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Holst, Sebastian C ; Landolt, Hans-Peter

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SLEEP-WAKE NEUROCHEMISTRY

Sebastian C. Holst [1], PhD, and Hans-Peter Landolt [2,3], PhD

[1] Copenhagen University Hospital, Rigshospitalet, Department of Neurology and Neurobiology Research Unit, Copenhagen, Denmark

[2] Institute of Pharmacology & Toxicology, University of Zürich, Zürich, Switzerland

[3] Zürich Center for interdisciplinary Sleep Research (ZiS), University of Zürich, Switzerland

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Addresses for correspondence:

Sebastian C. Holst

Copenhagen University Hospital,

Rigshospitalet, Department of Neurology and

Neurobiology, Research Unit

Copenhagen

Denmark

Tel. …

Fax …

e-mail: sebastian.holst@nru.dk

Hans-Peter Landolt

Institute of Pharmacology & Toxicology

University of Zürich

Winterthurerstrasse 190

8057 Zürich

Switzerland

Tel. + 41 - 44 - 635 59 53

Fax. + 41 - 44 - 635 57 07

e-mail: landolt@pharma.uzh.ch

Abstract

Behavioral states alternate between wakefulness and sleep, which is further subdivided into rapid-eye-movement sleep and non-rapid-eye-movement sleep. Waking and sleep states are highly complex processes that are elegantly orchestrated by fine-tuned neurochemical changes, including the neurotransmitters and neuromodulators glutamate, acetylcholine, γ-amino-butyric acid, norepinephrine, dopamine, serotonin, histamine, hypocretin, melanin concentrating hormone, adenosine, and melatonin. However, as highlighted in this brief overview, no single neurotransmitter or neuromodulator, but rather their complex interactions within organized neuronal ensembles, regulate waking and sleep states and drive their transitions. Dysregulation of and medications interfering with these neurochemical systems lead to sleep-wake disorders and functional changes of wakefulness and sleep. The neurochemical pathways presented here, thus, are aimed to provide a conceptual framework for the understanding of the effects of currently used medications on wakefulness and sleep.

Keywords

Neurotransmitters, neuropeptides, sleep, wake, glutamate, acetylcholine, γ-amino-butyric acid, norepinephrine, dopamine, serotonin, histamine, hypocretin, melanin concentrating hormone, adenosine, melatonin

1. Introduction

Based upon behavioral and (neuro)physiological characteristics derived from polysomnographic recordings, the three distinct vigilance states, wakefulness, rapid-eye-movement (REM) sleep, and non-rapid-eye-movement (NREM) sleep can be unambiguously defined in mammals. Wakefulness with eyes closed is typically associated with electroencephalographic (EEG) activity in the alpha range (8-12 Hz) and with high-frequency, desynchronized activity above 40 Hz. In a normal sleep episode, voluntary muscle control is gradually lost and NREM and REM sleep episodes alternate in a cyclic pattern. In NREM sleep, the EEG shows slow, high-amplitude activity reflecting widespread, synchronous oscillations of neurons exhibiting alternating periods of firing and silence (burst-pause firing pattern) (Steriade, McCormick and Sejnowski, 1993). The so-called EEG delta activity (< 4.5 Hz) is under tight homeostatic control and exhibits a declining trend in the course of the night, which reflects the dissipation of sleep need and the decline in sleep intensity (Achermann and Borbély, 2011). The EEG in REM sleep (sometimes also called paradoxical sleep) is partly reminiscent of EEG activity in drowsy wakefulness, yet it is characterized by muscle atonia with occasional muscle twitches and rapid eye movements.

Distinct neurotransmitter nuclei and neuronal pathways modulate and maintain the three vigilance states. First insights were reported by Constantin von Economo (1876-1931) who studied patients with a type of viral encephalitis that was never seen before, *Encephalitis lethargica* (Von Economo, 1930). Von Economo discovered that the encephalitis was associated with lesions to distinct brain areas in the midbrain and brainstem reticular formation. Lesions of the ventral periaqueductal gray (vPAC) and posterior hypothalamus were associated with severe hypersomnia, whereas lesions of the hypothalamic anterior preoptic area extending into the basal ganglia (BG) were associated with insomnia. These findings where the first in a series of fundamental studies eventually leading to the postulation of an “ascending reticular activating system” (ARAS) (Moruzzi and Magoun, 1949). The ARAS arises from a network of neuronal clusters in the brainstem, which activates forebrain, thalamus and cortex, mainly in wakefulness, but to some extent also in REM

sleep. Today, the ARAS is no longer seen as a loose reticular system, yet to consist of a network of individual nuclei expressing distinct neurotransmitters that promote arousal. The key modulatory neurotransmitters of the ascending activating system include acetylcholine, several monoamines (norepinephrine, serotonin, histamine, dopamine), and the slow-acting neuropeptide hypocretin (*aka* orexin). More recently, the fast-acting amino acid glutamate has been proposed to be the main regulator of arousal (Saper and Fuller, 2017). The known roles of these neurochemicals in promoting waking and sleep states provide a useful conceptual framework to understand the effects of medications on wakefulness and sleep. With the recent advent of powerful optogenetic and chemogenetic tools, experimental *in vivo* control of neuronal activity by stimulating or inhibiting distinct neuronal ensembles permitted exciting new insights into the causal underpinnings of brain state transitions. A comprehensive summary of these insights are beyond the scope of this article; this has been the topic of excellent recent overviews (Tyree and de Lecea, 2017; Luppi and Fort, 2018). Nevertheless, some recent progress in our current understanding of sleep-wake neurochemistry made by investigating sleep-wake circuits with optogenetic techniques will be covered.

2. The neurochemical underpinnings of wakefulness

2.1. Acetylcholine

Acetylcholine (ACh) releasing nuclei in the pedunculopontine and laterodorsal tegmental nuclei (PPT/LDT) of the pons project primarily to the basal forebrain, as well as to thalamic relay and reticular cells. This pathway is crucial for gating thalamic signaling to the cortex. A second cluster of ACh releasing neurons is located in the basal forebrain (BF), which projects widely to the cortex. ACh activates ionotropic nicotinic receptors and metabotropic muscarinic receptors. Nicotinic receptors are expressed in pre- and post-synaptic membranes. When they are activated, sodium and calcium ions rapidly enter the cells, leading to membrane depolarization. Muscarinic receptors are part of

the superfamily of G-protein-coupled receptors (GPCRs). Five types of muscarinic receptors are currently known. The M_1, M_3 and M_5 receptors are coupled with G_q proteins that activate phospholipase C. They are expressed in cortex, striatum, as well as other brain regions. M_2 and M_4 are coupled to $G_{i/o}$ proteins, and their activation inhibits adenylyl cyclase. These receptors are found, among other regions, in the BF where they act as auto-receptors, thought to control ACh synthesis and release. The cholinergic neurons in the central nervous system (CNS) are active mainly in wakefulness and REM sleep, and have a firing rate in the theta range (Lee, 2005). They promote cortical arousal and REM sleep, while reducing NREM sleep (Baghdoyan and Lydic, 1999; Nissen *et al.*, 2006; Zhang *et al.*, 2006).

Optogenetics recently improved our understanding of the role of ACh and other wake promoting neurotransmitters in controlling vigilance states. With this method, selected neurons are genetically modified to express channelrhodopsin-2 (ChR2), a light-activated non-specific cation channel. When ChR2 is activated by a light pulse, influx of cations such as Ca^{2+} is triggered and the ChR2 expressing cells are activated (Boyden *et al.*, 2005). Optogenetic activation of the cholinergic PPT/LDT neurons in mice during NREM sleep leads to a rapid transition into REM sleep, yet not into wakefulness (Van Dort *et al.*, 2015). On the other hand, optogenetic stimulation of PPT/LDT neurons during wakefulness is associated with moderate arousal (Van Dort *et al.*, 2015). Interestingly, activation of ChR2-expressing cholinergic neurons of the BF during wakefulness also promotes arousal, whereas activation during NREM sleep leads to transition, at roughly equal amount, into wakefulness and REM sleep (Han *et al.*, 2014). These observations suggest that the cholinergic neurons in the brainstem are more involved in regulating transitions into REM sleep, whereas the cells of the BF are more linked to general arousal.

Another recent study employing optogenetics could corroborate that the ACh neurons of the BF are contributing to arousal and the promotion of wakefulness (Xu *et al.*, 2015). Nevertheless, by stimulating also glutamatergic and γ-amino-butyric acid (GABA)-ergic neurons of the BF, it was found that the three neuronal cell groups exert similar effects on arousal (Xu *et al.*, 2015).

Interestingly, activation of BF GABA-ergic neurons substantially enhanced wakefulness. However, when BF glutamatergic and cholinergic neurons were activated, the effects on arousal were minor (Anaclet *et al.*, 2015; Kim *et al.*, 2015; Xu *et al.*, 2015; Chen *et al.*, 2016). In conclusion, although ACh neurons of the BF likely modulate wakefulness, it now appears that subgroups of BF GABA-ergic neurons may be more important than cholinergic neurons for the regulation of arousal.

2.2. Monoamines

The monoamines promoting arousal and maintaining wakefulness include norepinephrine (NE), serotonin (or 5-hydroxytryptamine [5-HT]), dopamine (DA), and histamine (His). The primary site of production and release of NE is the locus coeruleus (LC), of 5-HT the raphe nucleus (DRN), of DA the ventral tegmental area (VTA) and substantia nigra (SN), and of His the hypothalamic tuberomammillary nucleus (TMN). With the exception of the dopaminergic cell groups, all these nuclei fire at high rates in wakefulness, lower rates in NREM sleep, and are virtually silent in REM sleep (Jacobs and Fornal, 1999; Takahashi, Lin and Sakai, 2006; Takahashi *et al.*, 2010). They have widespread CNS projections and innervate cortex, brainstem, ventro-lateral preoptic nucleus (VLPO), thalamus and BF, making them ideally located to promote and sustain wakefulness.

2.2.1. Norepinephrine

The LC is an important small cell cluster involved in the regulation of arousal (Takahashi *et al.*, 2010). It is located in the brainstem and consists of roughly 25’000 NE neurons. NE can activate both the α and β families of adrenergic receptors, which are part of the GPCR superfamily. Adrenergic receptors are divided into three main types, α_1 (mainly G_q coupled), α_2 (inhibitory auto- and postsynaptic receptors, $G_{i/o}$ coupled) and β receptors (G_s coupled), which are all widely expressed in the CNS (Ramos and Arnsten, 2007). Optogenetic stimulation of the noradrenergic neurons in the LC is associated with immediate transitions from sleep to wakefulness and increased

locomotor activity (Carter *et al.*, 2010), highlighting the role of the LC in maintaining behavioral arousal. Recent evidence also suggests a role for NE in the sleep-driven macroscopic pathway referred to as the 'glymphatic system'. This system is governed by a flow of cerebrospinal fluid into the brain along para-arterial spaces, which enables the removal of macroscopic waste products from the brain parenchyma in NREM sleep (Iliff *et al.*, 2012; Xie *et al.*, 2013). Increased glymphatic function in NREM sleep results from an increased interstitial space volume fraction, which appears to be driven by reduced LC-derived NE-ergic tone (Xie *et al.*, 2013). However, the increase in interstitial space that enables glymphatic flow during sleep, may not solely be driven by NE. The size of the interstitial space can also be reduced by a wake-promoting cocktail of monoamines, ACh and hypocretin (Hctr), and even by altering the concentrations of potassium, calcium and magnesium ions in the cerebrospinal fluid (Ding *et al.*, 2016).

2.2.2. Serotonin

The major nuclei releasing 5-HT, the DRN, are located along the midline of the brainstem and reticular formation. Besides 5-HT neurons, the DRN also contains DA-ergic, GABA-ergic, glutamatergic and neuropeptide releasing neurons. Moreover, the DRN is innervated by GABA, glutamate, ACh, NE, His, Hctr and melanin concentrating hormone (MCH) expressing neurons originating from several other brain areas, rendering serotonergic influences on sleep-wake regulation highly complex in nature. Furthermore, the 5-HT receptors are sub-divided into seven distinct families: 5-HT_1 to 5-HT_7 receptors. Similar to the other monoamines, all 5-HT receptors except the ligand-gated 5-HT_3 ion channel, belong to the GPCR superfamily. The 5-HT_1 and 5-HT_5 receptors are coupled to $G_{i/o}$ proteins, the 5-HT_2 receptor is coupled to G_q protein, and the 5-HT_4, 5-HT_6 and 5-HT_7 receptors are coupled to G_s proteins. The many 5-HT receptor subtypes and the widespread effects of 5-HT in the CNS have made it challenging to elucidate the distinct roles for 5-HT in sleep-wake regulation (Landolt and Wehrle, 2009). Intriguingly, 5-HT_{1A} and 5-HT_{1B} receptor

knock-out mice have enhanced amounts of REM sleep (Boutrel *et al.*, 1999, 2002), whereas mutant mice without 5-HT_{2A} or 5-HT_{2C} receptors show enhanced durations of wakefulness and reduced NREM sleep (Frank, Stryker and Tecott, 2002; Popa *et al.*, 2005). Overall, the current evidence suggests that 5-HT transmission generally promotes wakefulness, inhibits REM sleep, and can interfere with slow wave sleep [for review, see (Monti, 2011)]. Optogenetic stimulation of DRN 5-HT neurons has been attempted and found to enhance patience in an anticipated-reward paradigm (Miyazaki *et al.*, 2014). With respect to sleep-wake regulation, optogenetic activation of DRN 5-HT neurons has not been examined. However, it was recently shown, that optogenetic activation of DRN DA neurons promote wakefulness and contribute to the regulation of sleep-wake states (Cho *et al.*, 2017).

2.2.3. Histamine

The TMN is a small His-releasing cell cluster found inferior to the hypothalamus. The TMN shows a projection pattern that is similar to the LC and the DRN, including strong reciprocal innervation with the VLPO (Sherin *et al.*, 1998). Three types of His-ergic GPCRs have been classified so far: H_1, H_2, and H_3 receptors that are coupled to G_q, G_s, and G_i proteins. It is well known that antihistamines are sedative, which is a common side effect of early H_1 receptor antagonists (e.g. diphenhydramine) in the treatment of allergies. The release of His within the TMN by optogenetic photo-stimulation activates H_3 auto-receptors and suppresses inhibitory GABA-ergic inputs to TMN (Williams *et al.*, 2014). Moreover, His from the TMN enhanced the inhibition of the VLPO. Combined, these observations support a role for His, in stabilizing wakefulness.

2.2.4. Dopamine

Psychostimulant and wake-promoting agents typically enhance DA-ergic neurotransmission [for review, see (Holst *et al.*, 2016)]. However, with respect to sleep-wake regulation, this

neurotransmitter has long been thought to be of limited importance, because DA-ergic neurotransmission in cats showed only minor alterations across the sleep-wake cycle. By contrast, more recent evidence in rats revealed that extracellular DA levels in the medial prefrontal cortex (mPFC) and parts of the nucleus accumbens (NAc) of the ventral striatum are high in wakefulness and REM sleep, and significantly lower in NREM sleep (Léna *et al.*, 2005; Dahan *et al.*, 2007). Thus, similar to the other monoamines, DA may indeed play an important role in regulating wakefulness and sleep. Five types of DA-ergic GPCRs are known. The D_1-like (D_1 and D_5) DA receptors are coupled to G_s protein and are mainly stimulatory. On the contrary, the D_2-like (D_2, D_3 and D_4) DA receptors are coupled to inhibitory G_i protein. Importantly, D_1 and D_2 receptors form functional heteromers with adenosine A_1 and A_{2A} receptors (see below), such that the binding of adenosine results in reduced dopaminergic signaling.

Both, distinct subtypes of DA and adenosine receptors are primarily expressed in the NAc, a brain region recently suggested to play a crucial role in sleep-wake regulation [for recent update, see (Luppi and Fort, 2018)]. The NAc is innervated by DA projections of the mesolimbic pathway originating in the VTA. By integrating signals from cortex, thalamus, amygdala and midbrain, the NAc is able to inhibit several other arousal pathways via GABA-ergic interneurons. Experimental inhibition and activation of the NAc, thus, promote wakefulness and sleep (Monti and Monti, 2007; Lazarus *et al.*, 2012; Holst and Landolt, 2015). Several studies interrogated DA-ergic neurotransmission by optogenetics, in particular with respect to reward and addiction. However, a recent study in rats, elegantly showed that destruction of DA afferents in the substantia nigra pars compacta (SNc), projecting via the nigrostriatal pathway to the dorsal striatum, enhanced wakefulness and induced sleep-wake fragmentation (Qiu *et al.*, 2016). The authors of this study then optogenetically stimulated the SNc DA neurons, which resulted in increased firing and enhanced sleep (Qiu *et al.*, 2016), suggesting that the nigrostriatal DA-ergic pathway promotes sleep. Taken together, it is likely that distinct roles for DA in sleep-wake regulation are region- and pathway-specific, and probably also dose-dependent.

2.3. Neuropeptides

Around 100 neuropeptides have been described in the human brain (Burbach, 2010). They typically act via GPCRs, have long duration dynamics at the synapse and do not cross the blood-brain barrier. These characteristics make them difficult targets for pharmacological interventions.

2.3.1. *Hypocretin*

Several neuropeptides, including Hcrt, MCH, galanin, oxytocin, neuropeptide Y, somatostatin, ghrelin and substance P, play important roles in regulating mood, reward, arousal and sleep (Richter, Woods and Schier, 2014). With respect to sleep-wake regulation, Hcrt and MCH deserve to be especially mentioned. These peptides are released from a small cluster of neurons found solely in the lateral hypothalamus (LH). The MCH contributes mainly to the promotion and maintenance of sleep (Monti, Torterolo and Lagos, 2013), and will be covered in the following section.

The Hcrt producing neurons project to all above-described nuclei of the arousal pathways, especially to LC, DRN, and TMN. They also release glutamate and play important roles in maintaining arousal and stabilizing the wake state. Similar to the other wake-promoting systems, the Hrct neurons are mainly active in wakefulness, especially when animals are exploring, and become silent in REM sleep and NREM sleep (Mileykovskiy, Kiyashchenko and Siegel, 2005). The Hcrt binds to two subtypes of GPCRs, referred to as Hcrt-1 and Hcrt-2 receptors. Activation of these receptors increases intracellular calcium levels. The loss of Hcrt neurons causes narcolepsy with cataplexy, which is characterized by “behavioral state instability,” most likely caused by an insufficient inhibition of the circuits regulating REM sleep and NREM sleep (Mochizuki *et al.*, 2004; España and Scammell, 2011). Supporting the wake-promoting role of Hcrt, optogenetic activation of Hcrt neurons triggers brief awakenings from both REM sleep and NREM sleep, an effect that is diminished

with increasing sleep pressure (Adamantidis *et al.*, 2007; Carter *et al.*, 2009). Combined, these findings suggest that sleep-dependent processes feed back to Hcrt neurons and inhibit their wake promoting actions.

3. The neurochemical underpinnings of sleep

3.1. Neuropeptides: Melanin concentrating hormone

Intermingled with Hcrt neurons in the LH are MCH-expressing neurons that also produce GABA and innervate many of the Hcrt-target regions, including the LC and the DRN (Kilduff and De Lecea, 2001). However, in contrast to Hcrt, the MCH projections are inhibitory and fire at high rates in REM sleep, are less active in NREM sleep, and remain almost silent in wakefulness [for recent review, see (Konadhode, Pelluru and Shiromani, 2015)]. Thus, MCH neurons likely promote REM sleep and inhibit wakefulness. Indeed, MCH-deficient mice spend less time in both, REM and NREM sleep (Willie *et al.*, 2008). On the contrary, when the MCH neurons are optogenetically activated, either REM sleep alone (Jego *et al.*, 2013; Tsunematsu *et al.*, 2014) or both, REM sleep and NREM sleep (Konadhode *et al.*, 2013) were found to be enhanced. Although more research is warranted, it appears evident that MCH neurons inhibit wakefulness and functionally oppose Hcrt neurons in regulating the transition between wakefulness and sleep states (Tsunematsu *et al.*, 2014).

3.2. Adenosine

Based upon the knowledge that the world's most readily consumed psychostimulant, caffeine, antagonizes adenosine receptors, adenosine and adenosine receptors have long been suggested to be important for sleep-wake regulation (Huang *et al.*, 2005; Rétey *et al.*, 2007; Landolt, 2008; Bodenmann *et al.*, 2012). Compelling evidence now suggests that the neuromodulator adenosine contributes to the regulation of the increase in sleep pressure during wakefulness and the decrease in sleep propensity during sleep. Four subtypes of G-protein-coupled adenosine receptors

have been identified to mediate adenosine's cellular effects: A_1, A_{2A}, A_{2B}, and A_3 receptors. Activation of A_1 and A_3 receptors inhibits adenylate cyclase (AC), whereas A_{2A} and A_{2B} receptors mediate their effects by increasing AC activity (Sebastião and Ribeiro, 2009). The A_1 and A_{2A} receptors are strongly expressed in the brain, yet their local expression patterns vary [for review, see (Urry and Landolt, 2015)]. By contrast, A_{2B} and A_3 receptors are expressed with only low abundance in cerebral structures, and their roles in sleep-wake regulation are not well established. Adenosine has many properties of a presumed endogenous sleep regulating substance. It has long been known that adenosine, when infused into the intra-cerebro-ventricular space, promotes sleep (Virus *et al.*, 1983). Moreover, extracellular adenosine levels in the brain of animal models are typically higher in the active phase (dominated by wakefulness) when compared to a phase of rest (dominated by sleep). Similarly, adenosine in the BF is increased by sleep deprivation and normalized by recovery sleep (Porkka-Heiskanen *et al.*, 1997; Porkka-Heiskanen, Strecker and McCarley, 2000). Nevertheless, lesion and pharmacological studies revealed that accumulation of adenosine in the BF is not necessary for sleep induction, nor are BF cholinergic neurons essential for sleep drive (Blanco-Centurion *et al.*, 2006). In conclusion, while adenosine contributes to sleep-wake regulation, a causal role for adenosine in the BF as a single regulator of sleep homeostasis has not been established.

3.3. γ-Amino-butyric acid

Constantin von Economo was the first to describe that lesions to the hypothalamic anterior preoptic area were associated with symptoms of insomnia (Von Economo, 1930). Today, it is widely accepted that the VLPO, as well as its neighbouring preoptic area region, the median preoptic area (MnPO), contain high densities of neurons that are active in and even a few minutes before initiation of NREM sleep. The VLPO/MnPO neurons fire less in REM sleep and are almost silent in wakefulness (Sherin *et al.*, 1996; Suntsova *et al.*, 2002). Lesions to the VLPO in cats dramatically reduce sleep (McGinty and Sterman, 1968). The VLPO neurons are well positioned to innervate arousal systems of

brainstem and hypothalamus, including the DRN, LC, LDT/PPT, SNc/VTA, TMN and Hcrt producing neurons in the LH (Saper and Fuller, 2017). The VLPO contains GABA-ergic as well as galanin-ergic projections, both associated with inhibitory transmission on effector targets (Luppi and Fort, 2018). Specific activation of GABA-ergic neurons in the VLPO enhances NREM sleep, while reducing wakefulness (Saito *et al.*, 2013).

Apart from VLPO/MnPO, GABA-ergic systems comprising new structures and pathways active in NREM sleep have been discovered in recent years, yet their specific roles in sleep-wake regulation are not yet well established (Luppi and Fort, 2018). The actions of GABA are mediated via ligand-gated ion channels referred to as $GABA_A$ and $GABA_C$ receptors, as well as via G_i protein-coupled $GABA_B$ receptors that promote potassium ion conductance upon their activation. The pharmacological properties of GABA receptors are well investigated, although extremely complex. The $GABA_A$ receptor is the target for the clinically widely used sleep-inducing medications, benzodiazepines and Z-drugs (non-benzodiazepine structure). This receptor subtype consists of a chloride ion channel composed of five subunits assembled from a symphony of α, β, γ, and other less frequent subunit variants (Rudolph and Möhler, 2006). In conclusion, GABA-ergic neurons of the VLPO, together with the MnPO appear to regulate inhibitory inputs to the ARAS and to promote the transition from wakefulness to sleep and the maintenance of NREM sleep.

3.4. Melatonin

The endogenous biological master clock regulating the daily sleep-wake cycle is localized in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN is a small neuronal cluster consisting of roughly 20’000 neurons (Gachon *et al.*, 2004). The SCN functions as an endogenous zeitgeber with a roughly 24-hour periodicity, operating virtually independently of prior sleep and wakefulness. The SCN is primarily entrained by light, which is detected by retinal ganglion cells and transmitted via melanopsin-releasing neurons of the retino-hypothalamic tract (Gooley *et al.*, 2001;

Berson, Dunn and Takao, 2002). Nevertheless, other zeitgebers such as temperature (Blake, 1967) and feeding (Richter, 1922) also modulate the endogenous clock. The actual timekeeping is maintained by interconnected SCN neurons and transcriptional/translational feedback loops of core and associated clock genes (Gachon *et al.*, 2004; Colwell, 2011).

The SCN has projections to the pineal gland and other hypothalamic nuclei, including the dorsomedial hypothalamic nucleus (DMH). The DMH appears especially important for the SCN in modulating sleep-wake timing, because abolition of DMH neurons also abolishes sleep-wake timing in experimental animals. The DMH extends strong glutamatergic and GABA-ergic projections to LH and VLPO, respectively, which allow the SCN to regulate sleep and wakefulness (Fuller, Gooley and Saper, 2006). An important regulator of SCN function is melatonin. Melatonin is often referred to as "the hormone of darkness". When compared to the biological day, its concentration is elevated about 10-fold during the biological night in both diurnal and nocturnal species. Melatonin binds to MT_1 (*aka* MT_{1A}) and MT_2 (MT_{1B}) melatonin receptors, which are GPCRs linked to $G_{i/o}$ proteins inhibiting the production of cyclic adenosine-monophosphate. Melatonin receptors are highly expressed in the SCN. Their signaling cascade of melatonin is complex and not yet fully understood (Hardeland *et al.*, 2011). Nevertheless, the FDA recently approved the MT_1/MT_2 receptor agonist ramelteon for treatment of insomnia. In addition, agomelatine, an MT_1/MT_2 receptor agonist and selective 5-$HT_{2B/C}$ receptor antagonist, shifts the phase of the circadian system and may improve sleep. Nevertheless, its usefulness for primary sleep disorders remains debated (De Berardis *et al.*, 2015).

The roles for melatonin in sleep-wake regulation were recently highlighted in a diurnal zebrafish model, in which the synthesis of melatonin was genetically abolished (Gandhi *et al.*, 2015). The mutant fish showed a general loss of circadian rhythmicity and strongly reduced sleep compared to normal fish when kept in constant dark conditions. These findings suggest that melatonin not only modulates circadian, but also homeostatic aspects of sleep-wake regulation. Interestingly, melatonin may induce the production of adenosine. Thus, when adenosine was activated in the fish mutants,

their wake-phenotype could be rescued, further strengthening the association of melatonin with sleep-wake a homeostasis. Despite the basic importance of these data, the simple nervous system of zebrafish may not be directly comparable to humans, and melatonin may play even more complex roles in mammalian sleep-wake regulation.

4. Synopsis and perspectives

Clinical observations and research spanning from sleep-wake disordered patients to genetically engineered animal models have consistently identified the ascending arousal pathways, the VLPO and the SCN as important players in the regulation of wakefulness and sleep (**Fig. 1**). In waking, distinct cell clusters of brain stem, BF, and hypothalamus producing Ach, GABA, monoamines and Hcrt activate the thalamus, hypothalamus, BF, cortex and spinal cord motor neurons, and inhibit the VLPO. In REM sleep, 'REM-on' brain stem nuclei containing ACh, glutamate and GABA promote activity in BF and cortex and induce muscle atonia and rapid eye movements. On the contrary, MCH-containing hypothalamic neurons suppress 'REM-off' brain centers, including the ventrolateral part of the periaqueductal gray matter (vlPAG), LPT, DRN and LC. In NREM sleep, GABA and galanin containing VLPO neurons inhibit arousal nuclei in brain stem, hypothalamus and BF. The endogenous sleep regulatory substance adenosine can actively excite sleep active cells of the VLPO.

In NREM sleep, DRN 5-HT-ergic and LC NE-ergic neurons inhibit cholinergic LDT/PPT cells. These DRN/LC neurons become silent in REM sleep, which enables the cholinergic LDT/PPT neurons to generate the hallmarks of REM sleep. GABA-ergic neurons participate in the mutual inhibition of REM-activating and REM-suppressing neurons. In REM sleep, the sublaterodorsal (SLD) nucleus and the precoeruleus (PC) area use ascending and descending projections, to activate the cortex and promote muscle atonia. In NREM sleep, vlPAG/LPT neurons inhibit these SLD/PC neurons.

The pathways presented above provide a conceptual framework of the neurochemical bases of sleep-wake regulation and the currently available pharmacological interventions to treat sleep-

wake disorders. Powerful new methods to interrogate sleep-wake regulating circuits have recently revealed additional molecular, cellular and network mechanisms and pathways in sleep-wake regulation, which may lead to an extension of the traditional views of sleep-wake neurochemistry (Tyree and de Lecea, 2017; Luppi and Fort, 2018). For example, the glutamatergic medial parabrachial nucleus in the dorsal pontine tegmentum regulates arousal (Fuller *et al.*, 2011) and the GABA-ergic parafacial zone in the pontomedullary junction promotes sleep (Anaclet *et al.*, 2012). Furthermore, human and animal studies employing different methodologies suggested diverse additional brain regions, neuronal structures and receptors as important regulators of wakefulness and sleep (Maquet *et al.*, 1997; Dang-Vu *et al.*, 2008; Murphy *et al.*, 2009; Dang-Vu *et al.*, 2010; Dittrich *et al.*, 2015; Holst *et al.*, 2017). These insights highlight the complexity of the mammalian brain and the sophisticated and fine-tuned regulation of wakefulness and sleep. Future progress is needed to pave the way for the development of novel rational sleep-wake therapeutics.

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Figure Caption

Figure 1. Outline of the neuroanatomical and neurochemical bases of sleep-wake regulation. **Wakefulness:** During waking, LDT/PPT cholinergic neurons and MA neurons innervate the thalamus and BF, MA neurons have a pronounced role, and in addition also directly innervate the cerebral cortex. Hcrt neurons of LH/PH reinforce the activity of this ascending arousal pathway and directly excite the BF. **NREM sleep:** NREM sleep is promoted by VLPO and MnPO nuclei, which inhibit the ascending arousal pathways. **REM sleep:** Cholinergic (ACh) neurons of LDT/PPT promote REM sleep. During REM sleep, NE and serotonergic (5-HT) neurons are inhibited. Entry into REM is inhibited by Hcrt and promoted by the VLPO. Black arrows indicate an excitatory connection. Gray inhibition lines indicate an inhibitory connection.

Abbreviations: 5-HT, 5-hydroxytryptamine; ACh, acetylcholine; BF, basal forebrain; DA, dopamine; Hcrt, hypocretine; His, histamine; LDT, laterodorsal tegmental; LH, lateral hypothalamus; MA, monoamine; MCH, melanin- concentrating hormone; MnPO, median preoptic; NE, noradrenergic; NREM, non-rapid-eye-movement; PPT, pedunculopontine tegmental; PH, posterior hypothalamus; REM, rapid-eye-movement; SCN, suprachiasmatic nuclei; VLPO, ventrolateral preoptic.

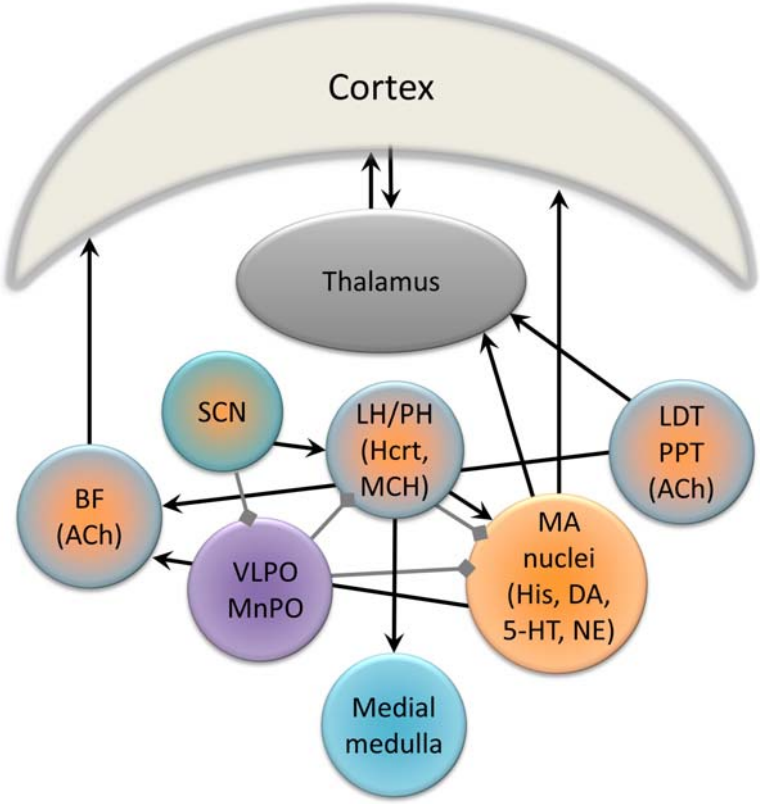
Cortex
Thalamus
SCN
LH/PH
(Hcrt,
MCH)
LDT
PPT
(ACh)
BF
(ACh)
MA
nuclei
(His, DA,
5-HT, NE)
VLPO
MnPO
Medial
medulla

Figure 1