Chapter 3

Hypothalamic Control of Sleep-Wake Circadian Cycle

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Abstract

Sleep-wake cycle is probably the most truthful signature of life. These unavoidable interchangeable states are together the matrix for all that occurs in physiology, and its rhythms are regulated by homeostatic and circadian processes involving different neuronal structures and distinct neural substrates. Hypothalamic regulation of sleep-wake cycle becomes of relevance as several neuropeptide-producing neurons involved in sleep and wakefulness regulation are located there. In this chapter, we provide a review of the hypothalamic regulation of sleep-wake cycle, focusing on the hypocretin system and melanin-concentrating hormone (MCH)-producing neurons located in the lateral hypothalamic area (LHA).

Keywords: hypothalamus, sleep-wake, circadian rhythm, hypocretin, orexin

1. Hypothalamus as a sleep-wake cycle regulator aside the RAS

The invention of the EEG by Hans Berger was a landmark in the history of sleep science. Until then, sleep was primarily considered to be a passive state, resulting from an exhaustion-modulated partial disconnection of sensory-motor circuitry from the higher-level neural regulators [1]. When early and after the first recordings of brain electrical activity, Berger established the alpha and beta waves as the EEG-dominant oscillations in healthy subjects [2]; he was proposing the electrophysiological definition of being awake. Later developments of Berger research allowed Frédéric Bremer, who was studying the physiology of the cerebellum and the neural control of muscular tone, to further investigate on the side effects of sleepiness after a lesion was produced on the hypothalamus. Although not precisely involved in sleep research, Bremer curiosity on exploring the functional effects of lower brain damages

further led him to perform cats' decerebration by which the forebrain was left in situ after a mesencephalic transection at intercollicular level. The results of this approach—the "cerveau isolé" model—leading to a persistent and indefinite condition with the brain deprived from the ascending sensory information, except for olfaction and optical ones, led Bremer to consider the hypothesis of sleep being a consequence of a complete deprivation of a sensory input arriving from the spinal cord. In this model, the cortical EEG pattern was dominated by a high-amplitude, low-frequency activity, like that observed in the slow-wave sleep (SWS). The following experiments, where the brain transection was performed at the level of the meeting point between the brain stem and the spinal cord, revealed very different results. In this "encephale isolé" model, an interchangeable oscillation between the sleep and the wake states, with an EEG pattern varying from the spontaneous low-frequency, high-amplitude activity usually observed in SWS, and high-frequency, low-amplitude activity, typical of wakefulness and rapid eye movement, was observed, not different from what can be noticed in a healthy condition. Although, at this time, Bremer was unaware of the reticular activating system (RAS), the assumption taken from his work that sleep was derived from a reduction in cortical tone while wakefulness resulted from the maintained sensorial flow to the brain served as the basis for later developments on sleep-wake cycle neurophysiology [3].

RAS was identified about 14 years later by Moruzzi and Magoun who significantly contributed to sleep-wake physiology by showing that brainstem reticular formation stimulation abolished EEG low-frequency activity and induced high-frequency activity in the cortical recordings [4]. Further experiments using the transection technique concluded that RAS underlies wakefulness, while its absence or its "silence" precipitates sleep [5]. These results were, however, obtained in acute experiments when EEG was assessed almost immediately after the brain damage. However, Villablanca [6] observed that, in the animals transected and maintained alive days or weeks after the surgical procedure, a waking-like EEG activity characterized by low-amplitude high-frequency waves was observed, suggesting that the forebrain could be involved in this partial recovery of the normal rhythm, in particular, its magnocellular region which contains cholinergic, GABAergic, and glutamatergic neurons. This allowed conceptualizing that the wake-state modulation may also be dependent regions located rostral to RAS, in particular, of the forebrain. Some studies showed that the electrical stimulation of the posterior hypothalamus and the basal forebrain in the isolated cat forebrain induced fast cortical EEG rhythms [7]. On the other hand, the cholinergic stimulation of these areas was shown to induce arousal, suggesting a role in the modulation of a wakening mechanism.

In a "diencephalic model," resulting from the removal of the cortex and striatum, leaving the thalamus, hypothalamus, and basal forebrain connected to the brain stem, animals became hyperactive, hyperreactive to sensory stimuli, and with a low-amplitude, high-frequency activity in the thalamus. In "athalamic animal" in which the thalamus was removed, they were also hyperactive and reactive to sensory stimuli, but they could not localize the stimuli and do not show very much awareness with only brief periods of low-amplitude, high-frequency activity.

To evaluate how close is the relationship between the structure and the elicited command to develop wake, we can infer using the latency of a stimuli to induce awake EEG. The stimulation of RAS-thalamic pathway is several times faster on inducing a wake-like pattern than stimulating basal forebrain or lateral hypothalamic/orexin pathways, thus meaning that for both regions, there is a need to project elsewhere to induce such a wake EEG pattern.

In the 1920s, during the influenza epidemic, a new type of encephalitis, attacking brain regions and regulating sleep and wakefulness, was described by Constantin von Economo. This disorder, which was eventually called encephalitis lethargica or von Economo's sleeping sickness, swept through Europe and North America, with some patients exhibiting severe insomnia, while others slept for 20 or more hours per day, arising only briefly to eat and drink. The postmortem autopsies of these patients indicated that those with an insomnia-like phenomenon had a damage in the anterior hypothalamus, whereas those with abnormally increased sleep periods showed an abnormal posterior hypothalamus. In view of that, an ascending arousal system originating in the brainstem that kept the forebrain awake was proposed and later described by Moruzzi and Magoun as the ascending reticular activating system. Later studies, during the 1980s, clarified the nature of this pathway.

Although Von Economo's work represented a crucial achievement for sleep research, the seminal studies of the hypothalamic-hypocretin system were performed by Lecea and Kilduff who characterized the mRNA-encoding hypocretin and identified that the neurons were responsible for its production [8]. Soon after their findings, the relationship between hypocretin/orexin neurons and narcolepsy was established with a mutation in the orexin-2/hypocretin-2 receptor observed in a narcoleptic dog [9]. Symptoms of narcolepsy, a disorder characterized by hypersomnolence and muscle weakness (cataplexy) triggered by emotion, were also associated to the absence of orexin/hypocretin [10] to the lack of orexinergic/hypocretinergic neurons [11] or orexin/hypocretin 2 receptor [12]. Cell bodies of those neurons are in the perifornical area and lateral hypothalamus (LH), responsible for RAS and tuberomammillar nucleus (TMN) neurons activation and are active during wake state and rapid eye movement (REM) sleep [13].

2. Orexinergic neurons, their receptors, and physio-pharmacological aspects of orexinergic system related to the sleep-wake cycle

Prepro-orexin protein is the precursor protein, generating the excitatory neuropeptides orexins A and B (hypocretins 1 and 2). Orexin A (hypocretin 1), with a structure of 33 amino acids and 3.5 kDa, is completely conserved among different mammals which reflects its physiological relevance. Orexin B (hypocretin 2) is a 28-amino acid peptide with 2.9 kDa with 46% similarity to orexin A [14]. Their neurons, located on the LH, project widely throughout the brain and spinal cord [15]. Orexin excites target neurons through two types of expressed G-protein-coupled receptors. Orexin 1 receptor (OX1R) is dominantly expressed in the locus coeruleus (LC) and orexin 2 receptor (OX2R) is dominantly expressed in the arcuate nucleus (Arc), ventrolateral hypothalamus (VMH), LH, and TMN. Both OX1R and OX2R are expressed in the raphe nucleus and ventral tegmental area (VTA).

Similar to other wake-promoting neurons, orexin neurons fire mainly during active wakefulness when orexin levels are highest and are silenced during NREM and REM sleep, concurring with the lowest levels of orexin [16].

Different neuronal pathways involving orexin and neurotransmitters affecting its activity were identified. Neuropeptide Y (NPY) and agouti-related peptide (AgRP) in the Arc project to orexin neurons [17]. Also, serotoninergic neurons in the median/paramedian

raphe nucleus and GABAergic neurons in the ventrolateral preoptic (VLPO) nucleus send axons to orexin neurons [18]. VLPO is of major importance on initiating and maintaining NREM sleep as their neurons are activated by the somnogens adenosine [19] and prostaglandin D2 [20], and VLPO damage reduces NREM and REM sleep [21]. Orexinergic neurons are also targeted by neuronal projections from the bed nucleus of the stria terminalis (BST), supraventricular zone, and dorsomedial hypothalamus (DMH) [18] and receive neuronal projections from the suprachiasmatic nucleus—the human master circadian clock [22]. A direct neuronal pathway between SCN and orexinergic neurons was not identified until now.

Since orexinergic neurons in LH are scarce and difficult to distinguish from other neurons just by morphology, a slice-path clamp technique, an electrophysiological method based on the expression of enhanced green fluorescent protein (EGFP) under the control of orexin promoter in transgenic mice, has been used in order to identify substances affecting orexinergic neuron activity [23, 24]. For instance, this allowed to assume the effects of distinct neurotransmitters on orexin neurons: glutamate receptor agonists AMPA and NMDA depolarize orexin neurons, while GABAA and GABAB receptor agonists muscimol and baclofen hyperpolarize those cells. Serotonin and noradrenaline hyperpolarize all orexin neurons through two receptors coupled to inhibitory Gi proteins (5HT1A and alpha 2A receptors, respectively) and subsequently activate protein-coupled inwardly rectifying potassium channels. Recent optogenetic methods allowed to confirm that the activation of serotoninergic neuron terminal inhibits orexin neurons either directly (via 5-HT1A receptor) or indirectly (via facilitation of GABAergic-inhibitory inputs) [25]. Dopamine also hyperpolarizes orexin neurons possibly by an indirect action through alpha 2A receptor [26], and glycine inhibits the activity of orexin neurons either directly and indirectly [27].

One complementary method to study the function of orexinergic neurons is to look for the physiological consequences of its ablation. Hara and coworkers generated transgenic mice, in which orexin neurons are ablated, and showed a phenotype similar to human narcolepsy [11], which also occurred in OX1r and OX2r knockout mice [28]. In transgenic mice, experimentally induced gradual ablation of orexin neurons using a specific "time-controlled death" technique was associated to a fragmentation of the usual sleep-wake cycle [29]. The anatomical proximity and the genetic co-localization of the orexin neurons regulating sleep-wake state have recently benefitted from optogenetics. Using this kind of approach, Adamantidis and collaborators showed that by increasing the activity of orexin neurons, there was also an increased probability of transition to wakefulness from either NREM or REM sleep [30]. On the other hand, results from Zhang group using the same kind of approach indicate that the acute inhibition of orexinergic neurons leads to a time-of-day-dependent induction of NREM sleep [31]. To overcome some difficulties related to the study of neuronal networks located deeper in the brain, several new-generation optogenetic tools are being developed with an expected great impact on the near future in the areas of chronobiology and sleep physiology.

3. Melanin-concentrating hormone (MCH) and MCH neurons

The melanin-concentrating hormone is a 19-amino acid peptide predominant in specific neurons with the cell body located in the lateral hypothalamus and incerto-hypothalamic area of mammals. Apart from the sleep-active neurons in the preoptic area, these groups of neurons are also active during sleep, especially in REM sleep [32]. MCH neurons project throughout the brain with a dense innervation of the cholinergic and monoaminergic arousal centers [33]. MCH decreases cAMP levels in the cell through the MCH receptor 1(MCHR1), a G-protein-coupled receptor linked to Gq, Gi, and Go subunits which are expressed widely in the brain [34], and cellular electrophysiological studies showed that MCH has both presynaptic and postsynaptic strong inhibitory effects [35, 36]. The evidence that MCHR1 is expressed in several areas of the brain including those which are part of physiological pathways within sleep-wake control mechanisms (hippocampus, subiculum, basolateral amygdala, shell of the nucleus accumbens, ventromedial nucleus, arcuate nucleus, tuberomammillary nucleus, dorsolateral pons including dorsal raphe, and locus coeruleus) [37] supports that MCH neurons must play an essential role on sleep-wake physiology.

Furthermore, while intracerebroventricular infusion of MCH peptide facilitates REM and NREM sleep [38], knockout of MCH is associated to a more active wakefulness state [39] and to a reduction on either REM or NREM sleep. Optogenetically selectively activated MCH neurons generally increase REM sleep duration [40–42]. Consistent results have shown that MCH neurons are strongly activated on REM sleep and de-activated during NREM, suggesting that MCH neurons promote REM sleep [32]. However, studies with timing-controlled ablation of MCH neurons revealed an increase in wakefulness and a reduction in NREM sleep, showing that MCH is also involved in the regulation of NREM sleep.

MCH neurons seem to inhibit some awake center neurons through GABAergic-inhibitory synapses onto histaminergic neurons of tuberomammillary nucleus. Recent work showed that the acute activation of MCH neurons, at the onset of REM sleep, extended the duration of this sleep stage but not that of the NREM sleep [42]. The inhibition of MCH neurons on the other hand reduces the frequency of theta rhythms from the hippocampus without interfering on REM sleep duration [41].

MCH neurons are excited by orexin, AMPA agonists, NMDA, and cannabinoid type-1 receptor agonists [43–45] and inhibit orexinergic and adjacent GABAergic neurons [46]. It is clear, however, that orexin may also inhibit MCH neurons via GABAa receptors [47]. Dopamine is also an MCH neuronal inhibitor either via alpha-2 receptor [48] or via D1- and D2-like receptors [49]. Furthermore, MCH neurons are inhibited by MCH itself and by GABA, noradrenaline, serotonin, acetylcholine, neuropeptide Y, and histamine [50]. This mutual inhibitory interaction between orexin neurons and MCH neurons in the LH is crucial for the regulation of sleep-wake physiological cycle [51–53].

4. Circadian regulation of sleep-wake cycles and some of its disturbances

Sleep disorders are complex phenomena. A detailed correlation of sleep-wake regulation and clinical states is beyond the scope of this chapter, but a few examples can help to bridge the basic science concepts to everyday clinical scenarios. Since the first description of the hypocretin/orexin system 20 years ago, a body of literature investigating the physiologic and pathophysiology role of this system, as well as the potential for drug development, has emerged. Disruption of this system has been linked to pathological sleep-wake states such as insomnia and narcolepsy. A role for the hypocretin/orexin system in other sleep disorders and in sleepiness associated with other neurological disorders has also deserved some investigation. Recent results indicate that subjects with head trauma or encephalitis may have moderately but significantly decreased hypocretin levels. A few selected subjects with Guillain-Barré syndrome, Parkinson's disease (PD), multiple system atrophy, and other neurodegenerative disorders have also been found to have shallow hypocretin levels. Importantly, central actions of orexin regulate motivated behaviors, stress response, and energy/glucose metabolism by coordinating regions of the central autonomic network and the endocrine system, these multiple actions of orexin being critical to maintaining life.

Considering these putative clinical targets, there has been an ongoing research in the development of selective hypocretin/orexin receptor agonists and antagonists. Recently, suvorexant became the first US Food and Drug Administration (FDA)-approved hypocretin/orexin receptor antagonist for the treatment of insomnia [54], and Nagahara and coworkers published a work on the first hypocretin/orexin agonist with good potency and pharmacological selectivity [55].

4.1. Primary hypersomnias

4.1.1. Narcolepsy

As previously mentioned, narcolepsy has been associated with changes in the orexinergic/hypocretinergic neurons. It is a disabling neurologic condition affecting around 1 in 2000 individuals, characterized by excessive daytime sleepiness, frequently running with sudden muscle paralysis (cataplectic attacks), and transitions from wakefulness into REM sleep [56]. Human narcolepsy is a genetically complex disorder and environmentally influenced. The association of HLA with human narcolepsy suggests that it may have an autoimmune origin. Available treatment strategies are mainly symptomatic and include amphetamine-like stimulants and antidepressants, being met with unsatisfactory results.

Canines with narcolepsy were found to have a mutation in the orexin-2 (hypocretin-2) receptor [57] while mice lacking the orexin peptide or the neurons containing orexin (hypocretin) displayed behavioral and EEG signs of narcolepsy [11, 58]. Human subjects with narcolepsy have been found to have a lack or very low levels of hypocretin neurons (with an 85–95% reduction in the number of neurons) and orexin-A in the CSF [59]. These findings have been corroborated by postmortem examination of brain tissue of subjects with narcolepsy,

depicting massive losses of orexin neurons [60]. It is not yet entirely clear what leads to this massive loss of the orexin neurons. By contrast, the number of melanin-concentrating hormone (MCH) neurons is not reduced in number, indicating that the cell loss is relatively specific for hypocretin neurons.

4.1.2. Idiopathic hypersomnia

Idiopathic hypersomnia is characterized by excessive daytime sleepiness, without sudden muscle paralysis (cataplectic attacks) nor abrupt transitions from wakefulness into REM sleep but with a dopaminergic and overall aminergic impairment associated with this condition. Some authors have described low but detectable levels of hypocretin in these patients [61], while others reported normal levels [62, 63]. Postmortem studies are not available yet.

4.2. Hypocretin studies in neurodegenerative disorders

4.2.1. Parkinson's disease

Sleep disturbances often occur in patients with Parkinson's disease (PD) and can even precede the motor symptoms, showing, in this way, the close relation at a central level between autonomic (non-motor symptoms) and sleep centers. Excessive daytime sleepiness has been reported in almost half of the PD patients [64, 65]. In postmortem brain studies, hypocretin-1 tissue concentrations in the prefrontal cortex were almost 40% lower in these patients, with the total number of hypocretin neurons being almost half compared with controls [66, 67]. A progressive loss of MCH neurons has also been described, increasing with the disease progression [67].

4.2.2. Multiple system atrophy

Sleep disturbances occur in 70% of patients with multiple system atrophy (MSA), a progressive neurodegenerative disease of undetermined etiology, characterized by parkinsonian features, cerebellar, autonomic, and urogenital dysfunction and corticospinal disorders [68]. The clinical features include reduced and fragmented sleep, excessive daytime sleepiness, rapid eye movement (REM), sleep behavior disorder (RBD), stridor, and sleep-disordered breathing [69, 70]. In these patients, Benarroch and coworkers found up to 70% reduction in the total number of hypocretin neurons in these populations of patients and described abundant glial cytoplasmic inclusions in the hypocretin distribution area [71].

4.3. Immune-mediated neurological disorders

4.3.1. Guillain-Barré syndrome

Guillain-Barré syndrome is a post-infectious polyradiculopathy affecting mainly the peripheral nervous system, frequently presenting also with autonomic nervous system failure symptoms. Not infrequently, these patients also show other signs of hypothalamic disturbance. Guillain-Barré syndrome has been the only disorder besides narcolepsy in which undetectable levels of

hypocretin have been consistently observed [63, 72]. Patients with the lowest levels tend to have a more severe and rapid disease course, running with tetraplegia and respiratory failure. The mechanism underlying the lack or very decreased levels of hypocretin in Guillain-Barré syndrome remains unknown, but an immune-mediated hypothalamic dysfunction has been hypothesized.

4.4. Orexin and sleep-related physical disorders: cardiovascular disease

Almost all bodily functions are dependent on the autonomic nervous system (ANS), which exerts precise control over visceral functions. Sleep disruption causes an increased activity of the sympathetic nervous system in association with an elevated blood pressure, and the risks of hypertension and cardiovascular disease are increased as a consequence of either strong acute or long-term sleep disruption [73]. The hypocretin/orexin system also contributes to the regulation of cardiovascular functions via the autonomic nervous system. Hypocretin/orexin neurons project to several brain regions involved in the regulation of cardiovascular activity, namely the paraventricular nucleus (PVN), nucleus tractus solitarius, and the rostral ventrolateral medulla (RVLM), all areas of the central autonomic network [74].

Over-activation of the hypocretin/orexin system has been implicated in the pathogenesis of hypertension. It has been shown that the central administration of orexins A and B increases arterial blood pressure and elicits tachycardia in animal models [74]. Conversely, orexin/ataxin-3 transgenic rats, lacking orexin neurons, have a significantly reduced sympathetic nervous system tone and a lower systolic blood pressure when compared with controls [75]. In addition, spontaneously hypertensive rats (SHRs) have increased levels of hypocretin/orexin [74] that, when blocked by the oral administration of almorexant or by intracerebroventricular injections of TCSOX229, led to a significant reduction of systolic blood pressure while not affecting arterial blood pressure in normotensive animals [76, 77]. These data suggest that hypocretin/orexin may play a significant role in the pathogenesis of hypertension. In humans, Dauvilliers and coworkers reported a lower cardiac activation associated with periodic leg movements during sleep in narcoleptic patients which was proposed to be related to changes in baroreflex sensitivity [78]. The same group found a large percentage of diastolic non-dippers, with 64% failing to achieve the 15% fall point on diastolic blood pressure [79], and recent data suggested that narcoleptic patients displayed a nighttime non-dipping blood pressure pattern with increased systolic blood pressure during nighttime REM sleep [80].

The blunted cardiac activation and sleep-related blood pressure fall in narcoleptic patients may be clinically relevant and may indicate an increased risk for cardiovascular events among attributable to a potentially clinically significant hypocretin/orexin deficiency.

5. Conclusion

In summary, despite being present throughout the animal kingdom, the precise sleep function is still relatively elusive. However, it is evident that sleep regulation is fundamental for survival having the hypothalamus a significant role in those modulatory processes through the orexin/hypocretin and the MCH neurons. Nevertheless, further studies on sleep physiology

are needed to determine the inner mechanisms associated with sleep-wake cycle and their regulatory processes.

Conflict of interest

The authors declare no conflict of interest.

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