Role of Orexin on Sleep: Interactions with Other Neurotransmitter Systems

Pablo Torterolo, Jaime Monti and S.R. Pandi-Perumal

Abstract In 1998, a group of phenotypically distinct neurons were discovered in the postero-lateral hypothalamus, which contained the excitatory neuropeptides orexin-A and orexin-B (also called hypocretin-1 and hypocretin-2). Orexinergic neurons project throughout the central nervous system (CNS) and are involved in the generation and maintenance of wakefulness. The sleep disorder narcolepsy, characterized by hypersomnia and cataplexy, is produced by degeneration of these neurons. We conducted a critical review of the literature on the interactions of the orexinergic system with other neurotransmitter systems involved in the generation and maintenance of wakefulness and sleep. Orexin has an excitatory action over the cholinergic, serotonergic, noradrenergic, histaminergic and dopaminergic neurons that constitute the activating system. Moreover, orexinergic neurons modulate the activity of γ-aminobutyric acid (GABA) and melanin-concentrating hormone (MCH) sleep-promoting neurons. Of note, orexin and MCH have opposite post-synaptic effects. In this respect, the orexinergic neurons are active during active wakefulness and "phasic" rapid-eye movement (REM) sleep, while the MCHergic cells are active during non-rapid-eye movement (NREM) sleep and "tonic" REM sleep. We hypothesize that the interactions of these opposite but complementary hypothalamic systems are critical for the generation of either wakefulness or sleep.

Keywords Hypothalamus · Peptides · REM sleep · Narcolepsy · Cataplexy · Melanin-concentrating hormone · Orexin (hypocretin)

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1 Introduction

Narcolespy is a disabling neurological condition affecting 1 in 2000 individuals. The pathophysiology of narcolepsy spins around two main axes: the difficulty in maintaining wakefulness (hypersomnia, mainly in the form of sleep attacks) and the increase in rapid-eye movement (REM) sleep. This is manifested either by the decrease in REM sleep latency or the intrusion of partial aspects of this state into wakefulness (cataplexy, sleep paralysis, hypnagogic hallucinations) (Mignot 2011). However, in spite of the presence of hypersomnia, night sleep is also disrupted.

Nowadays, based on animal and human research, it is known that the pathogenesis of narcolepsy with cataplexy occurs as a result of the degeneration of the orexinergic (hypocretinergic) neurons within the hypothalamus (Mignot 2011). However, questions remain to be answered in relation to the physiology of the orexinergic system. It was our purpose to review how the orexinergic neurons interrelate with other neurotransmitter systems in order to modulate waking and sleep states.

2 Waking and Sleep Promoting Neuronal Systems

It is presently known that the neuronal networks critical for the generation and maintenance of wakefulness (i.e. activating systems) are located within the dorsal and median raphe nuclei (DRN and MRN), laterodorsal and pedunculopontine tegmental nuclei (LDT-PPT), locus coeruleus (LC), nucleus pontis oralis (NPO), tuberomammillary nucleus (TMN), ventral tegmental area and ventral periaqueductal gray (VTA and vPAG), and basal forebrain (BFB) (Torterolo and Vanini 2010a). These neurons have ample projections towards the thalamus and/or the cortex, structures that support cognitive functions. The activating systems desynchronize the electroencephalogram (EEG), an electrophysiological sign of wakefulness (Torterolo and Vanini 2010a). Some of these neurons also project to premotor or motor nuclei, including the neuronal networks that control breathing, as well as the preautonomic and autonomic nuclei involved in the vegetative aspects of the waking state (Torterolo and Vanini 2010a).

Non-rapid-eye movement (NREM) sleep is induced by neurons located in the preoptic area of the hypothalamus, both in the ventro-lateral preoptic nucleus (VLPO) and the median preoptic nucleus (MnPO); these neurons are active during NREM sleep and inhibit the activating systems (Szymusiak and McGinty 2008; Torterolo et al. 2009; Benedetto et al. 2012). Other regions, such as the caudo-lateral peribrachial area, that may have a role in NREM sleep or in NREM-REM sleep transition, have been recognized also (Torterolo et al. 2011).

Adenosine is a neurotransmitter and a metabolic product involved also in the generation of sleep. Adenosine synaptic levels increase during prolonged wakefulness periods prompting sleep onset (Strecker et al. 2000). This substance promotes sleep by inhibiting activating systems located in the BFB, and disinhibiting the sleep-promoting VLPO (Strecker et al. 2000).

It is well-known that the neuronal network "necessary" and "sufficient" for REM sleep generation is located in the mesopontine tegmentum (Chase 2013; Luppi et al. 2013; Siegel 2005). Some of these mesopontine neurons have a dual role, such that they form part of the activating system and, in addition, have REM sleep generating functions. While monoaminergic (noradrenergic and serotonergic) neurons within this region are Wake-on but REM-off neurons and promote wakefulness, the cholinergic neurons are either Wake-on and REM-on or just REM-on, and are critically involved both in wakefulness and REM sleep occurrence (McCarley 2007). In fact, structural and mathematical models based on the activity of these neurons have tried to explain the dynamic of REM sleep generation (McCarley 2007).

The nucleus pontis oralis (NPO) in the cat (that comprises the peri-locus coeruleus α and a part of the medial pontine reticular formation) or its corresponding nucleus in the rat, which is called sub-laterodorsal nucleus, is constituted by glutamatergic and GABAergic neurons. The nucleus pontis oralis is considered to exert an executive control over the initiation and maintenance of REM sleep, and to be involved in the control of wakefulness as a part of the activating systems (Chase 2013; Siegel 2005; Luppi et al. 2007; Reinoso-Suarez et al. 2001). A single local injection of a cholinergic agonist, such as carbachol, results in the generation of a state with all the behavioral and electrographic signs of REM sleep that occurs with a very short latency (30 s to a few minutes), and can last for up to two hours (Baghdoyan et al. 1987).

Glutamatergic and GABAergic neurons distributed in different areas including the DRN, BFB, LDT-PPT, NPO and vPAG, play also a critical role in the control of the behavioral states (Torterolo and Vanini 2010a, b; Torterolo et al. 2001, 2002; Vanini et al. 2007; Xi et al. 2001). For example, the microinjection of GABA or a $GABA_A$ receptor agonist into the NPO results in a sustained period of arousal. This finding has led to the proposal that GABAergic neurons within this area are involved in the control of wakefulness as part of the activating systems (Xi et al. 2001).

Atypical neurotransmitters such as nitric oxide and the endocannabinoids play also a role in the control of behavioral states (Monti et al. 2013; Murillo-Rodriguez 2008).

The role of the different neurotransmitter systems involved in the control of wakefulness and sleep is summarized in Table 1. Most of these neuronal networks are strongly modulated by orexinergic neurons.

Table 1 Neurotransmitters/neuromodulators that have an active role in the generation of wakefulness and sleep

Neurotransmitter/neuromodulator	Location of the soma	Neuronal pattern of discharge
Wakefulness promoting		
Orexin	Postero-lateral hypothalamus	Wake-on
Acetylcholine	LDT-PPT and BFB	Wake/REM-on
Serotonin	DRN and MRN	Wake-on
Noradrenaline	Locus coeruleus	Wake-on
Dopamine	VTA and SNPc	Bursting discharge during wakefulness and REM sleep[b]
Histamine	TMN	Wake-on
GABA[a]	NPO	Probably Wake-on
Glutamate[a]	Mesopontine reticular formation	Probably Wake-on
NREM sleep promoting		
Melanin-concentrating hormone (MCH)	Postero-lateral hypothalamus	Active in NREM and tonic REM
Adenosine	Probably BFB, POA	[c]
GABA[a]	VLPO, MnPO	NREM-on and NREM-REM-on
REM sleep promoting		
Acetylcholine	LDT-PPT and BFB	Wake/REM-on and REM-on
Melanin-concentrating hormone (MCH)	Postero-lateral hypothalamus	Active in tonic REM sleep
Orexin	Postero-lateral hypothalamus	Active in phasic REM sleep
GABA[a]	vlPAG, DRN	Probably REM-on
Glutamate[a]	NPO	Probably Wake/REM-on

[a]The role of the GABAergic and glutamatergic neurons depends on the location of the neurons; only some examples are listed. [b]The pattern but not the frequency of discharge of the dopaminergic neurons changes along sleep and wakefulness. [c]The release of adenosine increases during prolonged wakefulness; however the origin (metabolic, neurotransmitter) of this substance is still unclear. *BFB* basal forebrain; *DRN* dorsal raphe nucleus; *LDT-PPT* laterodorsal and pedunculopontine tegmental nucleus; *MnPO* median preoptic nucleus; *MRN* median raphe nucleus; *NPO* nucleus pontis oralis; *POA* preoptic area; *SNPc* substantia nigra pars compacta; *TMN* tuberomammillary nucleus of the hypothalamus; *vlPAG* ventrolateral periaqueductal gray; *VLPO* ventrolateral preoptica area; ventral tegmental area

3 Orexinergic Neurons, Orexins and Receptors

Orexin-A and B (also called hypocretin-1 and 2) were discovered in 1998 by two independent groups (de Lecea et al. 1998; Sakurai et al. 1998). These neuropeptides are synthesized by a discrete group of neurons ($\sim$5000 in rodents, $\sim$11,000 in cats and 20–50,000 in humans) in the postero-lateral hypothalamus (Li et al. 2013;

Torterolo et al. 2006). Figure 1 shows the characteristics and distribution of the orexinergic neurons in the postero-lateral hypothalamus of the guinea pig. Orexins exert their biological function through two metabotropic receptors orexin-R1 and orexin-R2 (also known as hypocretin 1 and 2 receptors) that have broad, partially overlapping, but distinct patterns of distribution throughout the brain and body. The

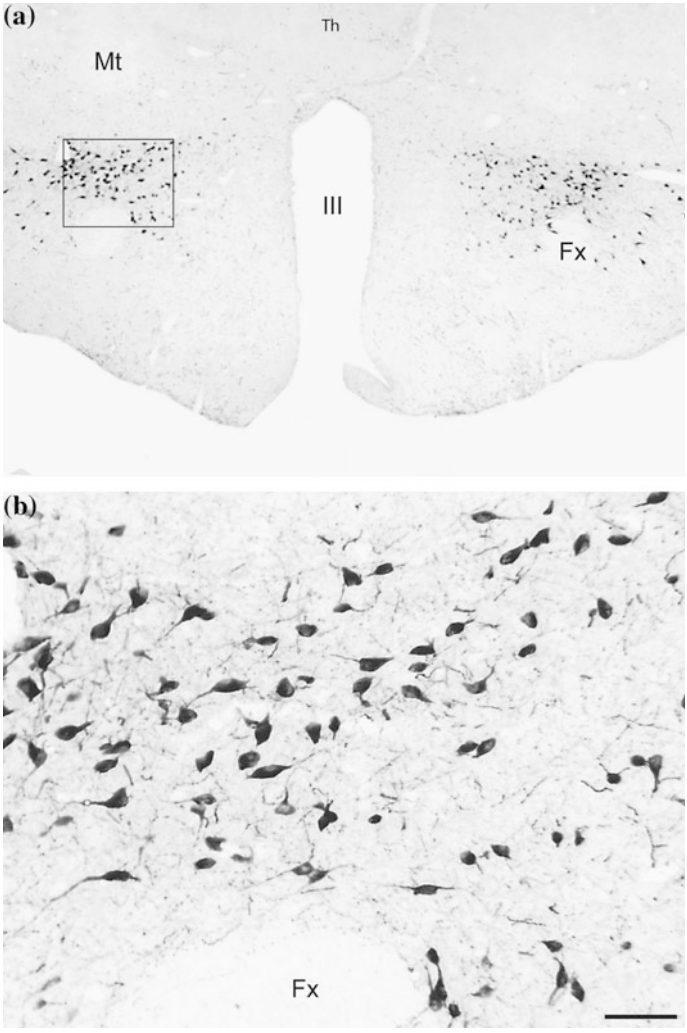


Fig. 1 Orexinergic neurons are located in the postero-lateral hypothalamus. **a** Photomicrograph that shows the distribution of the orexinergic neurons in a coronal plane of the tuberal region of a guinea pig hypothalamus. **b** The inset showed in (**a**), is exhibited in a higher magnification in (**b**). The orexinergic neurons are located close to the fornix. Calibration bar, 50 μm. Sections of 20 μm were processed employing the anti-orexin-B antibodies, ABC method and the DAB-H_2O_2 reaction to detect peroxidase activity

orexin-R1 has 10 to 100 times more affinity for orexin-A than for orexin-B; in contrast, orexin-R2 has the same affinity for both neuropeptides. Through these receptors, orexins produce an excitatory effect at postsynaptic sites (Mignot 2011; Li et al. 2013; van den Pol and Acuna-Goycolea 2006). It has been demonstrated that orexin increases Na^+-dependent current, non selective cation currents, and activates the Na^+/Ca^{++} exchanger. Depression of K^+ currents and increase in intracellular Ca^{++} was also demonstrated. At presynaptic sites orexin also increases the neurotransmitter release.

4 Projections of the Orexinergic Neurons

Orexinergic neurons project throughout the central nervous system (CNS) (Peyron et al. 1998); furthermore, the activity of the peripheral organs is also influenced by the orexins (Voisin et al. 2003). Orexinergic neurons have also the potential to mediate complex functions since they exhibit the morphology of prototypical "command" neurons, which are small groups of highly specialized cells that coordinate and integrate, in a complementary fashion, the activities of a vast number of neural and hormonal systems (Torterolo and Chase 2014).

Sensory and motor nuclei are directly innervated by orexinergic neurons (McGregor et al. 2005; Torterolo et al. 2007; Fung et al. 2001; Yamuy et al. 2004). Orexinergic neurons also project to the thalamus and cortex (Peyron et al. 1998), wherein they directly influence thalamo-cortical activities that support cognitive functions. Furthermore, dense concentrations of orexin-containing axon terminals are located in the TMN (Eriksson et al. 2001) as well as in brainstem areas such as the LDT-PPT, VTA, LC and DRN (Peyron et al. 1998; Chemelli et al. 1999; Date et al. 1999; Nambu et al. 1999), that participate in the control of wakefulness and REM sleep. We have demonstrated also that orexinergic neurons project to the NPO, that exerts executive control over the initiation and maintenance of REM sleep (Torterolo et al. 2013). Figure 2 shows orexinergic projections toward the LDT-PPT, LC and DRN in the cat.

Orexinergic neurons also project to the NREM generation areas such as the preoptic area (Peyron et al. 1998).

5 Orexinergic Neurons as a Part of the Activating Systems

The postero-lateral hypothalamic area where the orexinergic neurons are located, is the key brain site that, for decades, has been identified as being responsible for initiating, coordinating and maintaining goal-oriented survival-type behaviors such as fight, flight and food consumption among others. In addition, and in accord with the preceding concept, experimental studies have demonstrated that this area is critically involved in the control of sleep and wakefulness,

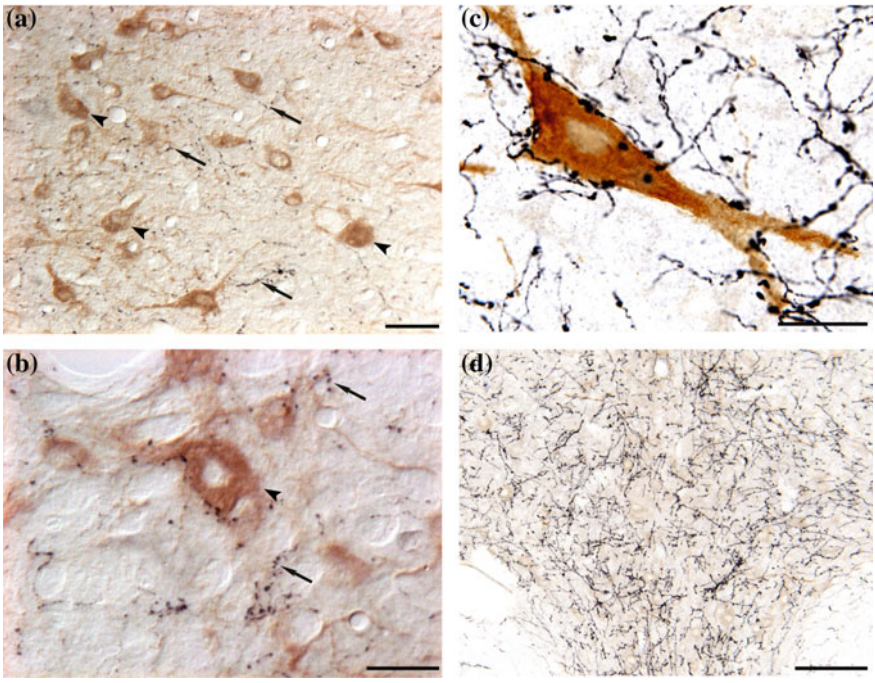


Fig. 2 Examples of the orexinergic projections. **a** and **b** Photomicrographs of the LDT-PPT of the cat. The sections were immunostained for orexin (in *black*, examples of orexienergic fibers/terminals are indicated by *arrows*) and for choline acetyltransferase (in *brown*, examples of cholinergic neurons are signaled by *arrowheads*). Sections were processed utilizing the ABC method and the DAB-H_2O_2 reaction to detect peroxidase activity. This reaction was enhanced with nickel to label orexinergic cells. *Calibration bars* **a** and **b**, 30 and 20 μm respectively. **c** Photomicrographs of the LC of the cat. The sections were immunostained for orexin (in *black*) and tyrosine hydroxylase (in *brown*). Sections were processed utilizing the ABC method and the DAB-H_2O_2 reaction to detect peroxidase activity. This reaction was enhanced with nickel to label orexinergic cells. *Calibration bar* 10 μm. **d** The photomicrograph shows the orexinergic fibers in the dorsal raphe nucleus; this is taken for another section of the experimental series shown in (**c**). *Calibration bar* 100 μm (Color figure online)

somatomotor activity as well as "pleasure" or "reward" (Torterolo and Chase 2014; Chase 2013).

Early studies revealed that the intraventricular injection of orexin induces wakefulness (Hagan et al. 1999; Piper et al. 2000). These data, as well as the fact that the lack of orexinergic neurons in narcoleptic patients induces hypersomnia (Mignot 2011), strongly suggested that this system promotes wakefulness. Genetically modified mice and optogenetic studies also confirmed the role of orexin and orexinergic neurons as a waking promoting substance and system (Chemelli et al. 1999; Hara et al. 2001; Adamantidis et al. 2007). However, by means of Fos technology (the Fos protein is a marker of neuronal activity), we demonstrated in

the cat that orexinergic neurons are not active during wakefulness per se (Torterolo et al. 2001) (Fig. 3). A detailed analysis of orexinergic neuronal activity shows that these neurons are strongly activated when animals are exploring an unknown environment (exploratory motor activity) (Torterolo et al. 2011). In the absence of motor activity during alert wakefulness, quiet wakefulness or quiet sleep, the orexinergic neurons are not activated to any significant extent (Torterolo et al. 2003). In fact, the number of Orexin+ Fos+ neurons was approximately 10 times greater during exploratory motor activity than during repetitive motor activity that occurred during forced locomotion, even though in both paradigms there was a comparable amount of motor activity (Torterolo et al. 2011). Therefore, neither wakefulness nor motor activity per se, were critical with respect to the activation of orexinergic neurons. Hence the orexinergic system is engaged when animals are performing

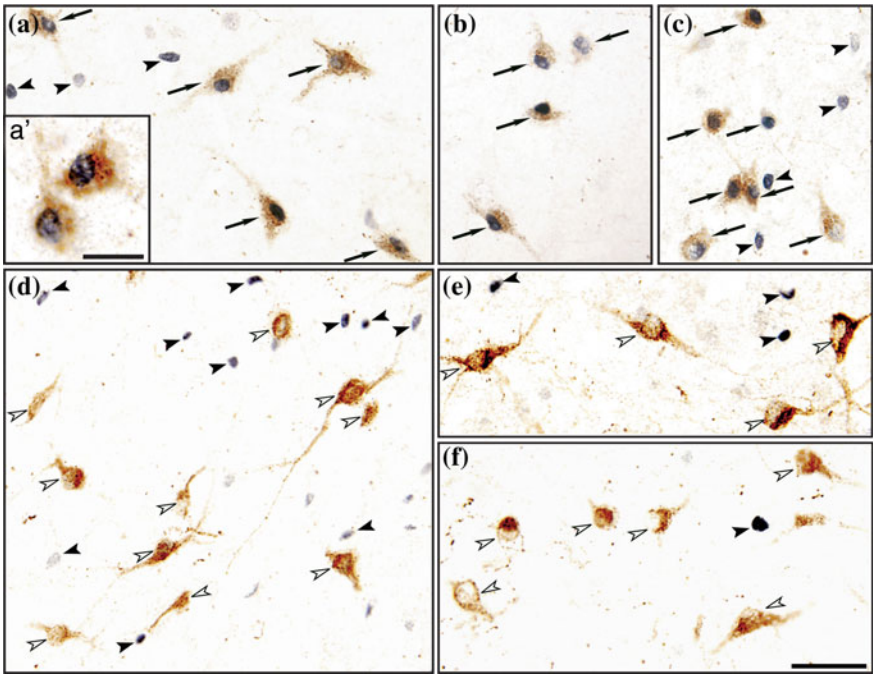


Fig. 3 Photomicrographs containing orexin and Fos immunoreactive neurons in the lateral hypothalamus of the cat during active wakefulness with motor explorative activity (**a**, **a′**, **b** and **c**), alert wakefulness without motor activity (**d**), quiet wakefulness (**e**) and NREM sleep (**f**). Orexinergic neurons are *stained brown*; Fos immunoreactivity, that is restricted to nuclei, is shown in *black*. *Arrows* indicate Orexin+ Fos+ cells; these neurons are exhibited with higher magnification in **a′**. Significant numbers of Orexin+ Fos+ neurons were observed only during active wakefulness with motor explorative activity. *Filled arrowheads* point to non-orexinergic Fos+ neurons, these neurons were observed in all of these behavioral states. *Empty arrowheads* indicate orexinergic neurons that did not express c-fos. *Calibration bars* **a**–**f** 50 μm; **a′** 20 μm. Modified from Torterolo et al. (2003) (Color figure online)

goal (reward)-directed behaviors. In agreement with our results, it was found that orexin knock-out mice were unable to work for food or water reward during the light phase (McGregor et al. 2011).

Recently, Chase presented the "Unified Survival Theory for the Functioning of the Orexinergic System" (Chase 2013). The basis of this theory is that the main role of the orexinergic system is to initiate, coordinate and maintain survival behaviors and survival-related processes.

In order to promote these waking effects during survival-related behaviors, orexinergic neurons activate different components of the activating systems such as the LC, DRN, LDT-PPT, TMN and BFB.

6 Orexinergic Neurons During Sleep

6.1 NREM Sleep

The orexinergic neurons, as a component of the activating systems (Torterolo and Vanini 2010a), do not actively participate in the occurrence of NREM sleep. In fact, there is a lack of Fos expression in these neurons during this behavioral state (Fig. 3). Subsequently, unit recordings confirmed our early findings (Mileykovskiy et al. 2005; Lee et al. 2005; Takahashi et al. 2008; Kolaj et al. 2008).

6.2 "Tonic" REM Sleep

The orexinergic neurons are considered to be REM-off neurons. The REM-off profile concept of the orexinergic neurons arose based upon electrophysiological recordings of identified orexinergic neurons during "tonic" REM sleep (see below) (Mileykovskiy et al. 2005; Lee et al. 2005; Takahashi et al. 2008).

6.3 "Phasic" REM Sleep

EEG activation, theta activity in the hippocampus and muscle atonia are the classic biomarkers for the identification of REM sleep. Accompanying these "tonic" signs are rapid-eye movements, muscle twitches, PGO waves, breathing irregularities as well as heart rate and blood pressure variations that constitute the phasic events of REM sleep. Other signs such as acceleration of the theta rhythm also correlate with these phasic events (Rowe et al. 1999).

Experimental evidence shows that while orexinergic neurons turn off during "tonic" REM sleep, at least a subset of these neurons discharge in bursts during phasic REM sleep (Mileykovskiy et al. 2005; Lee et al. 2005; Takahashi et al. 2008).

Studies in the cat, an animal that exhibits robust phasic periods of REM sleep (Ursin and Sterman 1981), strongly suggest that there is orexinergic neuronal activity during REM sleep, probably during the phasic events of this state. An increase in the number of Orexin+ Fos+ neurons was observed during REM sleep induced by carbachol microinjections into NPO (Torterolo et al. 2001). In this study, REM sleep was induced by carbachol microinjections in order to generate a state of sufficiently long duration to allow Fos protein to be synthesized in high concentration. During this state, 34 % of the orexinergic neurons were activated according to their Fos-expression (Torterolo et al. 2001). This result was in agreement with the findings by Kiyashchenko et al. (2002), who described an increase in orexin-A release during REM sleep, both in the hypothalamus and BFB in freely moving cats. Hence, this study also indicates that orexinergic neurons are active during REM sleep, probably in conjunction with phasic events.

Microinjection studies suggest also that administration of orexin into critical areas such as the medullary reticular formation of the rat or the NPO of the cat, can induce REM sleep-like atonia or REM sleep, respectively (Mileykovskiy et al. 2002; Xi et al. 2002; Xi and Chase 2010).

If orexinergic neurons are active during "phasic" REM sleep, they are likely to promote the phasic events of REM sleep (Torterolo and Chase 2014). In fact, orexins directly activate motor nuclei, breathing neuronal networks and sympathetic output that controls the cardiovascular system (Yamuy et al. 2004; Shirasaka et al. 2002; Zhang et al. 2005; Williams and Burdakov 2008), as well as the medial septum where the pacemaker for the hypocamppal theta rhythm is located (Gerashchenko et al. 2001). These are typical ergotropic or energy-expending behaviors that are induced by the orexinergic system (Chase 2013).

Then, orexinergic cells in the hypothalamus may be involved in the control of both active wakefulness and active components of REM sleep. As mentioned above, the orexinergic system plays an important role in the regulation of motor activity during motivational states. Is it possible that this system promotes motor activity during wakefulness and atonia during REM sleep? Actually, this pattern of duality of behavioral state control with opposite motor responses is reminiscent of the phenomenon of reticular response-reversal in the NPO (Chase and Babb 1973; Chase and Morales 1990). This phenomenon involves mechanisms that result in the facilitation of wakefulness and somatomotor activation during wakefulness, as well as the generation of REM sleep and its accompanying pattern of motor inhibition during this sleep state. The reticular response reversal determines the auditory stimulation promotion of somatomotor activation during wakefulness, while increasing the hyperpolarization of the motoneurons and atonia during REM sleep (Chase 2013). In fact, microinjections of orexin-A or orexin-B into the NPO of the cat increases the time spent in REM sleep and results in a decrease in the latency to the generation of this state (Xi et al. 2002). Juxtacellular application of orexin-A results in an increase in the excitability of NPO neurons, which is associated with the induction of REM sleep (Xi et al. 2003). In addition, orexin-A increases acetylcholine release in the NPO of the rat (Bernard et al. 2003, 2006). In this respect, it is known that acetylcholine levels within this region increase during REM sleep

(Kodama et al. 1990). On the other hand, an increase in wakefulness accompanied by a decrease in REM sleep has been observed also when orexin-A is microinjected into the NPO of the cat (Moreno-Balandran et al. 2008). Furthermore, the iontophoretic application of orexin-A into the NPO of the rat produces an inhibition of NPO neurons, which can be blocked by previous iontophoretic application of bicuculline, a $GABA_A$ receptor antagonist (Nunez et al. 2006). In fact, it has been shown that orexin increases GABA levels in the NPO of the rat, and orexin and GABA interact within this nucleus to promote wakefulness (Watson et al. 2008; Brevig et al. 2011). The presence of orexin-B receptors on GABAergic neurons within the NPO may be the cellular basis for this effect (Brischoux et al. 2008). The paradoxical or contradictory findings involving the REM sleep and wakefulness promoting actions of orexin within the NPO have been reconciled by Xi and Chase (Xi and Chase 2010). They demonstrated that the microinjections of orexin-A within the NPO generates REM sleep when applied during NREM sleep, but promotes wakefulness when applied during this behavioral state. Thus, the behavioral state of the animal at the time of the application of orexin determines whether REM sleep or wakefulness occur.

7 Afferents to Orexinergic Neurons

Orexinergic neurons receive inputs from several regions such as the allocortex, many hypothalamic nuclei, the periaqueductal gray matter, the DRN, and parabrachial regions, which suggests that these neurons integrate a variety of interoceptive and homeostatic signals (Yoshida et al. 2006). Interestingly, these neurons seem to be weakly influenced by exteroceptive sensory inputs (Mileykovskiy et al. 2005).

8 Effects of Different Neurotransmitters on Orexinergic Neurons

Table 2 summarizes the effects of the main neurotransmitters on orexinergic neurons. In mice, glutamate depolarizes orexinergic neurons acting through AMPA and NMDA receptors, while GABA inhibits these neurons though $GABA_A$ and $GABA_B$ receptors (Yamanaka 2006).

Neurotransmitters used by neurons that form part of the activating systems also modulate the activity of orexinergic neurons. They include the following:

Serotonin. The serotonergic neurons are localized at the raphe nuclei; the DRN is the region with the major concentration of these neurons (Monti 2010a, b). Serotonergic neurons of the DRN and MRN project to the thalamo-cortical systems. These cells are active during wakefulness and a group of them increase the firing rate in relation to automatic movements such as locomotion (Wake-on).

Table 2 Examples of the effects of neurotransmitter/neuromodulators onto orexinergic neurons (in vitro studies)

Neurotransmitter/neuromodulator	Cellular effects on orexineric neuron	References	Animal model
Orexin	despolarization by presynatic facilitation of glutamate release	Li et al. (2002)	Mouse
Acetylcholine	Carbachol depolarizes 27 % and hyperpolarizes 6 % of the neurons	Yamanaka (2006)	Mouse
	Acetylcholine has a predominantly excitatory effect	Bayer et al. (2005)	Rat
Serotonin	Hyperpolarizes through $5HT1_A$ receptors	Yamanaka (2006)	Mouse
Noradrenaline	- Hyperpolarizes through α2 receptors	Yamanaka (2006)	Mouse
	- Depolarization mediated by α1 receptor (in the presence of a α2-receptor antagonist)	Yamanaka (2006)	Mouse
	- Predominant excitatory effect	Bayer et al. (2005)	Rat
Dopamine	- D1 and D2 dopamine receptors have opposing effects on excitatory presynaptic terminals that impinge on orexinergic neurons	Alberto et al. (2006)	Rat
Histamine	- Almost no effect	Yamanaka (2006)	Mouse
GABA	Post-synaptic inhibition though $GABA_A$ and $GABA_B$ receptors	Yamanaka (2006)	Rat
Glutamate	Depolarize acting through AMPA and NMDA receptors	Yamanaka (2006)	Mouse
Melanin-concentrating hormone (MCH)	Attenuates orexin-A induced enhancement of spike frequency and the frequency of miniature excitatory postsynaptic currents	Rao et al. (2008)	Mouse
Adenosine	depresses the amplitude of evoked excitatory postsynaptic potential and the frequency of spontaneous and miniature excitatory postsynaptic currents	Liu and Gao (2007)	Mouse
Endocannabinoids	Agonist of CB1R hyperpolarized and reduced spontaneous firing	Huang et al. (2007)	Mouse

Serotonergic neurons decrease their firing rate during NREM sleep, reaching a nadir during REM sleep (REM-off). An in vitro study in mice has shown that serotonin hyperpolarizes orexinergic neurons through $5HT_{1A}$ receptors (Yamanaka 2006).

Noradrenaline. The noradrenergic neurons are located in the LC. These neurons project to the thalamus and cortex and have a Wake-on, REM-off profile in their firing rate (Torterolo and Vanini 2010). During wakefulness these neurons markedly increase their discharge rate in relation to new stimuli, and have been related with the modulation of attention. An in vitro study in mice has shown that noradrenaline hyperpolarizes orexinergic neurons through α_2 receptors (Yamanaka 2006), while a weak depolarization mediated by α_1 receptor was also observed in the presence of a α_2-receptor antagonist. On the contrary, in rats, noradrenaline has a predominant excitatory effect (Bayer et al. 2005).

Dopamine. These neurons are located in the VTA, the *substancia nigra pars compacta* and the vPAG (Monti and Monti 2007; Leger et al. 2006, 2010). Their projections are restricted to the striatum and the prefrontal cortex. Dopaminergic neurons are known to promote wakefulness and, due to their strong relationship with positive emotions, they may probably be related with the increase in vigilance produced during a motivational state. D1 and D2 dopamine receptors have opposing effects on excitatory presynaptic terminals that impinge on orexinergic neurons (Alberto et al. 2006).

Histamine. Histaminergic neurons are located in the tuberomammillary nucleus of the posterior hypothalamus. These neurons project to the thalamus and cortex and have a Wake-on, REM-off profile in their firing rate (Monti 2011). Interestingly, histamine has almost no effect on orexinergic neurons (Yamanaka 2006).

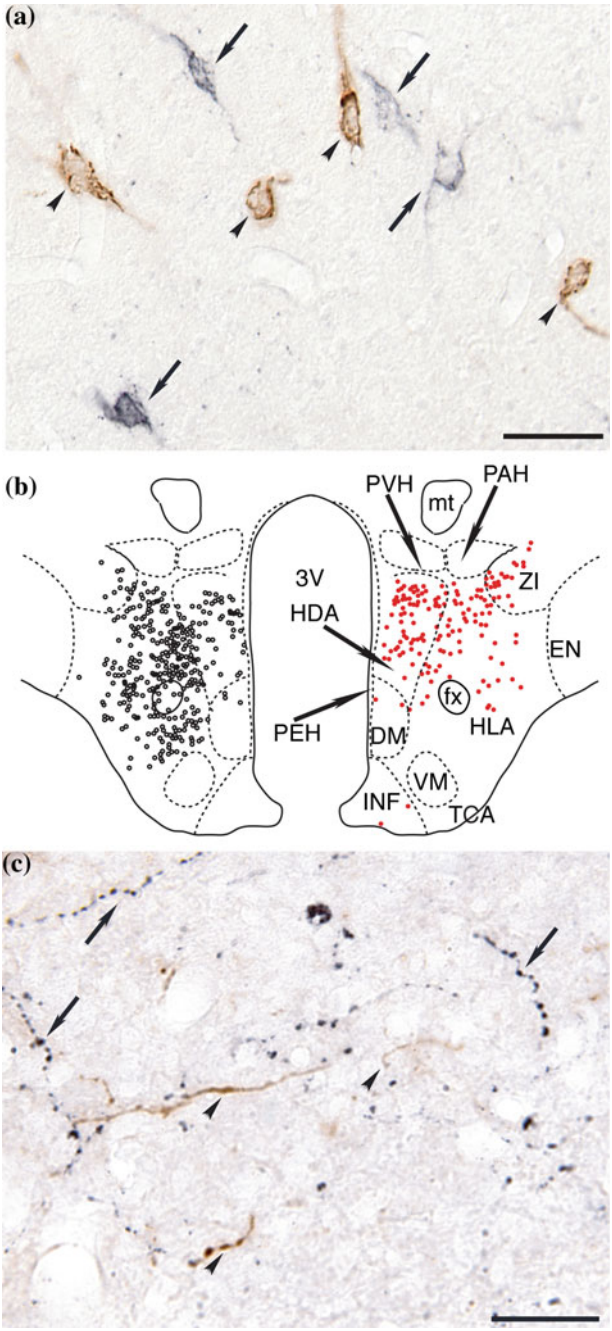
Acetylcholine. Cholinergic neurons are located in the LDT-PPT and the BFB. Ascending projections of the cholinergic neurons located in the LDT-PPT are directed toward the thalamus, while cholinergic neurons of the BFB project to the cortex and reticular nucleus of the thalamus (Torterolo and Vanini 2010). LDT-PPT cholinergic neurons project also to the NPO, the executive REM sleep generation area, that is critical for REM sleep generation (Chase 2013; Semba 1999). In mice, carbachol depolarizes 27 % and hyperpolarizes 6 % of the population of orexinergic neurons (Yamanaka 2006), and these effects are mediated by different muscarinic receptors. In rats, acetylcholine has a predominantly excitatory effect (Bayer et al. 2005).

The effects of adenosine, nitric oxide and endocannabinoids on orexinergic neurons are listed in Table 2.

The effect of the sleep-promoting factor, melanin-concentrating hormone (MCH) on orexinergic neurons will be reviewed below.

9 Orexin and MCH. Postero-lateral Hypothalamic Duality in the Control of Sleep and Wakefulness

Due to the importance of the MCHergic system in sleep physiology (Monti et al. 2013; Torterolo et al. 2011), it is relevant to examine the interactions between the MCHergic and the orexinergic system. A strong anatomical relationship exists between orexinergic and MCHergic neurons in the hypothalamus. As it is shown in Fig. 4,


(a)
(b)
PVH
mt
PAH
3V
HDA
ZI
EN
fx
PEH
DM
HLA
VM
INF
TCA
(c)

◀ **Fig. 4** Orexinergic neurons are intermingled with MCHergic neurons in the postero-lateral hypothalamus. **a** Photomicrographs of the postero-lateral hypothalamic area of the cat. The sections were immunostained for orexin (in *black*, *arrows*) and MCH (in *brown*, *arrowheads*). Sections were processed utilizing the ABC method and the DAB-H_2O_2 reaction to detect peroxidase activity. This reaction was enhanced with nickel to label orexinergic cells. *Calibration bars* 50 μm. **b** Location of MCHergic and orexinergic neurons in the postero-lateral hypothalamus of a representative cat. Camera lucida drawings of MCHergic (on the *left*, *black circles*) and orexinergic neuronal bodies (on the *right*, *red circles*) in the postero-lateral hypothalamus. The neurons are from the same hemi-hypothalamus (reflected in the figure). Camera lucida drawings were obtained from adjacent sections that were immunostained for MCH for Orexin-2, respectively; these sections were counterstained with Pyronin-Y. *DM* dorsomedial nucleus; *EN* entopeduncular nucleus; *fx* fornix; *HDA* dorsal hypothalamic area; *HLA* lateral hypothalamic area; *INF* infundibular nucleus; *mt* mammillothalamic tract; *PAH* paraventricular nucleus; *PEH* periventricular complex; *PVH* parvocellular nucleus; *TCA* area of the tuber cinereum; *VM* ventromedial nucleus; *ZI* zona incerta; *3V* third ventricle. Modified from (Torterolo et al. 2006). **c** Photomicrographs of the nucleus pontis oralis of the cat. The sections were immunostained for orexin (in *black*, *arrows*) and MCH (in *brown*, *arrowheads*). Sections were processed utilizing the ABC method and the DAB-H_2O_2 reaction to detect peroxidase activity. This reaction was enhanced with nickel to label orexinergic cells. *Calibration bars* 10 μm (Color figure online)

MCHergic neurons are intermingled with orexin-containing neurons in the postero-lateral hypothalamus, mainly at the tuberal and tuberomammillar levels (Torterolo et al. 2006). MCHergic fibers are in close relationship with orexinergic neurons and viceversa, which suggests the existence of reciprocal synaptic contacts between both types of cells (Torterolo et al. 2006; Guan et al. 2002). This fact, as well as the presence of orexinergic receptors on MCHergic neurons indicates the existence of an important functional interaction between both systems (Backberg et al. 2002). In this respect, orexin increases MCH mRNA expression in hypothalamic neurons, directly excites MCHergic neurons and increases glutamate release onto them (Bayer et al. 2002; van den Pol et al. 2004). Opposite, MCH modulates orexin-mediated effects on behavioral state and synaptic transmission in the lateral hypothalamus (Rao et al. 2008). The efficacy of glutamatergic synapses on orexinergic neurons is enhanced in MCHR1 knockout mice, and orexin A-induced firing is facilitated. On the contrary, in wild-type mice, MCH significantly attenuates orexin-A induced enhancement of spike frequency in orexinergic neurons, but not its basal activity. Furthermore, in these neurons, MCH attenuates orexin-1-induced enhancement of the frequency of miniature excitatory postsynaptic currents. These effects imply that MCH exerts a unique inhibitory influence on orexinergic signaling as a way to fine-tune the output of these neurons.

Interestingly, orexinergic and MCHergic neurons respond in a different way to most homeostatic signals such as glucose (Burdakov et al. 2005) or to waking-related neurotransmitters such as noradrenaline (Bayer et al. 2005). It should be noted that while orexinergic neurons of the rat express α_1 adrenergic receptors, MCHergic neurons express the α_2 adrenergic receptors, which are related to activation or inhibition of their targets, respectively (Modirrousta et al. 2005).

We have shown that both orexinergic and MCHergic neurons project to the NPO (Torterolo et al. 2009, 2013) (Fig. 4). In addition, fibers and terminals of both

systems are highly intermingled, which suggests the existence of important interactions between these systems within their mesopontine targets, similar to the anatomical and functional interactions that have been described within the hypothalamus (see above).

Several studies suggest that MCHergic neurons are involved in the generation of sleep, especially REM sleep (Monti et al. 2013; Torterolo et al. 2011). These neurons discharge in a reciprocal manner to orexinergic neurons across the sleep-wake cycle. MCHergic neurons have a high firing rate during tonic REM sleep, but do not increase their firing level during "phasic" REM sleep (Hassani et al. 2009). When MCH is microinjected into the cat NPO (executive REM sleep generation area), it produces an increase in the time the animals spend in REM sleep together with a decrease in the latency to this behavioral state (Torterolo et al. 2009). MCH also exerts its REM sleep promoting functions acting through the DRN (Lagos et al. 2009, 2011), where it shows an inhibitory role on serotonergic neurons (Urbanavicius et al. 2013; Pascovich et al. 2011). A REM sleep promoting effects of MCH have been observed also in the BFB (Lagos et al. 2012). In contrast, bilateral microinjections of MCH into the VLPO induces NREM sleep (Benedetto et al. 2013). Recent studies utilizing optogenetics confirmed the importance of MCH in promoting both NREM and REM sleep (Tsunematsu et al. 2014; Konadhode et al. 2013; Jego et al. 2013).

Interestingly, MCH blunts the central regulation of sympathetic tone, adaptive sympathetic reflexes and decreases metabolism (Saito and Nagasaki 2008; Messina and Overton 2007; Egwuenu et al. 2005). These are trophotropic or energy-conserving effects, which are opposite to the effects produced by the orexinergic system (see above).

The experimental evidence mentioned above is the basis for the hypothesis that MCHergic neurons are active during NREM seep as well as during tonic REM sleep and promote this "quiescent" behavioral state. On the contrary, orexinergic neurons are active during "survival-type" behaviors along wakefulness and during "phasic" REM sleep, and probably induce (at least partially) phasic episodes of this state. Hence, orexinergic neurons play an active role in energy-expending or ergotropic behaviors

10 Conclusions

The orexinergic neurons are a branch of the activating system; they project toward the thalamo-cortical system that supports cognitive functions, to the septum that is the pacemaker of the hippocampal activity, the premotor and motor nuclei including the breathing neuronal network, and the preautonomic and autonomic nuclei. By means of these projections these neurons are capable to integrate different aspects of the waking behavior. As proposed recently by Chase (2013), this system coordinates all facets of "survival" behavior including an increase in the vigilance state.

In addition, these neurons may be involved in the generation of the "active" or "phasic" aspects of REM sleep (Torterolo and Chase 2014).

Orexinergic neurons interrelate with other neuronal networks critical for the generation and maintenance of wakefulness and sleep. These neurons boost the activity of other branches of the activating systems in order to increase vigilance, especially during motivated-behaviors. In addition, it is possible that a group of hypocretinergic neurons promotes the "phasic" aspects of REM sleep.

Yin and yang is a Taoist symbol used to describe how polar or seemingly contrary forces are interconnected and interdependent in the natural world, and how they give rise to each other in turn. In a similar way, the orexinergic and MCHergic systems of the postero-lateral hypothalamus seem to produce either wakefulness or active (phasic) REM sleep (ergotropic or energy-expending function) or NREM sleep and tonic REM sleep (trophotropic or energy-conserving function), respectively.

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