1

NEUROANATOMY AND NEUROPHARMACOLOGY OF SLEEP AND WAKEFULNESS

Pablo Torterolo, Jaime M. Monti, and Seithikurippu Pandi-Perumal

ABSTRACT

Since the discovery of the ascending reticular activating system more than sixty years ago, the anatomy, electrophysiology, and neurochemistry of the neuronal networks involved in generating and maintaining wakefulness, that is, the activating systems have been characterized in detail. Furthermore, the neural areas critically involved in the generation and maintenance of rapid eye movement (REM) and non-REM (NREM) sleeps, which are called the hypnogenic systems, have also been delineated. The activating and hypnogenic systems deeply interact in order to induce the sleep/wakefulness cycle. These systems are modulated by the suprachiasmatic nucleus (SCN), the circadian rhythm pacemaker, as well as by various somnogenic substances such as adenosine and melatonin.

This chapter is a brief review on the neuroanatomy and functions of the activating and hypnogenic systems. The knowledge of neurobiological basis of these systems is crucial to understand the physiology of wakefulness and sleep, as well as to explain the pathophysiology of conditions such as insomnia, sleepiness, or abnormal behaviors during sleep (parasomnias). Additionally, the chapter highlights the concepts that can be easily applied to understand the neuropharmacology of sleep pathologies.

Pablo Torterolo
Department of Physiology, School of Medicine, Universidad de la República, Montevideo, Uruguay

Jaime M. Monti
Department of Pharmacology and Therapeutics, School of Medicine, Clinics Hospital, Universidad de la República, Montevideo, Uruguay

Seithikurippu Pandi-Perumal
Somnogen Canada Inc., College Street, Toronto, ON, Canada

Corresponding author: Dr. Pablo Torterolo, Departmento de Fisiología, Facultad de Medicina, Universidad de la República. General Flores 2125, 11800 Montevideo, Uruguay. Email: ptortero@fmed.edu.uy. Fax: 598 29243414-3338

1.1. INTRODUCTION

In humans, most mammals, and birds, three behavioral states can be readily distinguished: wakefulness (W), non-Rapid eye movement (NREM) sleep (also called slow wave sleep (SWS)), and rapid eye movement (REM) sleep. Polysomnography (PSG) is the basic tool used to differentiate these states. It consists of the simultaneous recording of various physiological parameters such as the electroencephalogram (EEG), the electromyogram (EMG), and eye movements; other bioelectrical signals can also be recorded in humans or experimental animals (Figure 1.1). The main features of the human PSG are summarized in Table 1.1.

During W, an optimal interaction with the environment allows the development of various behaviors necessary for survival. In humans, W is accompanied by awareness (consciousness) of the environment and internally generated stimuli such as hunger and thirst. The EEG recording of W is marked by the presence of high frequency and low amplitude (cortical activation, Figure 1.1) determined by the activity of thalamic and cortical neurons.

During sleep, there is a marked decrease in the interaction with the environment, an increase of the threshold for the reaction to external stimuli, and a decrease in somatomotor activity. Furthermore, animals adopt a distinctive position to conserve heat.

Presently, three NREM sleep phases (stages N1, N2, and N3 or SWS) are distinguished in humans according to the depth of the state. From stage W, normal adults enter in light NREM sleep (or stage N1). Stage N2 is characterized by the presence of sleep spindles and K-complexes, while the presence of low frequency (0.5–4 Hz) of high-amplitude delta waves characterizes the EEG during N3. Furthermore, tonic parasympathetic activity increases, determining characteristic changes in visceral activity. In the deeper stages of NREM sleep, cognitive activity (that is, dreams) is minimal (Dement and Kleitman, 1957; Pace-Schott, 2005).

REM sleep (or stage R) occurs periodically, and is always preceded by NREM sleep. REM sleep is a deep sleep stage although the EEG is similar to that of stage W; hence, it is also called "paradoxical" sleep. REM sleep is characterized by fast REMs that typically occupy 20–25% of total sleep in human adults. REM sleep occurs ~90 min after sleep onset. There are both *phasic* (episodic) and *tonic* (persistent) components in the stage R. Dreams occur mainly during REM sleep, which is also accompanied by muscle atonia as evidenced in the EMG channel (Figure 1.1), and phasic changes in autonomic activity. A shortened REM onset latency (REMOL; it is the interval between the sleep onset and the appearance of the first REM sleep episode) is a biological marker of primary depression. It is also considered to be a clinically significant pathological feature in other brain diseases.

In rats, a species commonly used in preclinical studies, W and sleep are defined by PSG as follows (Figure 1.1):

1) W, by the presence of low-voltage fast waves in frontal cortex, a mixed theta activity in occipital cortex, and relatively high EMG activity;
2) Light sleep, by the occurrence of high-voltage slow cortical waves

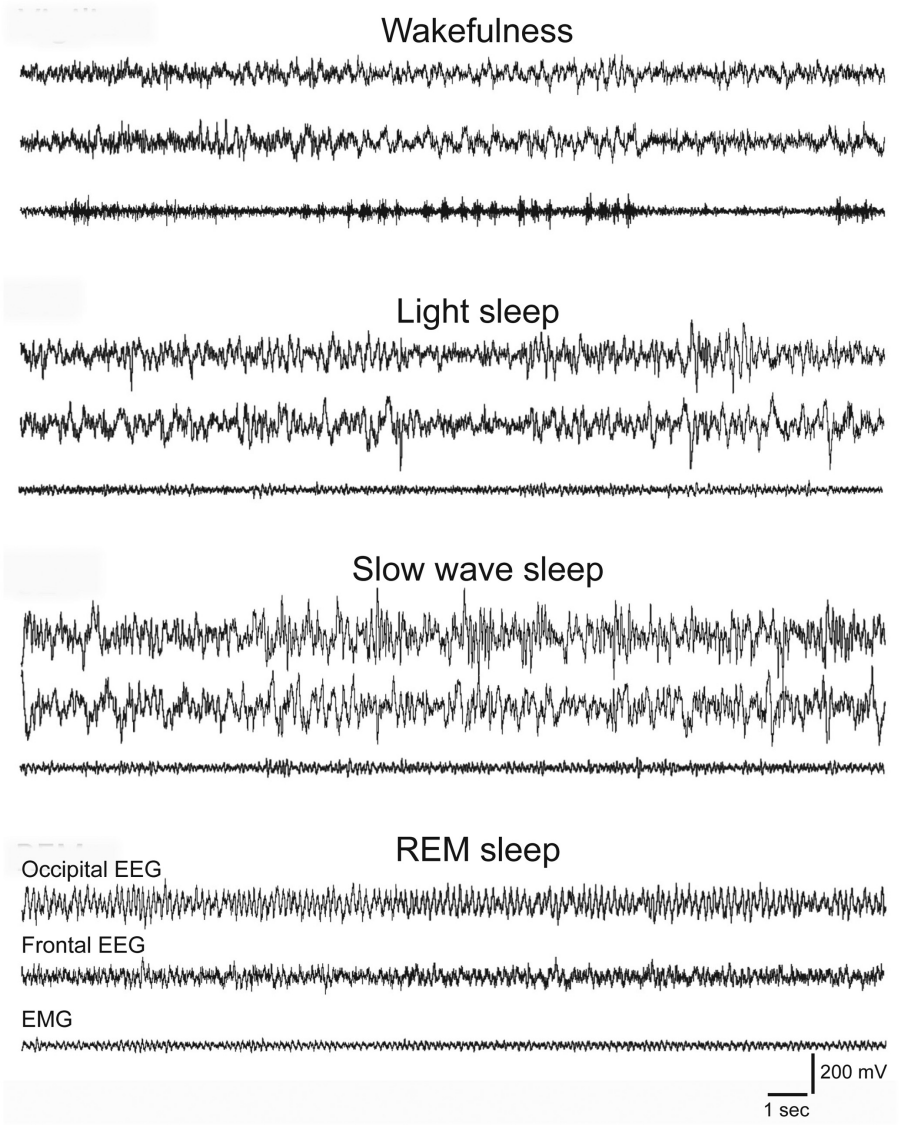

Figure 1.1: Polysomnographic recording during sleep and wakefulness in the rat. EEG, electroencephalogram; EMG, electromyogram.

Table 1.1: Electroencephalographic correlates of sleep stages.

		Characteristics			
Sleep stages	**TST (%)**	**EEG**	**EOG**	**EMG**	**Other variables**
Stage awake (relaxed wakefulness)		Alpha activity (8–12 Hz) or low-amplitude beta (13 35 Hz), mixed-frequency waves	REM (in sync or out of sync deflections), eye blinks	Relatively high tonic EMG activity	Alpha activity in occipital leads compared with central leads, eye opening suppresses alpha activity, movement artifacts
N1, formerly known as stage 1	2–5	Low-voltage, mixed-frequency waves (2–7 Hz range), mainly irregular theta activity, triangular vertex waves	SEMs, waxing and waning of alpha rhythm	Tonic EMG levels typically below range of relaxed wakefulness	Alpha ≤50%, vertex sharp waves in central leads, absence of spindles and K complexes
N2, formerly known as stage 2	45–55	Relatively low-voltage, mixed-frequency waves, some low-amplitude theta and delta activity	No eye movement	Low chin muscle activity	Sleep spindles (7–14 Hz) and K complexes occur intermittently
N3, formerly known as stages 3 and 4	5–20	≥20–50% of epoch consists of delta (0.5–2 Hz) activity	No eye movement	Chin muscle activity is lower than N1 and N2	Sleep spindles may be present
Stage REM	20–25	EEG is relatively low voltage with mixed frequency resembling N1 sleep	REM. Episodic rapid, jerky, and usually lateral eye movements in clusters	EMG tracing almost always reaches its lowest levels owing to muscle atonia	Phasic and tonic components, presence of sawtooth waves, alpha waves are 1–2 Hz slower than waves occurring during wakefulness and non-REM sleep

EEG, electroencephalography; EMG, electromyography; EOG, electrooculography; REM, rapid eye movement; SEMs, slow eye movements; TST, total sleep time.

interrupted by low voltage fast EEG activity;

3) SWS, by the occurrence of continuous high-amplitude slow frontal and occipital waves; light sleep + SWS is called NREM sleep;
4) REM sleep, by the presence of low-voltage fast frontal waves, a regular theta rhythm in the occipital cortex, and a silent EMG except for occasional myoclonic twitching.

Sleep in humans during the night shows four to five NREM-REM sleep cycles (the period from the sleep onset to the end of first REM episode or the period from the end of a REM sleep episode to the subsequent REM sleep episode). The average length of human sleep cycles is about 90–120 min. In contrast, the average sleep cycle duration of the rat is about 10 min (Trachsel et al., 1991).

1.2. COGNITIVE ACTIVITY THROUGH ACTIVATION OF THE THALAMUS AND THE CORTEX

Cognitive activities (consciousness and dreams) and the different EEG rhythms that support these functions are generated by the activity of cortical and thalamic neurons, which are mutually interconnected. Thalamic neurons have a complex electrophysiology that allows them to operate differently according to their level of polarization (Steriade et al., 1993). When hyperpolarized, the thalamic neurons that project to the cortex (thalamocortical neurons) oscillate at low frequency (0.5–4 Hz, delta rhythm), and tend to block the information toward the cortex that goes through the sensory pathways. This "oscillatory mode" of function synchronizes the cortical neurons and accompanied by other phenomena, generates the slow waves of NREM sleep. On the contrary, when these neurons are relatively depolarized, they enter in the "tonic mode" of function. In this condition, the thalamocortical neurons transmit sensory information toward the cortex in a reliable way. This mode of function occurs during W and REM sleep.

Therefore, the thalamus is critical for the generation of slow waves and spindles that characterize NREM sleep. When the thalamus is lesioned as it occurs in the "fatal familial insomnia," the generation of these electrographic signs is blocked and sleep is prevented (Montagna, 2005).

Neurons that form part of the activating system, that is, the neuronal system that generates and maintains wakefulness are summarized in Figure 1.2. The activating neurons project directly to the thalamus and/or the cortex (Jones, 2005). They depolarize thalamic neurons in order to produce the thalamic tonic mode and desynchronization (activation) of the EEG that accompanies the behavioral awakening. Part of the activating system (the cholinergic nuclei) is also active during REM sleep and activates the corticothalamic system during this behavioral state.

1.3. THE ACTIVATING SYSTEM

Which are the neural mechanisms involved in the generation and maintenance of the behavioral states?

In the 1930s, before REM sleep discovery, Bremer proposed that the baseline state of the brain was sleep (Bremer, 1935). His proposal was based on experimental transections at the level of the intercollicular region of the midbrain, in a prepa-

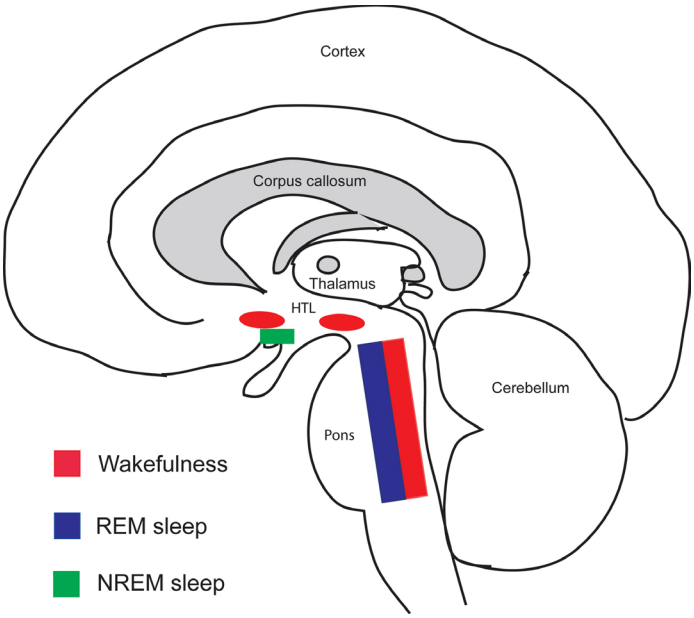

Figure 1.2: Activating (labeled in red) and hypnogenic (labeled in blue for REM sleep and green for NREM sleep) systems are shown. HTL, hypothalamus.

ration known as "cerveau isolé" ("isolated forebrain"). Animals with this injury had an EEG similar to that observed during NREM sleep. Since these animals had lesions in the ascending sensory pathways at the level of the midbrain, at that time it was considered that sleep occurred because sensory inputs to the diencephalon and telencephalon were reduced. In other words, sensory activity was viewed as necessary to maintain W. It was considered that the sensory blockade accompanied by ill-defined neuronal "fatigue," was the cause of sleep. This concept was known as the passive hypothesis of sleep (or deafferentation).

In 1949, Moruzzi and Magoun published their seminal work entitled: *Brainstem reticular formation and activation of the EEG* (Moruzzi and Magoun, 1949). This work is considered one of the most influential contributions in the field, and it inspired numerous investigations in the following decades. The reticular formation (RF) is located in the central area of the brainstem. Neurons in this region are not grouped in nuclei but are arranged in a complex mesh or network. This region is characterized by its high connectivity, receiving afferents from different sources and sending efferents to different sectors of the central nervous system (CNS) (Jones, 1995). Moruzzi and Magoun (1949) performed electrical stimulation at different rostrocaudal levels of the RF. Their main finding was that electrical stimulation of the RF activates the EEG during anesthesia, that is, from an EEG with high-amplitude and low-frequency waves (SWS-

like), the stimuli induced a high frequency, low amplitude EEG as in W. The activation of the EEG was widespread (throughout the neocortex) and in lightly anesthetized animals, it was accompanied by behavioral awakening.

The role of the midbrain and pontine RF in generating and maintaining W was confirmed later making use of different approaches. This area along with its ascending projection was called "reticular ascending activating system" (RAAS). Of note, lesions of this area in patients and experimental animals generate a coma condition (Lindsley et al., 1950; Plum and Posner, 2000).

At the time of its discovery, the RAAS did not contradict the "passive" hypothesis of sleep, but complemented it. It was believed that sleep is initiated by the progressive inactivation of the RAAS, where the decrease in sensory input played a major role. This hypothesis was named "passive inactivation of the RF" or "reticular sleep hypothesis."

The activation of the RAAS promotes the thalamocortical activation (as evidenced by EEG desynchronization) that supports cognitive awakening. The arousal reaction is accompanied by motor, autonomic, and endocrine changes. Then, the RF would also modify directly or indirectly, the activity of motoneurons, autonomic preganglionic neurons, and hypothalamic endocrine-related neurons. In this review, we will emphasize aspects related to the cognitive awakening and EEG activation. Therefore, only the upward or ascending (thalamocortical) component of the RAAS will be considered.

The identification of specific neuronal groups that use different neurotransmitters was the beginning of a new era for understanding the RF and the RAAS. Furthermore, different experimental approaches allowed obtaining details of the physiology of the activating system. Nowadays, the following concepts have been established: (i) The RAAS is composed of various neuronal groups that differ in their neurotransmitters; (ii) the neurons from specific regions of the posterior and lateral (perifornical) hypothalamus and basal forebrain, that are considered the rostral extension of the RF of the brainstem, behave as activating nuclei. Thus, the RAAS, the posterior and posterolateral hypothalamus, and the basal forebrain constitute the activating system; (iii) the neuronal groups that make up the activating systems project through a dorsal pathway toward the specific and non-specific thalamic nuclei, and/or a ventral pathway passing through the lateral hypothalamus, and basal forebrain toward the cerebral cortex.

The different components of the activating system are discussed below.

1.3.1. Mesopontine Glutamatergic Neurons

Anatomical and functional studies have shown that the main component of the RAAS is located within the mesopontine RF. With respect to the glutamatergic neurons, they do not form a specific group but are distributed throughout the mesopontine RF intermingled with specific neuronal groups. Regarding their functional activity during the sleep–wakefulness cycle, mesopontine W/REM-on, REM-on, or W-on glutamatergic neurons have been identified (Boucetta et al., 2014).

It has been contended that ketamine, a *N-methyl-D-aspartate* (NMDA) glutama-

tergic antagonist, inhibits W and produces sedation, hypnosis, or pharmacological coma; part of these effects could be produced by reducing the synaptic effects of mesopontine glutamatergic neurons (Wolff and Winstock, 2006).

1.3.2. Noradrenergic Neurons of the Locus Coeruleus

The locus coeruleus (LC) is a noradrenergic nucleus located in the mesopontine dorsolateral region. The ascending projections of this nucleus are part of the dorsal pathway to the thalamus, and are also included in the ventral pathway that project directly to the cerebral cortex. LC noradrenergic neurons show their maximum firing rate during W; it decreases during NREM sleep and is minimal during REM sleep (Aston-Jones and Bloom, 1981). This profile of activity is in agreement with the pattern of release of noradrenaline in the cerebral cortex as measured by microdialysis (Berridge and Abercrombie, 1999). It should be mentioned that during W, these neurons markedly increase their firing rate following a new stimulus, but the response is reduced after habituation, which led to the proposal that this neuronal group regulates attention (Foote et al., 1991). Interestingly, a1 antagonists including prazosin facilitate the generation of sleep, while a2 agonists such as dexmedetomidine inhibit the activity of LC neurons and are used as sedatives (Nishino and Mignot, 1997; Nelson et al., 2003).

1.3.3. Midbrain Dopaminergic Neurons

The substantia nigra pars compacta (SN) and the ventral tegmental area (VTA) are located in the midbrain. Both regions are characterized by the presence of dopaminergic neurons; while dopaminergic neurons of the SN project to the dorsal striatum, VTA neurons project to the prefrontal cortex and nucleus accumbens (ventral striatum) (Oades and Halliday, 1987). The firing rate of dopaminergic neurons and extracellular concentration of dopamine in the prefrontal cortex increases during reward-related stimuli (Mirenowicz and Schultz, 1996; Feenstra, 2000). Dopamine agonists and antagonists increase and decrease W, respectively (Monti and Monti, 2007; Monti and Jantos, 2008). Presently available evidence tends to indicate that these dopaminergic neurons are involved in the arousal that accompanies reward and motivation. Cocaine and amphetamines inhibit dopamine reuptake and induce its release, respectively. As expected, their administration produces an increase of W. In addition, these drugs are strong positive reinforcers, which is because of their addictive power. Notwithstanding this, drugs that increase synaptic dopamine levels are in the first-line for the treatment of hypersomnia (Nishino and Mignot, 1997).

1.3.4. Serotonergic Neurons of the Rostral Raphe Nuclei

Serotonergic neurons of the dorsal (Figure 1.3A) and median raphe nuclei are located within the mesopontine midline (Jacobs and Azmitia, 1992; Monti, 2010b, a). These neurons project toward the thalamus and cortex. Serotonergic neurons discharge more frequently during W, decrease their activity during NREM sleep, and virtually turn off during REM sleep (McGinty and Harper, 1976). A similar pattern of

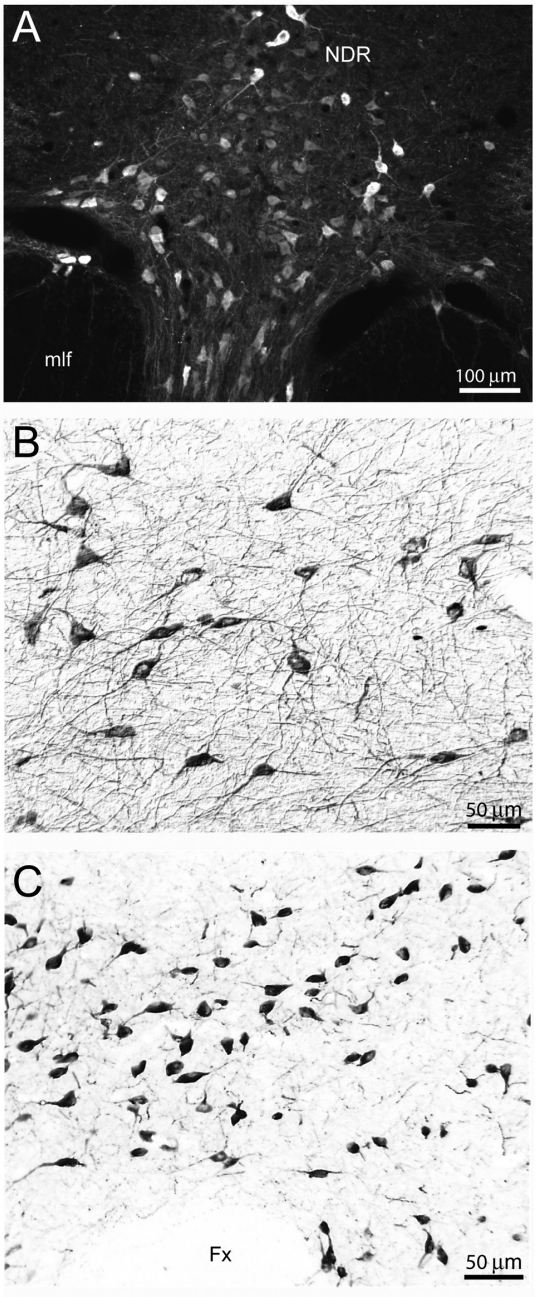

Figure 1.3. (A) Photomicrographs illustrating serotonergic neurons of the dorsal raphe nucleus of the rat; (B) cholinergic neurons of basal forebrain of the cat, and; (C) hypocretinergic neurons of the guinea pig. These neurons were revealed following immunohistochemistry procedures.

activity has been observed with respect to the release of serotonin as measured by microdialysis (Portas et al., 2000). Subgroups of these serotonergic neurons are activated during stereotyped movements that take place when the experimental animal is moving or grooming (Jacobs and Fornal, 2008). In turn, electrical stimulation of the dorsal raphe nucleus produces a marked EEG activation (Dringenberg and Vanderwolf, 1997). It has been proposed that serotonergic neurons play a permissive role in the generation of REM sleep such that they must be inhibited for REM sleep to occur (McCarley, 2007). Local GABAergic neurons would be involved in this inhibition (Torterolo et al., 2000). Since there are several types of receptors for serotonin, the effect of serotonergic drugs on sleep is complex (Monti and Jantos, 2008).

1.3.5. Cholinergic Neurons of the LDT-PPT

Mesopontine cholinergic neurons are located in the laterodorsal and pedunculopontine tegmental nucleus (LDT-PPT). These neurons project directly to the thalamus (Satoh and Fibiger, 1986). Cholinergic neurons are activated during W in close relation to the cortical activation. They are inhibited during NREM sleep and re-activated during REM sleep (Boucetta et al., 2014). In the thalamus, acetylcholine acts on muscarinic and nicotinic receptors in order to produce cortical activation (Curro Dossi et al., 1991). In humans, increasing synaptic levels of acetylcholine by acetylcholinesterase inhibitors produces W and cortical activation, while REM sleep precipitates if this drug is applied during NREM sleep (Gillin and Sitaram, 1984). These data suggest a bimodal role of cholinergic neurons, promoting both the generation of W and REM sleep. Of note, drugs that increase synaptic levels of acetylcholine, such as physostigmine, reverse the state of general anesthesia produced by *sevoflurane* in humans (Plourde et al., 2003).

1.3.6. Mesopontine GABAergic Neurons

GABAergic neurons, terminals, and receptors are distributed throughout the mesopontine region. In contrast to the effects of hypnotics that enhance GABAergic neurotransmission and facilitate sleep, the application of GABAergic receptor agonists into the NPO generates W (Xi et al., 1999). Furthermore, local increase of GABA levels in the NPO prolongs the time necessary to induce general anesthesia, while isoflurane anesthesia reduces GABA levels within the NPO (Vanini et al., 2008).

1.3.7. Histaminergic Neurons of the Posterior Hypothalamus

Neurons using histamine as a neurotransmitter are located only in the tuberomammillary nucleus of the posterior hypothalamus, and project to the thalamus and cortex (Monti, 2011). The firing rate of the histaminergic neurons decreases when passing from W to NREM sleep and is minimal during REM sleep (Takahashi et al., 2006).

The information provided by "knockout" mice lacking histidine decarboxylase (enzyme involved in the synthesis of histamine) is revealing; these animals are unable to stay awake when they are placed in a new environment (Parmentier et al., 2002). Drugs that increase synaptic levels of

histamine augment cortical activation and W (Kalivas, 1982). In humans, drugs that antagonize the H1 receptor including pyrilamine and diphenhydramine and have been prescribed as anti-allergic, cause drowsiness as a side effect (Roth et al., 1987).

1.3.8. Hypocretinergic Neurons of the Posterior Hypothalamus

In 1998, two independent research groups identified hypocretins almost simultaneously by different techniques (de Lecea et al., 1998; Sakurai et al., 1998). Hypocretin 1 and 2 (also called orexin A and B) neuropeptides are synthesized by a small group of neurons located exclusively in the dorsal, posterior, and lateral hypothalamic region (de Lecea et al., 1998; Sakurai et al., 1998) (Figure 1.3C). These neurons use the hypocretins as neurotransmitters and project diffusely throughout the CNS, including mesopontine areas critical for waking and sleep generation (Figure 1.4). Hypocretins act on two types of metabotropic receptors exerting presynaptic and postsynaptic excitatory effects.

The intracerebral or intraventricular administration of hypocretins facilitates the generation of W (Piper et al., 2000). In turn, several experimental approaches have shown that these neurons are primarily activated during motivated W; their activity is reduced during NREM sleep and is almost absent during tonic REM sleep; however, hypocretinergic neuronal activity seems to increase in the presence of the phasic components of REM sleep.

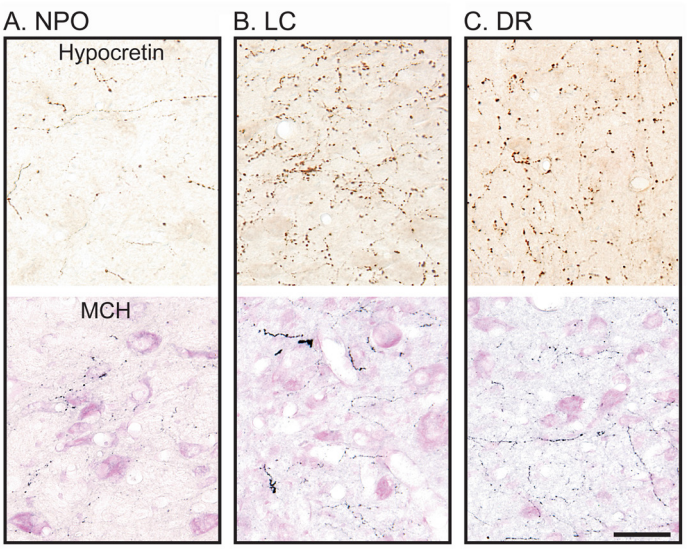

Figure 1.4. Hypocretinergic (above) and MCHergic (below) fibers in the nucleus pontis oralis (A. NPO), locus coeruleus (B, LC), and dorsal raphe nucleus (C, DR) of the cat. The sections were prepared for immunohistochemistry to detect hypocretin-1 and MCH. The sections treated with MCH antibodies (below) were also counterstained with pyronin-y. Calibration bar, 100 mm. This figure highlights the strong interconnection among the neurons that are critically involved in the generation of sleep and wakefulness.

(Torterolo et al., 2001b; Kiyashchenko et al., 2002; Torterolo et al., 2003; Lee et al., 2005b; Mileykovskiy et al., 2005; Torterolo et al., 2009c; Torterolo and Chase, 2014). The medical importance of this system boosted when Nishino et al., (2000) showed the absence of hypocretin 1 in the cerebrospinal fluid of narcoleptic patients; degeneration of these neurons is the pathological basis of narcolepsy-cataplexy.

1.3.9. Basal Forebrain Cholinergic Neurons

These cholinergic neurons are located in the area known as basal forebrain (anterior to the hypothalamus), which includes the nucleus basalis of Meynert (Figure 1.3B). The main projections of these neurons are to the neocortex, hippocampus, and reticular thalamic nucleus (Semba, 2000). Chemical and electrical stimulation of this region generates cortical activation and W, whereas its inactivation produces NREM sleep (Belardetti et al., 1977; Cape and Jones, 2000). During W and REMS, there is an increase in the firing rate of basal forebrain cholinergic neurons which is correlated with EEG activation and an increase in the release of the acetylcholine at cortical levels (Marrosu et al., 1995; Lee et al., 2005a). During W, these neurons regulate sensory information processing, attention, and learning. Of note, cognitive disorders characteristic of Alzheimer's disease are related to lesions of this neuronal group (Coyle et al., 1983; Vitiello and Borson, 2001).

1.3.10. Role of Wake-Promoting Neurons in the Different Types of Wakefulness

There is an important anatomical and functional relationship between the activating neuronal groups (Figure 1.4), which tends to indicate that these neurons act in tandem to generate and maintain W. W is a heterogeneous process. Thus, it is not the same as state of W when caused by nociceptive stimulation, by intense motor activity, or just during relaxed activity. There is evidence showing that the relative activity of the different components of the activating system varies with the type or level of W. For example, experimental studies using Fos protein as an index of neuronal activity have shown that the hypocretinergic neuronal activity increases during W with motor activity related to the motivation to explore a new environment, but not during quiet wakefulness or forced locomotion (Figure 1.5) (Torterolo et al., 2001b; Torterolo et al., 2003; Torterolo et al., 2009c). Moreover, serotonergic neurons are active during W related to stereotyped and automatic motor activity, while LC noradrenergic neurons would be critical in the increased surveillance that occurs following a new stimulus (Foote et al., 1991; Jacobs and Fornal, 2008).

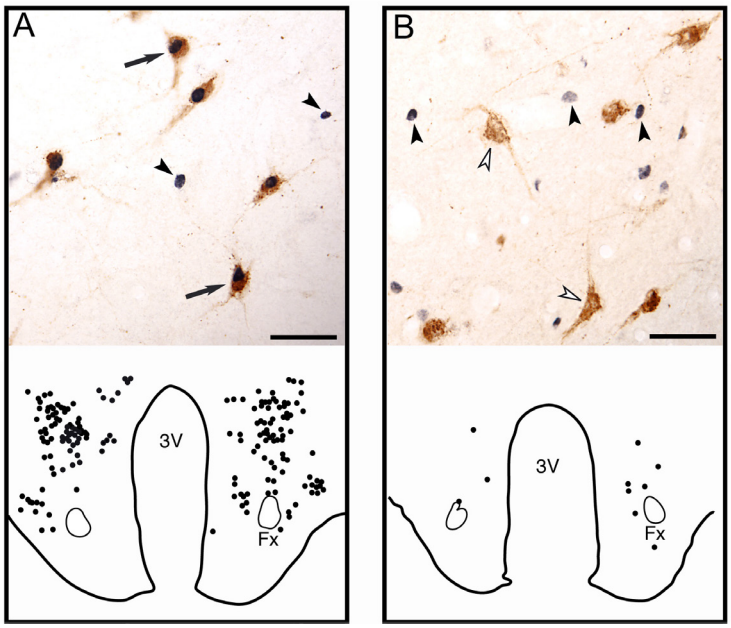

Figure 1.5. Photomicrographs illustrating hypocretin and Fos (a marker of neuronal activity) immunoreactive neurons from the posterolateral area of the hypothalamus. (A) Above, hypocretinergic neurons that express c-Fos (arrows) during active wakefulness with motor exploratory activity. Hypocretinergic neurons are stained in brown, Fos immunoreactivity, which is black, is restricted to nuclei. Hcrt- Fos+ neurons (arrowheads) are also intermingled with Hcrt+ Fos+ neurons. Below, camera lucida drawing of a representative hypothalamic sections of the same animal. The distribution of Hcrt+ Fos+ neurons is represented. Each mark indicates one labeled neuron (3V, third ventricle; Fx, fornix). (B) Above, group of hypocretinergic neurons during quiet wakefulness. The hypocretinergic neurons shown did not express c-Fos (unfilled arrowheads, i.e., not active), although Hcrt- Fos+ neurons are intermingled with these neurons (filled arrowheads). Below, the distribution of Hcrt+ Fos+ neurons in a representative section for the same animal is shown. This figure highlights the fact that hypocretinergic neurons are active during active wakefulness, but not during quiet (relaxed) wakefulness. Modified from Torterolo et al. (2001b).

1.4. HYPNOGENIC SYSTEMS

1.4.1. NREM Sleep: Preoptic Area

The neuronal groups critical in the generation and maintenance of NREM sleep are located in the preoptic area (POA) of the hypothalamus (Kumar, 2004; Szymusiak et al., 2007; Torterolo et al., 2009a; Benedetto et al., 2012) (Figure 1.2). These neurons, increase their firing rate during NREM sleep, and have been identified in the medial, median, and ventrolateral region of the POA. Electrical stimulation of the POA and adjacent basal forebrain induces NREM sleep and inhibits the activating system; in fact, GABAergic neurons of the POA project toward the activating system (McGinty and Szymusiak, 2005).

In turn, neurons from the activating system inhibit hypnogenic regions (Gallopin et al., 2000). This reciprocal inhibition between activating and hypnogenic neurons is critical for the transition between sleep and W.

1.4.2. REM Sleep Generation

The necessary and sufficient neuronal networks critical for the generation and maintenance of REM sleep are located in the mesopontine RF, where the RAAS is located (Figure 1.2) (Siegel, 2011). In fact, the LC noradrenergic neurons, and the dorsal raphe nucleus serotonergic neuron are active during W but turn off their firing during REM sleep (REM off neurons). Conversly, cholinergic neurons of the LDT-PPT increase their firing rate during REM sleep, thus contributing to the cortical activation of this state (McCarley, 2007; Boucetta et al., 2014). These cholinergic neurons also project to the NPO that is the executive area for REM sleep generation. Then, acetylcholine is released within this area to induce REM sleep. This effect is mimicked by microinjection of carbachol, a mixed (nicotinic and muscarinic) cholinergic agonist, into the NPO of a cat. Carbachol generates a long duration (up to 2 h) REM sleep episode with a very short latency (down to 30 s) (Figure 1.6). Physiologically, it is considered that cholinergic neurons of the LDT-PPT activate glutamatergic neurons of the NPO. In turn, these neurons activate different groups of neurons that execute REM sleep functions such as atonia, REMs, EEG activation, autonomic unsteadiness, and so forth. For example, REM sleep atonia depends upon glutamatergic neurons of the NPO that project to the magnocellular medullary RF and depolarize premotor glycinergic neurons (Chase, 2013). These neurons produce the postsynaptic inhibition of motoneurons during REM sleep.

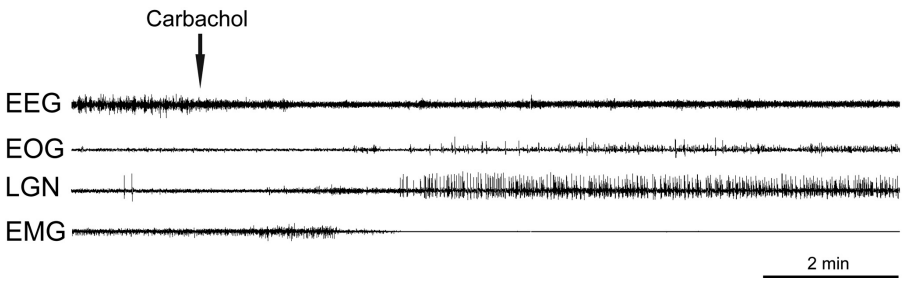

Figure 1.6. Polygraphic recording during the onset of an episode of REM sleep induced by a microinjection of carbachol into the nucleus pontis oralis of the cat. REM sleep is signaled by the appearance of ponto-geniculo-occipital (PGO) waves in the lateral geniculate nucleus (LGN), a decrease in muscle tone, REMs, and EEG desynchronization. This episode of REM sleep was maintained for approximately 2 h. An arrow signals the beginning of the microinjection of carbachol into the nucleus pontis oralis. EEG, electroencephalogram; EOG, electrooculogram; EMG, electromyogram; NGL, electrogram of the lateral geniculate nucleus (visual thalamus).

GABAergic neurons of the mesopontine area (dorsal raphe nucleus, LDT-PPT, ventrolateral periaqueductal gray, and so forth) also play an important role in the generation of REM sleep (Torterolo et al., 2000, 2001a; Torterolo et al., 2002a; Torterolo et al., 2002b; Vanini et al., 2007; Torterolo and Vanini, 2010). In fact, there are models which propose that REM sleep generation depends upon the activity of mesopontine GABAergic neurons (Lu et al., 2006; Luppi et al., 2007).

The hypothalamus also participates in the generation of REM sleep. As mentioned before, histaminergic neurons are REM-off. Moreover, hypocretinergic neurons decrease their firing rate during "tonic" REM sleep; however, Fos protein and microdialysis studies conducted in cats suggest that these neurons may be active during the "phasic" components of REM sleep, and may contribute to the induction of twitches, REMs, autonomic instability, and so forth (Torterolo and Chase, 2014). The role of the hypothalamic melanin-concentrating hormone (MCH) will be described in the next section.

1.4.3. Melanin-Concentrating Hormone: A Sleep Promoting Factor

There are neurons in the posterolateral hypothalamus (intermingled with the hypocretinergic neurons) and incertohypothalamic area that utilize the neuropeptide MCH as a neuromodulator (Torterolo et al., 2006; Torterolo et al., 2011a; Monti et al., 2013), and project throughout the CNS, including the mesopontine RF, where the RAAS is located (Figure 1.4) (Bittencourt et al., 1992). These neurons fire scarcely during W, increase their firing rate during NREM sleep, and reach a maximum during REM sleep. Since, the administration of MCH into the cerebral ventricles, preoptic area, basal forebrain, dorsal raphe nucleus, LC, and NPO facilitates the generation of NREM sleep and/or REM sleep, it is possible that MCHergic neurons inhibit the activating systems and/or activate the hypnogenic nucleus in order to promote sleep (Verret et al., 2003; Lagos et al., 2009; Torterolo et al., 2009b; Lagos et al., 2012; Benedetto et al., 2013; Monti et al., 2015a). Recent studies have demonstrated that MCH inhibits dorsal raphe nucleus serotonergic neurons (Devera et al., 2015); this finding may explain, at least in part, the promotion of REM sleep induced by MCH (Lagos et al., 2009). The suppression of the serotonergic activity by MCH could explain also the pro-depressive effect of this neuropeptide (Lagos et al., 2011; Lopez Hill et al., 2013; Urbanavicius et al., 2014).

1.5. TRANSITION FROM WAKEFULNESS TO NREM SLEEP

The physiological transition between W and sleep is regulated by a circadian and a homeostatic component (Borbely, 1982). Like all circadian rhythms, sleep and W are regulated by commands from the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN receives photic information directly from the retina, and regulates the activity of both the hypnogenic and activating systems (Mistlberger, 2005). Furthermore, through indirect modulation of the sympathetic system, the SCN regulates the release of melatonin from the pineal gland during the night (Pandi-Perumal et

al., 2008). Melatonin has a weak sleep-promoting effect.

The homeostatic component also regulates the sleep–wakefulness cycle, that is, prolonged W facilitates the generation of sleep. Different lines of research have shown that sleep-promoting substances including adenosine, are released and accumulated during W (Basheer et al., 2004; Huang et al., 2011). Adenosine promotes sleep by inhibiting the activating systems and stimulating the hypnogenic systems. Of interest, caffeine promotes W by blocking the receptors for adenosine (Nishino and Mignot, 2005).

1.6. TRANSITION FROM NREM SLEEP TO REM SLEEP

There is limited knowledge about the neuronal basis involved in the transition from NREM sleep to REM sleep. However, a role for the caudolateral peribrachial region during this transition has been proposed (Torterolo et al., 2011b).

1.7. BRIEF SYNOPSIS OF THE NEUROPHARMACOLOGY IN SLEEP PATHOLOGY

Knowing the neurobiological basis of W and sleep provides the clinician with the frame to understand sleep pathologies and the pharmacological approaches to their treatment.

Sleep pathological conditions are characterized by either a lack of the necessary amount (or quality) of sleep that is called insomnia, or by an excess of sleep, that is hypersomnia. Another type of syndromes is caused by the appearance of abnormal behaviors during sleep and is called parasomnias.

The chronic insomnia disorder in adult patients occurs no less than three times per week, for at least three months and is characterized by an inability to fall or stay asleep, and daytime complaints such as somnolence and fatigue (American Academy of Sleep Medicine, 2005). Medications approved for this disorder include benzodiazepine (BZD) receptor allosteric modulators, BZD (triazolam, temazepam, and flurazepam) or non-BZD (zolpidem, eszopiclone, and zaleplon) agents. These drugs promote sleep, at least in part, by reducing the activity of the activating systems. Melatonin and the melatonin receptor agonist ramelteon are also used for the treatment of an insomnia disorder; these drugs make the most of the sleep-promoting effect of natural melatonin. Low-dose doxepin (a tricyclic antidepressant) is also employed for the treatment of sleep disorders. Its mechanism of action is mainly related to the blockade of histamine H1 receptor. Finally, the dual orexin (hypocretin) receptor antagonist (DORA) suvorexant that blocks the effect of endogenous hypocretin (a wake-promoting neuromodulator), has been recently approved by the FDA for the treatment of insomnia disorders.

Briefly, drugs currently used for the treatment of chronic primary insomnia address sleep onset latency (zolpidem immediate-release, zaleplon, and ramelteon) and/or sleep maintenance (temazepam, flurazepam, zolpidem extended-release, eszopiclone, and low-dose doxepin). However, during their administration, N3 sleep and REM sleep do not regain normal lev-

els or can be even further reduced. With respect to suvorexant, the compound increases N2 sleep and REM sleep in patients with insomnia disorder (Monti et al., 2015b).

Hypersomnia disorder is a term used for a group of disorders in which the primary characteristic is excessive daytime sleepiness in the presence of normal or longer than normal nocturnal sleep (Larson-Prior et al., 2014). Among these disorders, the most common is narcolepsy. The management of this pathology includes several behavioral approaches and pharmacological treatment. For excessive daytime sleepiness, modafinil is in the first line pharmacological treatment. This drug blocks the dopamine transporter (DAT) and increases dopamine synaptic levels, which is central in its wake-promoting effect; in fact, genetic ablation of the DAT abolishes the wake-promoting effect of modafinil. However, there are other possible sites of action of this drug (Wisor, 2013). Amphetamine-like drugs such as methylphenidate are also used to reduce sleepiness.

Parasomnias are unpleasant or undesirable behavioral phenomena that occur during the sleep period. There are different types of parasomnias (Pandi-Perumal et al., 2014). One of them is the REM sleep behavioral disorder (RBD). During RBD, the REM sleep atonia does not occur and the patients act out their dreams. Severe lesions can occur during the REM without atonia episodes. About 90% of patients with chronic RBD respond well to clonazepam (0.5–2 mg) administered half an hour before sleep time (Mahowald and Schneck, 2009). Clonazepam is a BDZ whose mechanism of action for the RBD is still unknown.

1.8. CONCLUSIONS AND FUTURE DIRECTIONS

A detailed knowledge of the anatomy and physiology of the activating and hypnogenic systems is important to understand and treat sleep pathologies. A recent achievement in relation to the activating systems has been the unveiling of the pathogenesis of narcolepsy. This pathology is caused by the degeneration of hypocretinergic neurons (Mignot, 2011), which prompted paraclinical studies such as the titration of hypocretin-1 in the cerebrospinal fluid for diagnostic confirmation of narcolepsy, and therapeutic advances such as intranasal hypocretin-1 administration for the treatment of some aspects of the disease (Baier et al., 2008).

KEYWORDS

- **REM**
- **reticular formation**
- **hypothalamus**
- **basal forebrain**
- **sleep**
- **MCH**
- **acetylcholine**
- **dopamine**
- **hypocretin**
- **histamine**
- **norepinephrine**
- **serotonin**

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