndrome (RLS) and periodic limb movement disorder (PLMD). In RLS, the patient feels discomfort, even pain in the legs when preparing to go to bed or being immobile for longer periods, while in PLMD sleep is interrupted by violent leg or arm movements, even convulsions. In these conditions, DA agonists of the antiparkinsonian type have been efficient (see [131]). A prominent symptom of narcolepsy is cataplexy (attacks of muscle atonia). D_2-type agonists aggravate the cataplexy in narcoleptic dogs if administered systemically or locally into the substantia nigra/VTA area, suggesting involvement of altered DA transmission (see[132]). However, narcolepsy is primarily a disorder of the hypocretin/orexin system (see below). In patients with the Parkinsonian syndrome (PD), daytime sleepiness is common, and includes a tendency to REMS at sleep onset (a narcoleptic tendency). Dopamine agonist therapy for PD is somehow related to these symptoms [133].

Glutamate and GABA

Glutamate is the most common excitatory transmitter in the brain, and is likely to have the greatest direct impact on neuronal activity. The ascending activating reticular system is probably glutamatergic [33]. Glutamate is the transmitter in pontine networks mediating motor and autonomic activation, in the thalamocortical projection responsible for cortical activation, in intracortical networks and in corticofugal pathways. The role of the other transmitters considered in the regulation of vigilance is primarily to modulate the excitability of neurons.

Gamma-aminobutyric acid (GABA) is the most prominent inhibitory neurotransmitter in the brain. $GABA_A$ is a ionotropic receptor linked to the opening of a Cl^- ion channel, and $GABA_B$ a metabotropic receptor linked to the opening of K^+ channels. The membrane effect of $GABA_A$R activation depends on the chloride equilibrium potential for the cell type in question, which in turn is dependent on the activity of several cation-chloride transporters. The most important is KCl cotransporter KCC2, which is absent from thalamic RE neurons, which thus can be depolarized by GABA instead of hyperpolarized [134]. Reversed postsynaptic potentials between reticular neurons can thus trigger spike bursts and sustain rhythmic activity [135]. In most other structures in the adult brain, $GABA_A$Rs only mediate inhibition.

The ubiquitous localization of GABAergic neurons in the brain implies multiple targets of action [33]. Their role in thalamocortical synchronizations was mentioned above [37]. The sleep-active GABAergic neurons in the VLPO and adjacent BF may have a crucial role in the induction and maintenance of sleep by inhibiting histaminergic and other wake-promoting cell groups [33, 104, 136]. Reciprocal connections between VLPO and other wake-promoting cell groups are apparently important in sleep-wake and wake-sleep transitions. Acetylcholine, noradrenaline and serotonin inhibit the VLPO, but it is insensitive to histamine [137]. Finally, in the DR and LC nuclei, GABA release increases during REMS, indicating that GABAergic inhibition in these nuclei is actively involved in the initiation and maintenance of REMS [138, 139].

The $GABA_A$ receptor complex also binds ethanol and

the benzodiazepine drugs, which act in synergy to GABA. Concerns have been raised about the use of benzodiazepines as hypnotics: rapid aquisition of tolerance with ensuing danger of addiction, altered sleep structure and ataxia. The very short-acting benzodiazepine drugs may also cause amnesia and confusion, especially in elder subjects. To limit side effects, other types of drugs acting on the $GABA_AR$ have been developed, like zopiclone, zolpidem and gaboxadol, and GABA transporter antagonists like tiagabide [140]. Taking into account that GABAergic projection from the VLPO may be a crucial factor in the induction of sleep, successful manipulation of this system could be of extreme importance for the treatment of insomnia.

Hypocretin/orexin

Hcrt/OX actions are mediated by two receptor types (OX_1R and OX_2R), both of which are sensitive to both peptides, although OX_1R is tenfold more sensitive to Hcrt2/OX-B than to Hcrt1/OX-A [48]. Both receptors mediate excitation [48, 47, 141]. Mice with a constitutional absence of the Hcrt/OX gene display sudden attacks of immobility during night-time waking behavior [142]. In dogs with hereditary narcolepsy, a mutation has been found in the OX_2R gene [143]. Autopsy findings from narcoleptic humans showed deficiency in neuron number in the lateral hypothalamus [144], and gliosis in areas with OX_2Rs [49]. In narcolepsy patients, OX-A levels in the CSF are low compared with controls [145]. These findings indicated that the narcolepsy syndrome stems from any deficiency of the Hcrt/OX system. To some extent, Hcrt/OX passes the blood-brain barrier, and repeated administration to narcoleptic dogs was able to prevent cataplectic attacks (see [49].

Physiologically, Hcrt/OX is related to the maintenance of motor activity (including feeding [48]) and to tonic arousal [49]. Hcrt/OX excites numerous cell groups related to waking or motor activation (see [49]). When injected icv, a selective OX_2R agonist increased wakefulness and inhibited sleep in rats [141].

Sleep factors

Research into the neurochemical reasons for deterioration of performance and the need for recovery sleep after prolonged waking started in the early 20^{th} century. CSF [146] or brain homogenate [147] from sleep-deprived dogs caused excess sleep when infused into the CSF of recipient animals. As ultrafiltration or heating destroyed the somnogenic action of CSF, the 'hypnotoxin' was assumed to be a protein [146].

Cytokines

A breakthrough in the identification of sleep factors was the extraction of 'sleep-inducing factor S' from CSF and brain homogenates of goats and sheep [148]. The extract induced excess sleep in rats and rabbits, and was purified and found to be a small peptide, present in the brain at concentrations around 30 pmol/g brain, and somnogenic in picomolar concentrations [149]. Extraction of a similar sleep-inducing substance from large volumes of human urine showed 'factor S' to be a glycopeptide resembling bacterial peptidoglycan [150], later identified as muramyl peptide [151]. Study of the muramyl peptides which induced formation of interleukin-1 (IL-1), an endogenous pyrogen in macrophages, showed that IL-1 also induced sleep, and that its somnogenic and pyrogenic effects were mediated by separate mechanisms[152]. Other cytokines, like tumor necrosis factor alpha (TNFα), interferon alpha-2 and IL-6 were also found to be somnogenic (see [153]). It was apparent that the response of the brain to SD resembled, in many ways, the host defense to microbial infection [153].

Growth hormone axis

Growth hormone is released in pulses mainly during NREMS, and delay, advance or interruption of sleep phase shifts the main GH pulse accordingly [154–156]. SD decreases GH secretion in human and mammals [156, 157]. Systemic administration of GH to animals increases especially REMS [157a], while administration of antiserum to endogenous GH decreased both NREMS and REMS in rats [158].

Pituitary secretion of GH is controlled by two hypothalamic peptides: growth-hormone-releasing hormone (GHRH) and the inhibitory somatostatin (SRIH). The relationship between GHRH and sleep is reviewed in [159]. Icv-injected GHRH dose-dependently promoted NREMS and suppressed REMS in rats and rabbits [160]. Hypophysectomy abolished the effect on REMS, but not that on NREMS, indicating that the latter was not mediated by GH. GHRH antagonist and antibody blocked the effect of GHRH on sleep, and the antibody also blocked the sleep rebound after SD. A GHRH-GH-IGF-deficient dwarf mouse strain has a reduced amount of NREMS [161]. As icv pretreatment with GHRH antibodies also suppressed the NREMS increase caused by IL-1, it was apparent that the sleep-inducing effect of GHRH is at least partly mediated by IL-1. After SD, hypothalamic GHRH messenger RNA (mRNA) was increased, indicating a role in the homeostatic regulation of NREMS [162, 163]. In humans, effects depend on time and administration (see [156]). Repeated boluses of GHRH during early sleep increased NREMS/SWS [164]. Oral administration

of the GHRH analog MK-677 during 7 days resulted in an increase in SWS and also in REMS (see [156]). SRIH inhibits GH secretion and also reciprocally inhibits GHRH neurons. Icv infusion of SRIH increased and pharmacological depletion of SRIH decreased REMS in rats [165]. The SRIH analog octreotide also increased REMS in rats when injected systemically [166, 167], apparently by inhibiting GHRH neurons [168]. Homeostatic involvement in REMS regulation in rats is supported by the increase in SRIH mRNA in the hypothalamus after selective REMS deprivation [157]. Microinjections of SRIH antagonist into the LC decreased REMS, indicating LC as a possible target site [169]. In young humans, intravenous SRIH had only minor effects, but in the elderly, REMS was decreased (see [156]).

PRL, VIP and more

In human, prolactin (PRL) is secreted mainly during the second half of sleep [170], and also has intrinsic circadian rhytmicity [171]. PRL affects neuronal activity in rat hypothalamus [172]. PRL-immunoreactive neurons are found in the lateral hypothalamus, and innervate hypothalamic areas, LC and DR (review in [173]). Injection of PRL systemically or into the hypothalamus of rats and rabbits increased REMS, but in rats only during the light period [173]. Antiserum to PRL suppressed REMS in rats [158], and REMS was reduced in genetically PRL-deficient mice [174].

Vasoactive intestinal peptide (VIP) is one of the PRL-inducing factors. Icv-injected VIP substantially increased REMS in rats, and was able to restore REMS during PCPA-induced insomnia or during REMS suppression by the protein synthesis inhibitor chloramphenicol [175]. VIP also restored REMS to PCPA-treated cats [175], but while it increased baseline REMS in cats, it was unable to counteract the suppression induced by chloramphenicol [176]. REMS increase caused by VIP injection was prevented by pretreatment with PRL antiserum, which indicated that the effect of VIP on REMS is mediated by PRL (see [173]). In humans, repeated intravenous (iv) VIP boluses stimulated REMS only if the dose was high enough to stimulate PRL [177].

Stress can impair sleep through the sympathetic nervous system, but also through CRH, which promotes waking (see [178, 179]). Both the GH and the HPA axis affect glucose metabolism. Glucose tolerance is adversely affected by SD in healthy humans [180]. Leptin levels are inversely related to sleep duration, and ghrelin levels increased by SD, leading also to an increase in appetite [181]. Sleep is fragmented and SD recovery diminished in leptin-deficient *ob/ob* mice, pointing to a relationship between sleep disruption and metabolic disruption [182]. The effect of exogenous leptin on sleep has not been tested. Ghrelin stimulates GHRH release (see [183]). While nocturnal bolus injections of ghrelin increased NREMS in humans [184], feeding took precedence over sleep in ghrelin-stimulated rats, possible because ghrelin promotes waking by stimulating the Hcrt/OX system or the HPA axis [183].

Adenosine

The hypnogenic properties of adenosine (ADE) were first recognized when it was found that icv injections of ADE induced sleep-like behavior in the cat [185]. Further research showed that systemic administration of ADE, its analogs or inhibitors of its metabolism increase especially NREMS in rodents [186]. The most widely used psychoactive stimulant, caffeine, is an ADE receptor antagonist, and while ADE affects cell function through several mechanisms, only the membrane receptors are affected at physiological concentrations, indicating receptor blockade as the main action [187].

Membrane receptors for ADE are G-protein-coupled, and divided into classes A_1, A_{2A}, A_{2B} and A_3 (for reviews see [188–190]. A_1Rs (and A_3Rs) inhibit adenylyl cyclase, while A_2Rs have the opposite effect. Postsynaptic A_1Rs are coupled to stimulation of K+ channels, resulting in hyperpolarization and inhibition of neural activity [191, 192]. Another target for A_1Rs is phospholipase C, which leads to mobilization of intracellular Ca^{++} [193]. A_1Rs are widely distributed in the central-nervous system (CNS), and inhibit especially cholinergic neurons from the BF and LDT/PPT [37, 192]. Locally increased ADE concentrations could promote sleep by inhibiting the cholinergic neurons [36, 37]. This does not exclude the possibility that ADE also presynaptically inhibits GABAergic inputs on sleep-active neurons in the VLPO [194, 195]. The excitatory A_{2A}Rs have more restricted distribution, comprising the striatum, and at least in rodents also the leptomeninges [196]. Presynaptic A_{2A} Rs can enhance the release of other excitatory transmitters. They can also enhance ADE uptake into neurons and thus modulate the extracellular availability of ADE [197].

The metabolism of ADE is linked to energy metabolism, which is relevant to the relation between neuronal activity and sleep need [198]. When ATP is broken down, excess ADE is transported to the extracellular space by equilibrative nucleoside transporters (ENTs) [190, 199, 200]. Under conditions of adequate supply of oxygen and glucose, ADE kinase is the most effective regulator of intracellular ADE levels, and prevents extracellular concentrations from rising [201]. When energy is depleted, ADE deami-

nase becomes important in regulating extracellular ADE concentrations [202]. ADE can also be formed extracellularly from ATP [203]. The extracellular concentration of ADE is also influenced by equilibrative and concentrating transporters (ENTs and CNTs) [204]. Nitrobenzylthioinosine (NBTI aka NBMPR), which blocks the more sensitive type of ENT (ENT1), increases extracellular ADE concentrations in vivo [8], which indicates that under normal conditions there is a net uptake of ADE into cells due to an inward concentration gradient.

The case for ADE as a sleep factor has been reviewed in [37, 205, 206]. ADE infused by *in vivo* microdialysis into the BF of cats increased sleep [207]. Extracellular ADE levels in various brain areas in the cat were about 20% higher during spontaneous wakefulness compared with sleep [8]. However, when waking was prolonged to 6 h by SD, adenosine levels in the BF increased to 200% of baseline, and were restored only during recovery sleep. Infusion of NBTI increased ADE similarly to the SD and also resulted in recovery sleep. These findings formed solid evidence for ADE as a homeostatically sleep-regulating substance with a special role in recovery sleep, and with a specific action in the BF, possibly on its cholinergic, wake-promoting neurons [8]. In the cortex, ADE initially increased during SD. But in all other tested brain regions, ADE did not rise [208]. Corresponding changes were found in rats [209, 210]. Microdialysis infusion of ADE into the BF of rats and cats decreases the discharge rate of wake-related neurons [211, 212].

Why does extracellular ADE increase in the BF during prolonged waking? Possibilities include increased intracellular breakdown, inhibition of ADE kinase, ADE deaminase or S-adenosyl-homocysteine hydrolase (SAH hydrolase), decreased transporter activity or increased extracellular formation of adenosine. Systemic injection of the selective inhibitor of ADE kinase ABT-702 increased SWS and decreased REMS in rats [213]. Quantitative trait loci analyses in mice showed that the accumulation of slow EEG waves during wakefulness (indicating increased sleep pressure) was related to a genomic region containing the genes of ADE deaminase and SAH hydrolase [214]. Most variants of ADE deaminase in the human genome are related to severe immunodeficiency, but the most frequent asymptomatic one (G-to-A transition at nucleotide 22) is related to more SWS and fewer awakenings than in persons with the majority isoform [215]. However, during experimental SD in rat, no changes were found in the activity of ADE kinase, ecto- and endonucleotidases [216] or ADE deaminase [217]. Decreased ENT1 activity should increase extracellular ADE like NBTI does [218]. In the rat, 6 h of SD decreased NBTI binding in BF, which might mean decreased ENT1 activity [219]. As inhibition of extracellular AMP hydrolysis does not significantly affect ADE levels [220], the main source of ADE is probably intracellular, and possibly related to energy consumption. Experimental energy depletion by infusion of dinitrophenol (DNP) into the BF dose-dependently increased ADE, pyruvate and lactate levels, and subsequently caused excess sleep, similar to the effect of SD [210]. Infusion of DNP outside the cholinergic BF area had the same local metabolic consequences, but there was no subsequent increase in sleep, which constitutes evidence that SD causes recovery sleep by local energy depletion in the BF, resulting in accumulation of ADE.

Nitric oxide (NO) can inhibit neuronal energy production, and NO donors cause large increases in extracellular ADE in neuron cultures [220]. NO concentrations in brain undergo state-dependent modulation during the sleep-wake cycle [221,222]. Inhibitors of NO synthase decrease spontaneous sleep [223], while NO donors have the opposite effect [224]. Bilateral application of the NO synthase inhibitor L-NAME into the lateral forebrain reduced NREMS [225], and microdialysis infusion of NO donor into BF increased ADE and caused subsequent sleep increase [226]. Of the three NO synthases, neuronal (nNOS), endothelial (eNOS) and inducible (iNOS), iNOS is normally present in brain only in trace amounts [227], and is usually activated only by immunological or stressful challenge. In recent experiments from our laboratory, SD of rats caused induction of iNOS specifically in the BF [9]. Specific inhibition of iNOS completely abolished recovery of NREMS. On the contrary, inhibition of nNOS inhibited only REMS recovery and not that of NREMS. Without previous SD, nNOS inhibition slightly reduced both NREMS and REMS, while iNOS inhibition was without effect on NREMS, indicating that induction of iNOS occurs only after SD, and not in the spontaneous sleep-wake cycle. In all these experiments [9, 226], accumulation of ADE in the BF area was a prerequisite for recovery sleep to occur. A recent study showing that in nNOS knockout mice spontaneous REMS was decreased, but in the iNOS knockouts REMS was increased, also indicates that nNOS is involved primarily in REMS production [228].

An interesting question is how the hypnogenic effect of ADE is accomplished. Experimental evidence indicates involvement of at least A_1Rs [37, 206]. Infusion into BF of A_1R antagonist decreased sleep, whereas drugs acting on A_{2A}Rs were inefficient in this area. Microdialysis of A_1R agonist into BF decreased, and A_1R antagonist increased the firing frequency of wake-active neurons [212], and antisense oligonucleotides to A_1R mRNA reduced NREMS and its

recovery after SD [229]. Prolonged SD upregulated A_1R mRNA, but not that of $A_{2A}Rs$ [230]. ADE can activate PLC in the cell membrane, causing a rise in intracellular Ca^{++} [193], and this seems to be limited to cholinergic neurons [37]. This can induce translocation of the transcription factor NF-κB, which in turn can induce A_1R expression, which would maintain A_1R-mediated signalling efficiency during prolonged SD [37, 205]. The site specificity of the A_1R-mediated hypnogenic effect to the BF implies inhibition of wake-maintaining neurons in this area, which are considered to mainly be cholinergic [36, 37].

However, in A_1R knockout mice, spontaneous sleep/wake patterns and recovery after SD were not different from wild-type controls [231]. Icv A_1R agonist was ineffective in $A_{2A}R$ knockout and wild-type mice, while $A_{2A}R$ agonists increased sleep in the wild-type [232]. $A_{2A}Rs$ are abundant in the leptomeninges of rodents [196]. Infusion of $A_{2A}R$ agonists into the subarachnoid space close to the BF dose-dependently increase NREMS and REMS, whereas A_1R agonists inhibited sleep [233]. In the adjacent VLPO, where GABAergic sleep-active neurons have been found [136], Fos was expressed after subarachnoid infusion of $A_{2A}R$ agonist [234], while GABA release was increased in the histaminergic TM nucleus, indicating that $A_{2A}R$ activation can cause GABAergic inhibition of at least the histaminergic waking system [235]. Study of VLPO slices indicated that ADE could block inhibitory GABAergic input to this area, thus disinhibiting sleep-active VLPO neurons [195]; however, $A_{2A}R$ activation can also directly excite one type of VLPO neurons [236]. The same leptomeningeal area surrounding the BF is also the target for the hypnogenic action of prostaglandin D2 (PGD2) [233, 237]. Local infusion of PGD2 into this subarachnoid space increased local ADE, and the hypnogenic effect could be antagonized by an $A_{2A}R$ antagonist. In $A_{2A}R$ knockout mice, the hypnogenic effect of the PGD2 infusion was reduced to 40%, and recovery sleep after SD was inhibited [232]. There is thus evidence for involvement of both A_1Rs and $A_{2A}Rs$ in the hypnogenic effect of ADE, but through different brain areas. It is possible that the A_1Rs in the BF are more involved in normal homeostasis, and the $A_{2A}Rs$ in sleepiness caused by leptomeningeal inflammation [37].

Genetics of sleep

A complex behavior like sleep must depend on numerous proteins produced by hundreds of genes. Although changes in protein composition and activity may occur despite no previous change in gene transcription, it is safe to assume that mutations or transcriptional changes in key genes should affect sleep. Studying genes might reveal facts about sleep function, reasons for individual differences and clues to sleep disorders. A number of recent review articles illustrate the current state of this rapidly developing field [238–243].

From genotype to phenotype

One strategy is to try to identify genes that change expression as a function of sleep-wake state ('molecular genetics'), especially as a function of SD and recovery sleep. Another aspect is to test the effect of modifying a gene on the sleep phenotype by disruption, induced overexpression or antisense technology ('reverse genetics').

The first targeted approaches consisted in comparing the gene expression (measuring mRNA) of enzymes or receptors related to neurotransmission between spontaneously waking and sleeping, or sleep-deprived and recovery sleeping animals. Increased expression of immediate-early genes (IEGs) like *c-fos* and *NGFI-A* in brain during SD indicated that a large number of target genes might be affected [244]. For instance, REMS deprivation induced tyrosine hydroxylase expression in the LC of rats [245]. Increasingly large-scale random searches have subsequently been made using the techniques of subtraction library, differential display and complementary DNA (cDNA) microarrays. Screenings of rat and *Drosophila* genomes have revealed interesting similarities, indicating highly conserved sleep regulation and brain function during rest/sleep [17].

Identified waking-related target genes fall into several distinct groups (extensive lists in [239, 241, 246]). In addition to IEGs (immediate early genes), these comprise target genes related to transmitter systems, neuropeptides, energy metabolism, heat shock proteins and chaperones, plasticity-related genes and others. Many genes related to long-term memory or plasticity are upregulated during waking. The expression of several of these is dependent on noradrenergic innervation, and impaired by chemical denervation with DSP-4 [247]. NA released during waking is thus an essential component in synaptic plasticity and the formation of memory. Fewer genes have been found to be expressed selectively during sleep. Sleep-related genes include components of the translational machinery and genes involved in the formation of long-term memory.

Manipulation of genes to change the function ('reverse genetics') has been used in experimental sleep research (extensive review in [238]). Prion protein knockout mice showed loss of circadian rhythm,

fragmentation of sleep, and a stronger rebound after SD than wild-type mice [248], a follow-up of the finding that fatal familial insomnia is due to a point mutation of the prion gene [249]. Transgenic mice oversecreting GHRH showed selective suppression of NREMS [161], part of the evidence that GHRH regulates NREMS [159]. Numerous studies have been made using constitutional knockouts of various transmitter receptors, and diverse cytokines. Some examples have been given in the previous paragraphs. A problem with constitutional knockouts is the functional compensation that takes place during development. Conditional knockout techniques which avoid this will evidently be useful in the future.

Antisense targeting of mRNAs, finally, has been used in a few sleep studies (some mentioned in [238]). Thus nonviral DNA transfer of antisense was used to change 5-HT tranporter function and to investigate subsequent changes in 5-HT receptor function in rats [250]. Microdialysis perfusion of A_1R antisense nucleotide into the BF of rats decreased waking, supporting the notion that in the BF, adenosine induces sleep through A_1Rs [229]. Icv administration to rats of antisense to CRH decreased waking in the dark period, in agreement with the notion that CRH is involved in promotion of waking [178].

From phenotype to genotype

A different strategy is to find genes that underly individual traits and differences in sleep as well as sleep disorders, by trying to identify the underlying mutations or polymorphisms ('forward genetics'). The reader is referred to the article by Kimura and Winkelmann in this issue [251].

For a genome-wide search, quantitative trait loci (QTL) analysis and mutagenesis in animals have been applied (see [13, 238, 240]). Loci have thus been determined in mice for amounts of NREMS and REMS, for homeostatic responses to SD and for theta rhythm [13, 238]. In narcoleptic dogs, QTL analysis made it possible to map and identify the responsible OX2 receptor gene [252]. The technique of chemical mutagenesis, while successful in circadian research in mouse, will probably be of more utility in sleep research when applied to non-mammalian organisms (fruit flies, zebra fish).

Proteomics and sleep

Recently, an evaluation of proteomic changes in the brain after SD was made [253]. In the BF, screening of 969 proteins showed 89 that were differentially produced in wake and sleep. Eleven of these proteins were cytoskeletal or synaptic proteins, further illustrating the relationship between sleep and synaptic plasticity leading to improved memory traces (for review, see [254]). Tubulin and GAP43 showed post-translational modification after 6 h SD.

Summary

Wakefulness is maintained through the action of several brain areas and their specific transmitters, acting in parallel. On the other hand, while sleep is a vitally important behavior, its induction is apparently dependent on a circumscribed mechanism in the preoptic/basal forebrain area, controlling the waking centers through GABAergic output. Extracellular adenosine release due to local energy depletion in the basal forebrain is a prerequisite for recovery sleep after periods of sleep deprivation. One triggering factor may well be nitric oxide, released locally in the BF due to activation of iNOS, forming a parallel between the response of the brain to infection and its response to sleep deprivation. Cellular, molecular and proteomic studies are expected to shed more light on the functions of sleep and the mechanisms leading to performance impairments during sleep loss.

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