

The waking brain: an update

Jian-Sheng Lin · Christelle Anacleit ·
Olga A. Sergeeva · Helmut L. Haas

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Abstract Wakefulness and consciousness depend on perturbation of the cortical soliloquy. Ascending activation of the cerebral cortex is characteristic for both waking and paradoxical (REM) sleep. These evolutionary conserved activating systems build a network in the brainstem, mid-brain, and diencephalon that contains the neurotransmitters and neuromodulators glutamate, histamine, acetylcholine, the catecholamines, serotonin, and some neuropeptides orchestrating the different behavioral states. Inhibition of these waking systems by GABAergic neurons allows sleep. Over the past decades, a prominent role became evident for the histaminergic and the orexinergic neurons as a hypothalamic waking center.

Keywords Wake · Sleep · Cortical activation · Histamine · Orexin

Activation of the cerebral cortex

The cerebral cortex is active day and night, but we are not always aware of its activity. During slow wave sleep, the electroencephalogram (EEG) is dominated by high-voltage δ -waves (0.5–3 Hz) indicating a high degree of cortical inactivation or synchronization. Consciousness depends on

external perturbation that causes a radical change in the cortical mode of function visible in the EEG as cortical activation or desynchronization, with low voltage and fast frequency (mainly β and γ , 20 and 60 Hz). This is achieved by ascending afferents leading to cortical activation during waking or paradoxical sleep (synonym REM sleep). Both of these behavioral states are conscious though in different ways.

Moruzzi and Magoun [1] demonstrated cortical arousal in the cat by stimulating and lesioning the brain stem reticular formation and formulated the concept of the ascending reticular activating system (ARAS), that reaches the cortex through the non-specific thalamus, the medial and intralaminar nuclei, as well as through extrathalamic pathways. During the following decades, stimulations, lesions, and brain transections in combination with electrophysiological recordings (from EEG to single cells) have been used to determine the structures involved in the regulation of sleep and waking. Acute preparations of high brainstem transection [2] or isolated forebrain display continuous slow synchronous high-amplitude activity, similar to that seen during deep slow wave sleep. These studies led to the conclusion that the cerebral cortex does not possess an intrinsic mechanism for its own activation and have identified four brain regions that can activate the cortex: (1) the thalamus, medial and intralaminar nuclei; (2) the basal forebrain (substantia innominata and adjacent areas); (3) the monoaminergic nuclei of the brainstem; (4) the posterior hypothalamus.

The hypothalamus, though long suspected to play a role in sleep-waking regulation, has been relatively neglected in the past and will be treated with preference here in the network formed by the four regions listed above. The thalamus, basal forebrain, and brainstem have been extensively reviewed in this context [3–11].

J.-S. Lin · C. Anacleit
INSERM-U628, Integrative Physiology of Brain Arousal
Systems, Claude Bernard University, 69373 Lyon, France

O. A. Sergeeva · H. L. Haas (✉)
Department of Neurophysiology, Heinrich-Heine-University
Düsseldorf, POB 101007, 40001 Düsseldorf, Germany
e-mail: haas@uni-duesseldorf.de

The ascending reticular activating system

The ARAS-concept [1] (Fig. 1) has been supported and complemented, especially at the cellular and electrophysiological levels, mainly by Steriade and co-workers [5, 6, 9, 12]. The excitatory inputs to the thalamus and other subcortical relay structures include cholinergic neurons of the mesopontine tegmentum, aminergic neurons in the brainstem and hypothalamus, and glutamatergic neurons located in the large brainstem reticular core [4, 8, 13, 14]. The reticulothalamocortical pathway is not the only system involved, however, as cortical EEG desynchronization can reappear following extensive destruction of the mesencephalic reticular formation [15] or its thalamic relay [16–18] indicating the existence of extrathalamic systems, capable of activating the cortex, that have drawn more attention recently: the magnocellular substantia innominata, the adjacent basal forebrain and the cholinergic and GABAergic corticopetal neurons [6–8], as well as the posterior hypothalamus with the histamine and orexin systems [19, 20].

The basal forebrain

Cholinergic neurons of the basal forebrain discharge tonically during both wakefulness and paradoxical sleep [6, 18]. They can excite cortical neurons directly and suppress the thalamic reticular nucleus oscillation generating the

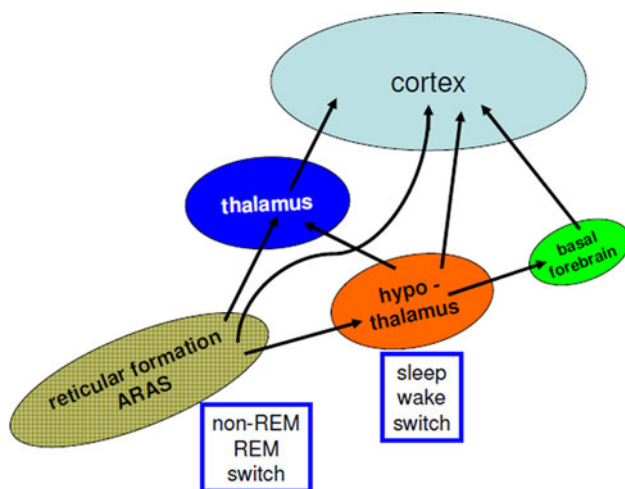


Fig. 1 Ascending activation of the cortex. The ascending reticular activation system (ARAS) reaches the cortex through a ventral pathway (hypothalamus, basal forebrain), through the aminergic nuclei (containing catecholamines, acetylcholine, and serotonin) and a dorsal pathway, the thalamic relay. Switching between paradoxical sleep (REM-sleep) and slow wave sleep occurs in the reticular formation, whereas the switch between sleep and waking lies in the hypothalamus

cortical spindles and drowsiness or light slow wave sleep [21]. In keeping with this, electrical stimulation of certain basal forebrain sites elicits cortical acetylcholine release and cortical desynchronization, while chemical inactivation or unilateral lesion in the basal forebrain cholinergic zone decrease cortical fast rhythms and increase slow activity [18, 22, 23]. Like thalamocortical neurons, basal forebrain cholinergic neurons can relay excitation (e.g., from glutamatergic, noradrenergic, and histaminergic neurons) from the lower brain reticular structures to the cortex [7, 10, 24]. GABAergic ascending neurons in the basal forebrain also project to the cortex [7] and might act in synergy with the cholinergic neurons in cortical activation, likely by ascending disinhibition, since they largely innervate inhibitory cortical neurons [25]. Thus, there is little doubt that the substantia innominata and the adjacent basal forebrain as a whole, including cholinergic, GABAergic, and perhaps further, non-identified neurons, play an important role in cortical activation both during waking and paradoxical sleep and in the modulation of different cortical rhythmic activities. However, the basal forebrain is not indispensable for the long-term maintenance of fast, low-voltage cortical activity, since, in the cat, extensive destruction of the basal forebrain, including the adjacent lateral preoptic areas, does not abolish cortical activation [26]. Ibotenic acid lesioning of the cholinergic zone within the basal forebrain results in a transitory reduction in waking lasting 1–2 days, after which the sleep-wake cycle returns to the pre-lesioning level [27].

Monoaminergic systems

An intense interest in the diffuse ascending projections from the brainstem monoaminergic neurons arose in the 1960s from the histochemical demonstration of their locations and projections [28] and the pharmacological intervention on monoaminergic transmission in major psychiatric disorders, schizophrenia, and depression. These diseases include disturbed sleep-waking regulation. Inhibition of catecholamine synthesis results in decreased waking and behavioral somnolence. Moreover, psychostimulants, such as amphetamine or cocaine, lead to an accumulation of catecholamines, causing a waking state and behavioral excitation.

Noradrenergic and serotonergic (but not most of the dopaminergic) neurons discharge tonically during waking, decrease their activity during slow wave sleep, and cease firing during paradoxical sleep [5, 9, 29–31]. In mice, locus coeruleus noradrenergic neurons show the earliest activation at wake onset among the known waking systems (137). In the cat, lesioning of the ventral tegmental area and the substantia nigra, containing dopaminergic ascending

neurons, induces behavioral unresponsiveness and akinesia, but is not associated with loss of cortical activation [32].

A group of diffusely projecting dopaminergic cells in the ventral periaqueductal grey matter expresses *c-fos* after natural or forced wakefulness and their lesioning results in increased sleep. These cells may provide the long-sought ascending dopaminergic activation [33]. Large lesions of the mediodorsal tegmentum, including the locus coeruleus and the ascending noradrenergic pathway, impair waking [11], while electrolytic or chemical lesioning confined to the locus coeruleus region [34] or selective DSP-4 lesioning of locus coeruleus ascending projections to the cortex [35] does not cause a major deficit of waking. Ascending dopaminergic neurons, as a whole, can thus play an important role in waking, notably locomotion, motivation and cognitive activities, whereas the brainstem noradrenergic neurons (groups A1–A7, including neurons in the locus coeruleus) are permissive in paradoxical sleep, and modulate behavioral and cortical arousal, as well as qualitative and cognitive aspects of waking, such as perception [5, 8, 11, 14, 29, 31, 36] or the expression of immediate early genes [37]. Although dorsal raphe serotonergic neurons possess widespread ascending projections to the cortex and discharge tonically during waking [5, 30], and although serotonin may participate in cortical activation when associated with acetylcholine [38], pharmacological depletion of serotonin using parachlorophenylalanine or lesioning/inactivation of the dorsal raphe nucleus results in insomnia [39]. The postsynaptic action of serotonin on many neuronal targets is dominated by the strong inhibition through 5-HT 1A receptors, while the waking amines, such as noradrenaline or histamine display predominantly excitatory or excitation potentiating actions [7, 8, 10, 14, 40, 41]. A modulation of the sleep-wake cycle by serotonin is evident but it seems not directly and centrally involved in cortical activation and waking (Fig. 2).

The posterior hypothalamus is a waking center

The posterior hypothalamus is a heterogeneous structure made up of different neuronal populations containing diverse neurotransmitters (histamine, dopamine, glutamate, GABA) and neuropeptides [orexins, melanin concentrating hormone (MCH), galanin, enkephalins, substance P, thyrotropin releasing hormone (TRH)]. This region has extensive reciprocal anatomical connections with many brain regions, notably those involved in sleep-wake control such as the cortex, thalamus, preoptic-anterior hypothalamus and other forebrain structures, the brainstem cholinergic, and monoaminergic nuclei [42–45].

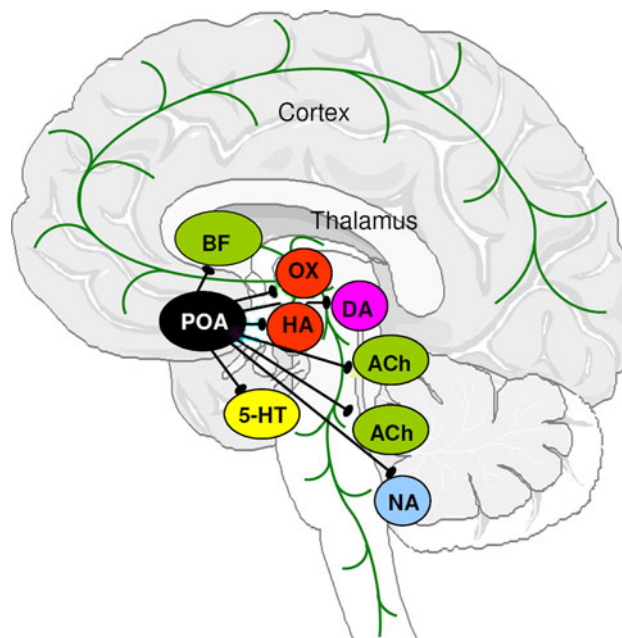


Fig. 2 The waking brain. Schematic localization of the brain's waking systems and their inhibition. NA (noradrenaline, locus coeruleus); ACh (acetylcholine, pedunculo-pontine nucleus, PPN; lateral dorsal tegmentum, LDT; basal forebrain, BF); DA (dopamine, periaqueductal grey, PAG), 5-HT (serotonin, dorsal raphe, DR); HA (histamine, tuberomammillary nucleus, TMN), OX (orexins, periventricular nucleus, PVN); POA: preoptic area, GABAergic neurons which inhibit all the waking systems, *black lines* indicate inhibitory pathways

The posterior hypothalamus has only recently been recognized as a major waking center in spite of early indications (Fig. 2). After the influenza epidemic of 1918, von Economo identified hypothalamic lesions in the anterior or in the posterior hypothalamus correlating with insomnia and hypersomnia (encephalitis lethargica [46]). Subsequent studies in cats, monkeys, and rats have confirmed that electrolytic lesioning of the posterior hypothalamus causes somnolence, hypersomnia, or coma [4]. Nauta defined a waking center in the posterior hypothalamus and a sleep center in the preoptic/anterior hypothalamus on the basis of lesion studies in the rat. He suggested a reciprocal interaction between these two hypothalamic centers in the alternation of sleep and wakefulness [47]. The posterior hypothalamus as a waking center is also supported by the fact that electrical stimulation of this region in the normal [48] or mesencephalic transected [22] cat causes EEG desynchronization.

This role of the posterior hypothalamus has recently received increasing interest with the identification of widespread hypothalamo-cortical projection systems [49–51] and electrophysiological studies revealing several types of neurons, discharging with neocortical activation [42, 52–54], suggesting a source for driving cortical arousal.

Sakai et al. [55] have identified three types of tonic unitary activity in the cat: type-I neurons, discharging during waking and paradoxical sleep, and type-II neurons with a significantly higher discharge rate during paradoxical sleep than during waking and slow wave sleep. Both patterns are encountered diffusely in the posterior hypothalamus. Type-III neurons displaying paradoxical sleep-off or waking-specific discharge have been identified in the tuberomammillary nucleus and the ventrolateral area of the posterior hypothalamus. Thus, the posterior hypothalamus, like the thalamus and the basal forebrain, represents a major component of the ascending activating system.

As electrical lesions [4] destroy not only cellular somata but also fibers en passage, more recent studies [15, 56] have used chemical agents such as excitatory amino acids (kainic or ibotenic acid) to induce selective cell death following over-excitation of neurons. Cellular destruction, under anesthesia, of large areas in the cat posterior hypothalamus including the most caudal part and the hypothalamo-mesencephalic junction produces hypersomnia including both paradoxical sleep and slow wave sleep lasting 1–2 days, accompanied by narcoleptic episodes, i.e., direct onsets of paradoxical sleep from waking (sleep onset REM); while lesions restricted to the rostral part of the posterior hypothalamus, sparing the hypothalamo-mesencephalic junction produce a significant decrease in waking and an increase in slow wave sleep lasting for 1–3 weeks.

Muscimol (GABA_A-receptor agonist) injections can acutely inactivate different hypothalamic loci and deliver functional information on their role in sleep-wake states. In normal freely moving animals, muscimol microinjection into the preoptic/anterior hypothalamus or the hypothalamo-mesencephalic junction provokes increased waking and hyperactivity. In sharp contrast, the same injection in the rostral and middle parts of the posterior hypothalamus induces a pronounced and long-lasting increase in deep slow wave sleep, accompanied by a reduction in, or suppression of, paradoxical sleep. When the injection is performed in the caudal part, the increase in deep slow wave sleep is followed by an increase either in waking or paradoxical sleep, depending upon the exact injection site. In the latter case, paradoxical sleep can even occur directly from waking as narcolepsy (sleep onset REM) [57].

The rostral and middle parts of the posterior hypothalamus, so far the sole brain region associated with such a pronounced hypersomnia after inactivation by muscimol, are therefore the main hypothalamic waking territory. Under physiological conditions, this region must be inactivated to allow the appearance and maintenance of sleep likely by the local release of GABA that inhibits the wake on neurons. A selective increase in GABA during slow wave sleep is indeed seen in the cat posterior hypothalamus [58].

Further support for the central role of the posterior hypothalamus in the maintenance of waking comes from a number of observations in insomniac cats: insomnia caused by inhibiting the synthesis of serotonin by para-chlorophenylalanine is reversed by muscimol injection in the TM and adjacent areas with restoration of slow wave sleep and paradoxical sleep with short latency [57]. Similarly, lesioning of the preoptic and anterior hypothalamus results in long-lasting insomnia and hyperthermia, both effects being reversed by muscimol microinjection into the TM and adjacent areas with restoration of both slow wave sleep and paradoxical sleep [59].

During the long-lasting and total waking state following the enhancement of dopaminergic transmission by amphetamine, slow wave sleep (but not paradoxical sleep) is restored at short latency by microinjection of muscimol into the TM area. The wake-promoting drug modafinil, which causes long-lasting quiet waking without behavioral activation, acts through dopamine D2 receptors in the ventral tegmental area [60] and other arousal systems but not in TM histamine neurons. This waking state is reversed by local injection of muscimol [19, 61, 62]. The histamine neurons display an unusually low sensitivity to D1 and D2R agonists but are highly sensitive to L-Dopa, which they can take up and convert to dopamine [63].

Thus, inactivation of the posterior hypothalamus induces hypersomnia in normal cats and restores sleep in various models of insomnia, suggesting a key role of this region in the maintenance of cortical activation and the waking state. Posterior hypothalamic neurons are likely in a state of hyperactivity during insomnia and offer themselves as targets for medication. Nelson et al. [64] reproduced the muscimol microinjection and also injected gabazine in the rat posterior hypothalamus TMN which antagonized propofol anesthesia and loss of the righting reflex. They attribute a key role to this region for the action of GABAergic anesthetics. The posterior hypothalamus also controls sympathetic and behavioral functions, such as thermoregulation, cardiovascular and respiratory regulation, locomotion, emotional reactions, and feeding behaviors [4, 65–68]. It therefore seems likely that, during waking, cortical EEG activity and the concomitant behavioral signs of arousal are coordinated at the level of the posterior hypothalamus, thus organizing an integral functional activation of the brain.

Neuronal substrates involved in arousal in the posterior hypothalamus

The posterior hypothalamus contains different categories of neurons: those involved in the control of cortical activation and waking display arborizing projections, allowing

the modulation of large brain areas. The dopaminergic A11 group has massive hypothalamic projections and sends fibers to the mesopontine tegmentum [69], which plays an important role in the cortical activation during waking and paradoxical sleep [5, 9, 21, 70]. Muscimol microinjection in the dorsolateral and perifornical regions, which, in the cat, contain both type-I and type-II tonic neurons [55] induces, with a certain latency, continuous deep slow wave sleep accompanied by suppression of paradoxical sleep [57]. These populations, including orexin and MCH neurons, send out widespread ascending and descending projections [51].

Experimental data obtained from our laboratories as well as the results from other groups suggest a major role of the histaminergic neurons located in the tuberomammillary nucleus and ventrolateral part of the posterior hypothalamus in waking. The sedation caused by classical antihistamines (H1-receptor antagonists) has long been known as an undesirable side-effect in the treatment of allergy [71]. Only after histamine was recognized as a transmitter in the brain [72–77] a block of histaminergic transmission was made responsible for the drowsiness caused by antihistamines [78] and many drugs used in the treatment of neuropsychiatric diseases that bind to the H1-receptors.

The histaminergic system in brain

Histamine is synthesized from histidine by histidine-decarboxylase; its levels in the brain measured by microdialysis display a circadian rhythmicity in accordance with the firing of histamine neurons during waking [79]. Extracellular histamine levels in the preoptic/anterior hypothalamus follow the oscillations of different sleep stages (wakefulness > non-REM sleep > REM sleep). Sleep deprivation does not affect histamine levels, suggesting the relay of circadian rather than homeostatic sleep drive [80]. Philippu and Prast [81] have demonstrated a direct correlation between histamine levels in the hypothalamus and behavioral state by electroencephalography. Synthesis and release of histamine are controlled by feedback through H3-autoreceptors located on somata and axonal varicosities [82]. Inactivation of histamine in the extracellular space of the CNS is achieved solely by methylation through neuronal histamine N-methyltransferase [83].

The tuberomammillary nucleus contains ca. 3,000 neurons in the rat and about 64,000 in man, and is the only source of neuronal histamine in the adult vertebrate brain and histamine is its main transmitter. Further transmitters (or their synthetic enzymes) expressed within tuberomammillary nucleus neurons include GABA, galanin, enkephalins,

TRH, and substance P. Histamine neurons have widespread projections to the whole brain.

Histaminergic neurons present morphological and electrophysiological properties similar to those seen in other aminergic neuron populations [84]. They fire slow and regular at a membrane potential of about -50 mV with 1–4 action potentials per second [85]. The action potentials have a significant contribution from Ca^{2+} channels triggering a strong afterhyperpolarization. The excitatory arm of the pacemaker cycle includes dendritic Ca^{2+} potentials and a non-inactivating Na^{+} current. The Ca^{2+} -currents are likely instrumental for histamine release from dendrites and axons; they are blocked by H3-autoreceptor activation. In behaving cats, rats and mice, the firing is more variable during waking and absent upon drowsiness and during sleep [52, 55, 86, 87]. This is the most wake-selective firing pattern identified in the brain to date.

Tuberomammillary neurons are influenced by many transmitters and other humoral signals. Excitatory and inhibitory synaptic potentials (EPSPs and IPSPs) evoked by stimulations of several locations are mediated by glutamate and GABA. Monoaminergic, cholinergic, and peptidergic fibers innervate the tuberomammillary neurons. Many peptides function as signaling molecules in the hypothalamus where they are involved in endocrine and homeostatic functions. They can be co-expressed and differentially released with other neurotransmitters; in many neurons, however, they represent the main transmitter or hormone.

Aminergic and some peptidergic neurons are mutually connected, mostly through excitation, occasionally also inhibition, forming an orchestra that is to a certain extent self-organizing. The orexin neurons give the signals for sleep-waking architecture and the histaminergic neurons are the dominant cell group with respect to cortical arousal and wake quality. Multifold arborizing histaminergic axons reach the entire central nervous system through two ascending and one descending bundle [72, 88–92]. The highest density of histaminergic fibers is seen in the hypothalamus. In the posterior part, the fibers often make close contact to the brain surface. The septal nuclei and those of the diagonal band receive a very strong innervation.

Four metabotropic histamine receptor types (H1R–H4R) have been cloned so far. H1R, H2R, and H3R are expressed in abundance in the brain. All histamine receptors display constitutive activity. The sedative effects of antihistamines (H1-antagonists) have prompted early suggestions of histamine as a waking substance [78]. Neuronal excitation is achieved by activation of H1R, Gq/11-proteins and phospholipase C, the formation of the two second messengers DAG and IP3, as well as intracellular Ca^{2+} release, which can trigger: (1) opening of cation channels, causing

depolarization; (2) activation of an electrogenic Na–Ca-exchanger (NCX), causing depolarization; (3) formation of NO and cyclic GMP; (4) opening of Ca²⁺-dependent potassium channels, resulting in a hyperpolarization [93]. Blocking a potassium leak conductance through direct G-protein action can shift the thalamic relay mode towards an open state and cortical activation [94]; or directly excite cortical neurons [95]. Activation of a tetrodotoxin insensitive Na-current is proposed for the excitation of cholinergic septal neurons [96] and a mixed cation channel for the excitation of dorsal raphe serotonergic neurons [97]. Firing is also increased in the suprachiasmatic nucleus [98] and cholinergic basal forebrain neurons [24].

The histamine H₂ receptors, β -adrenergic receptors, serotonin 5-HT₂ receptors, among others, are coupled to Gs-protein, adenylyl cyclase and PKA, which phosphorylates proteins and activates the transcription factor CREB. The direct action on neurons is usually excitatory or excitation potentiating. Through this signaling pathway these transmitters block a Ca²⁺-dependent potassium conductance, which is responsible for long-lasting after-hyperpolarizations and the accommodation of firing. This effect modulates the response of target neurons (e.g., in cerebral cortex and hippocampus): an identical stimulus can thus elicit a response consisting of few or many action potentials depending on the aminergic activation. Such a potentiation of excitation is perfectly suited to raise attention.

H₃ receptors function as autoreceptors on histaminergic cell somata, dendrites, and axons (varicosities) where they provide a negative feedback to restrict histamine synthesis and release. Importantly, as heteroreceptors, they are also located on many non-histaminergic axons where they modulate the release of glutamate, GABA, noradrenaline, and acetylcholine. H₃ receptors are coupled to G_q and high-voltage activated Ca channels, a typical mechanism for the regulation of transmitter release (Fig. 3). A high degree of molecular and functional heterogeneity through different transcriptional and post-transcriptional processing (splice variants) is prototypic for the H₃R [82, 99]. H₃R-related drugs are being developed largely for the treatment of sleep disorders [100].

Histamine and waking

The above data strongly indicate that histaminergic neurons activate or facilitate large brain areas through postsynaptic H₁- and H₂-receptors, thus contributing to cortical activation. Indeed, treatments that impair histamine-mediated neurotransmission enhance cortical slow activity and increase sleep. For instance, the blockade of histamine synthesis with α -fluoromethylhistidine markedly reduces

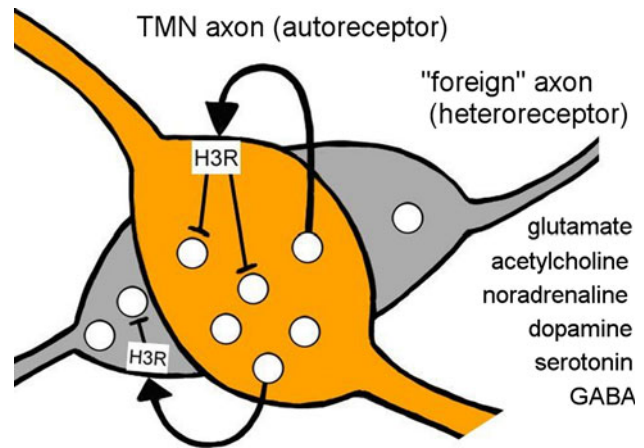


Fig. 3 The histamine H₃R as an autoreceptor mediating negative feedback on histamine synthesis and release, and, importantly, as a heteroreceptor suppressing the release of many other transmitters from their varicosities. H₃R antagonists and partial agonists are widely developed for several neuropsychiatric indications including sleep disorders

histamine levels, decreases waking, and increases slow wave sleep in the cat [19] and rodents [101–103]. In contrast, enhancement of histaminergic neurotransmission by inhibiting histamine degradation promotes waking, reviewed in [19, 75, 102]. The absence of histamine synthesis in histidine decarboxylase knockout mice impairs the cortical EEG and has deleterious effects on both sleep and wake quality, causing permanent somnolence and behavioral deficits. Consequently, mice that lack brain histamine are unable to remain awake when high vigilance is required, at lights off, or when they are placed in a new environment [103] (Fig. 4). Like orexin neurons [104], histamine neurons may also be involved in CO₂-mediated arousal; they are activated by short-term hypoxia [105] and are excited by mild acidification (Sergeeva, unpublished observations). Taken together, histaminergic neurons have a key role in maintaining the brain awake. They promote wakefulness through their direct widespread projections to the cerebral cortex and indirectly via their subcortical targets in the thalamus, basal forebrain, and brainstem [14, 75, 92].

Orexinergic/hypocretinergic neurons

Orexins/hypocretins are two peptides (Ox-A, OxB/HCr₁, HCr₂) derived from proteolytic cleavage of a precursor peptide encoded by the prepro-orexin(hypocretin) gene. The orexin-containing neurons are almost exclusively located in the perifornical area of the dorsolateral hypothalamus, therefore, just dorsorostral to the histaminergic tuberomammillary nucleus. Like histamine neurons, they project all over the brain [106]. They bind to two

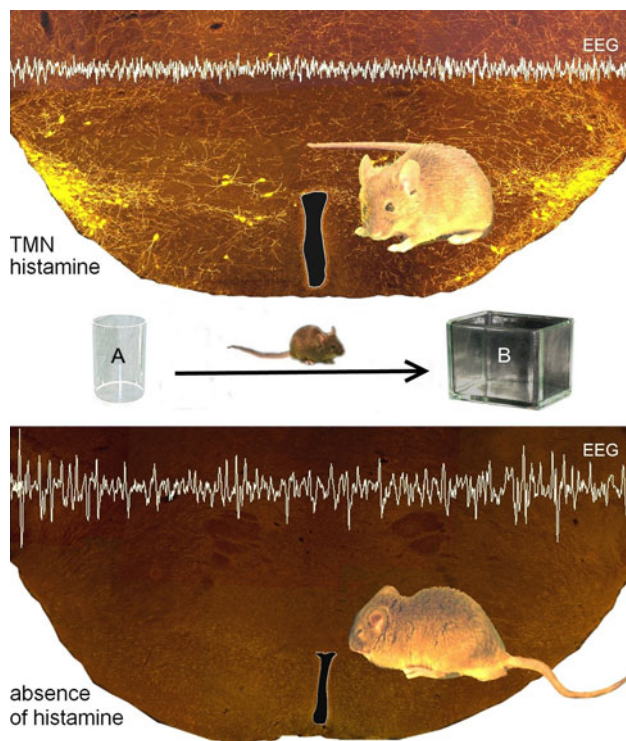


Fig. 4 Effects of an environmental change on the cortical EEG and behavioral states in wild-type (*upper*) and knockout-mice lacking histamine synthesis (*lower*). The environmental change (*middle*) consists of transferring the mice from their habitual home cage (A) to a new cage (B). *Upper* A wild-type mouse with an intact brain histaminergic system. This mouse placed in the new environment remains highly awake, as indicated by the alert behavior and waking EEG. *Lower* A knockout mouse without histamine. This mouse falls asleep a few minutes after being placed in the new environment, as shown by the sleeping behavior and EEG signs of slow wave sleep. Histaminergic neurons play a key role in maintaining the brain in an awake state in the presence of behavioral challenges. Modified from Parmentier et al. (2002) *Journal of Neuroscience* 22:7695–7711

G-protein-coupled receptors (OX1/2-R, HCrt1/2-R) [107–111]. Orexin neurons also contain excitatory glutamate and inhibitory dynorphin [112]. Orexin receptors are expressed in numerous targets throughout and even outside the nervous system. The name orexins, indicating a function in food intake, was first envisaged [113]; however, it soon became apparent that these peptides fulfil important roles in the regulation of behavioral state and sleep architecture [114, 115] and serve many physiological functions [108]. Deficiency of the orexins is the cause of narcolepsy-cataplexy whereas their hyperactivity, for instance after sleep deprivation or metabolic challenges [116, 117], predisposes to addiction and compulsion (see [118]).

Orexin neurons display a wake-active discharge pattern, clearly correlated to muscle tone and posture change, with a significant decrease from active waking to quiet waking and from quiet waking to slow wave sleep [119–121]. In the rat, the discharge rate of orexin neurons during active

waking is more than 4.5 times that of quiet waking, indicating that their main activity is to promote behavioral activation during waking [119, 120]. Cerebrospinal fluid Ox-A level [122] or *c-fos* expression in orexin neurons [123] increase after forced waking or behavioral activation. Finally, central application of orexins elicits active arousal and hyperactivity in rats, an effect prevented by SB-334867 [124, 125]. Taken together, we suggest that Ox-neurons promote locomotion and behavioral arousal and thus contribute to the maintenance of waking by enhancing locomotion [126].

Orexins are also involved in higher brain functions; they can facilitate memory performance and synaptic plasticity [127, 128]. An OX2R-dependent increase of GABAergic transmission in septo-hippocampal pathways may promote arousal via hippocampal disinhibition and theta rhythm [129] and a behavioral-state-dependent large-scale oscillatory brain activity associated with heightened synaptic plasticity and memory processing during REM-sleep, exploratory behavior, and stress [130]. Long-term potentiation of synaptic transmission (LTP) in the hippocampus [131], a cellular correlate of learning and memory is enhanced by orexin infusion into the rat dentate gyrus or locus coeruleus in vivo, while stimulus-induced LTP of Schaffer collateral-CA1 synapses in dorsal hippocampal slices as well as spatial memory in a water maze task is inhibited by orexins. OX-A induces an endogenous form of LTP at excitatory Schaffer collateral-CA1 synapses (LTPOX), relying on co-activation of metabotropic amino acid and biogenic amine receptors [132].

The respective roles of orexin and histamine-systems for waking

The histaminergic neurons are currently regarded as a downstream system driven by the orexin neurons through their dense axon arborizations in the tuberomammillary nucleus. However, recent studies show that the behavioral and sleep-wake phenotypes of histidine-decarboxylase (HDC, histamine-synthesizing enzyme)^{-/-} mice are distinct from those of orexin knockout^(-/-) mice [126, 133]. While both mouse strains display sleep fragmentation and increased paradoxical sleep, they present a number of marked differences:

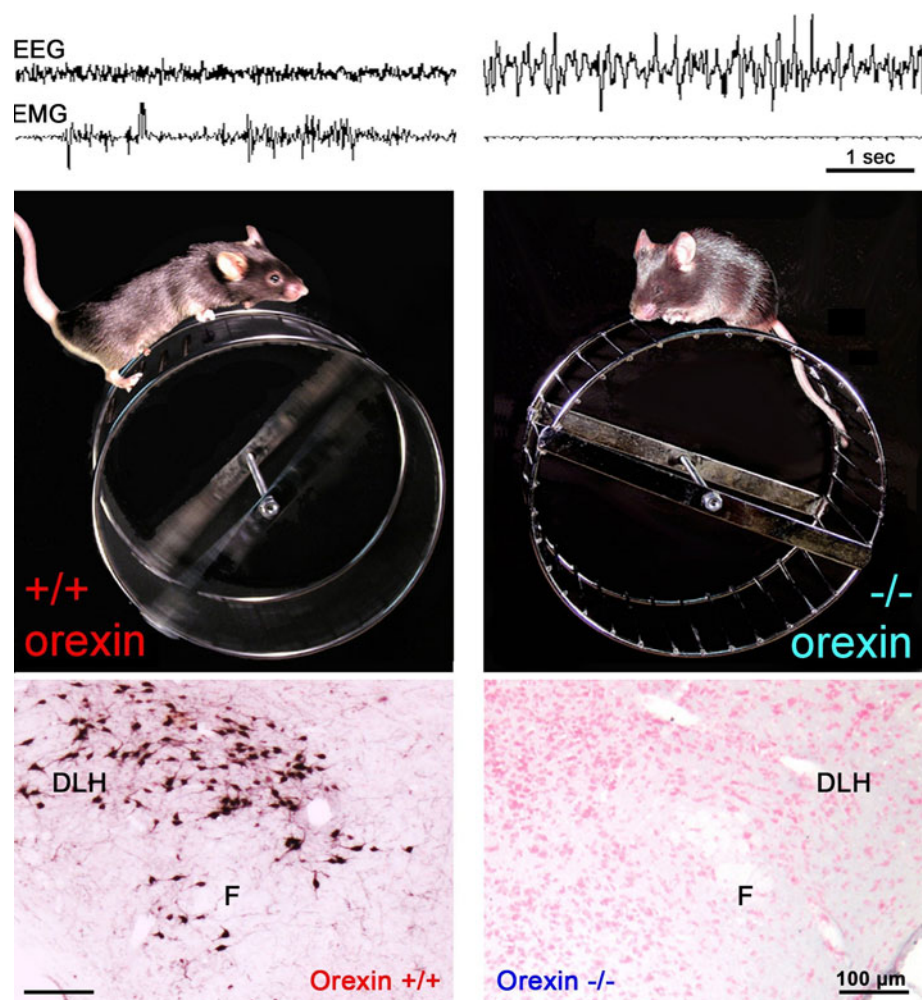
(1) The paradoxical sleep-increase in HDC^{-/-} mice is seen during lightness, whereas that in Ox^{-/-} mice occurs during darkness; (2) Contrary to HDC^{-/-}, Ox^{-/-} mice have neither waking deficiency around lights-off, nor an abnormal EEG and respond to a new environment with increased waking; (3) Only Ox^{-/-}, but not HDC^{-/-} mice, display narcolepsy and deficient waking when faced with a motor challenge. Wild-type, but not littermate Ox^{-/-} mice, when

placed on a wheel, voluntarily spend their time in turning it, and as a result, remain highly awake (Fig. 5); this is accompanied by dense c-fos expression in many areas of their brains, including Ox-neurons in the dorsolateral hypothalamus. The waking and motor deficiency of Ox $-/-$ mice is due to the absence of Ox as intraventricular dosing of Ox-A restores their waking amount and motor performance. SB-334867 (Ox1-receptor antagonist, i.p.) impairs waking and locomotion of wild-type mice during the test.

Thus, histamine- and orexin-neurons, with their reciprocal interactions, exert a synergistic and complementary control over waking, the histaminergic system being mainly responsible for cortical activation (EEG) and cognitive activities and the orexinergic system being more involved in the behavioral arousal during waking, including muscle tone, posture, locomotion, food intake, and emotional reactions. Orexin deficiency is in most cases the direct cause of narcoleptic episodes in humans (DREMs, direct onsets of REMs from wake or SOREMs, sleep onsets REMs) and cataplexy [134], whereas decreased histaminergic transmission likely accounts for the somnolence and

excessive daytime sleepiness seen in this disease and other sleep disorders [103, 126, 135, 136]. The advent of optogenetic stimulation has opened new ways to study the impact of defined neuronal populations on behavior. Carter et al. [137] reported recently such activation of orexin/hypocretin neurons causing increased waking and c-Fos expression in locus coeruleus and the tuberomammillary nucleus, but this effect was lost after sleep deprivation, presumably being overwhelmed by homeostatic mechanisms (see below). Interestingly, the increase in waking was unchanged in HDC-KO mice lacking histamine: the histaminergic system is an important, but only one of many targets of the widely arborizing orexin/hypocretin axons. There clearly is redundancy within the wake-active systems. They act together like an orchestra and are able to compensate for the failure of some of their players. For instance, 3 weeks after triple saporin-induced lesions of the cholinergic forebrain, the tuberomammillary nucleus and the locus coeruleus in the rat, the remaining phenotype is a wake deficit during the light to dark period [138], similar to that identified in HDC-KO mice [133]. The acute effects of

Fig. 5 Different behavioral performance and ability to maintain waking between wild-type and orexin knockout mice when faced with a motor challenge demonstrated using simultaneous electroencephalogram and electromyogram monitoring (upper). When wild-type mice (middle left) were placed on a wheel, they voluntarily spent their time in turning it and, as a result, remained highly awake. In contrast, orexin knockout mice (middle right) usually tried to adapt a position to stay immobile, thus falling asleep. Note the absence of orexin neurons in the knockout mice (lower). Modified from Anaclet et al. (2009) *Journal of Neuroscience* 29:14423–14438



such lesions, notably those on the neocortical EEG, remain to be investigated.

The search for substances increasing alertness led to the discovery of the wake-promoting action of montirelin (a non-hydrolyzable TRH analogue, which showed beneficial action in canine narcolepsy [139]). TMN neurons express two known TRH receptors, are excited by TRH and montirelin, and the wake-promoting action of montirelin is missing in histamine-deficient mice [133]. Thus, the histaminergic system represents an attractive target for wake-promoting medication in narcolepsy [136] and e.g., in Parkinson's disease [140], where most arousal centers undergo degeneration while the histaminergic system remains intact and an H3-receptor inverse agonist increases alertness [100, 140]. Interestingly, modafinil exerts only a minor action in PD [140] in accordance with the degeneration of dopaminergic neurons and subsequent down-regulation of D2R.

Other posterior hypothalamic neurons regulating sleep-wake alternation

The posterior hypothalamus is a heterogeneous structure also from a functional point of view. In addition to its well-recognized role in wake, it has long been suggested that this region exerts hypothalamic control over the brainstem paradoxical sleep-generating mechanisms and may play an important role in cortical activation not only during wakefulness but also during paradoxical sleep, reviewed in [3, 11, 55].

In addition to the histaminergic wake-specific and orexinergic wake-active discharge patterns, neurons firing selectively and tonically during paradoxical sleep were also identified in the cat and more recently in the rat posterior hypothalamus [55, 141]. This pattern is driven, at least in part, by neurons containing MCH in the rat [141]. Although MCH-containing cells are found in the same area where orexin cells are located, the perifornical area of the dorsolateral hypothalamus [49, 142], the fact that they discharge in a reciprocal manner to orexin or histamine neurons [141] suggests that they might play a different role than orexin or histamine neurons in sleep-wake regulation. However, intracerebroventricular supply of MCH increases paradoxical sleep supporting a role in promoting this behavioral state, whereas mice lacking MCH-R1 also show enhanced paradoxical sleep [143]. Thus, the hypothalamic mechanisms involved in cortical activation during paradoxical sleep remain to be clarified, but it seems important to determine the possible interactions between MCH-containing cells and orexin and histamine neurons to further understand the hypothalamic control on sleep-wake alternation via the three widespread projecting systems.

Interaction between the waking systems and sleep-generating mechanisms

The waking systems are inactivated to allow sleep. Sleep-wake alternation results from an interaction between the waking systems and the brain's sleep-generating mechanisms. The best defined brain structure for sleep generation is the preoptic-anterior hypothalamus, which contains dense populations of sleep-active neurons discharging at a high rate during slow wave sleep [26, 144]. A lesion of this region causes severe insomnia [59]; reviewed in Szymusiak [145]. Later on, it was proposed that the ventrolateral [146], median [147] or dorsolateral [148] preoptic area and adjacent regions generate sleep mainly through GABAergic inhibition of the aminergic and peptidergic (orexins) waking systems.

Early studies also suggest that waking occurs by direct or indirect inhibition of the preoptic area by the waking systems. Microinjection of histamine in this area enhances waking in the cat [149]. In vitro studies demonstrated direct and indirect inhibition of sleep-active preoptic neurons by the ascending activating neurotransmitters noradrenaline, serotonin, acetylcholine [150], and histamine [151].

The classical view on the orchestration of sleep-wake alteration by interactions between sleep-generating and wake-promoting structures is currently challenged or modified: sleep-active neurons are also identified in wake-promoting structures like the tuberomammillary nucleus and the adjacent posterior hypothalamus whereas wake-specific neurons are also found in the preoptic and the adjacent basal forebrain. Moreover, at the transition from wake to slow wave sleep, sleep-active neurons discharge not before, but after, cessation of activity of the wake-specific neurons, indicating that release of the inhibition by wake-promoting systems plays a major role in sleep generation [144, 152, 153].

Circadian and homeostatic regulation of sleep and waking

A two-process model of sleep-waking regulation proposes the factors C (circadian pacemaker) and S (sleep propensity increasing with the duration of waking, homeostasis) [154, 155]. A search for endogenous substances interacting with this regulation continues for decades, in particular for promoting sleep [156]. Adenosine has been identified as a candidate that accumulates in the brain during strong nervous activity, during prolonged waking. It causes sedation [157] and likely induces sleep [158, 159]. Adenosine A1 receptors are positively coupled to various potassium channels, negatively to Ca²⁺-channels and cyclic AMP,

exerting post- and presynaptic inhibition at many sites in the brain, specifically in the cholinergic basal forebrain [160]. Interestingly, the histaminergic neuron's firing is unaffected by adenosine [161]. Adenosine A2A receptors are more localized, and they mediate excitation of sleep-active neurons in the preoptic area. Both these adenosine receptors are blocked by caffeine, resulting in arousal, especially at times when endogenous adenosine has accumulated during sleep deprivation [159, 162]. Whereas adenosine levels correlate with low energy reserve [163], high levels of the energy-rich adenosine-triphosphate (ATP) can mediate an increased excitability through closure of K_{ATP} -channels and, after release to the extracellular space, to direct excitation through ionotropic and metabotropic purine receptors of the P2 type, e.g., in histaminergic neurons [161].

In summary, multiple waking systems operate together to ensure the complex vital function wakefulness. The different activating and inhibiting systems form a complex distributed network. Disturbances of sleep and waking are frequent, socially and economically relevant. Understanding the regulation and the neural mechanisms opens the way for successful intervention: recent efforts in drug discovery concern the waking center in the posterior hypothalamus.

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