

# The orexin system: Roles in sleep/wake regulation

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Orexin system: roles in sleep/wake regulation

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Running title: Orexin and sleep/wake state

**Abstract**

The neuropeptides orexin A and orexin B, produced in hypothalamic neurons, are critical regulators of sleep/wake states. Deficiency of orexin-signaling results in narcoleptic phenotype in humans, dogs, and rodents. Recently, accumulating evidence has indicated that the orexin system regulates sleep and wakefulness through interactions with neuronal systems that are closely related with emotion, reward, and energy homeostasis. In this review, we will discuss the current understanding of the physiology of the orexin system especially focusing on its roles in the regulation of sleep/wakefulness states.

Keywords: orexin, hypothalamus, narcolepsy, sleep, wakefulness

## **Introduction**

Orexin was first identified as endogenous peptide ligands for two orphan G-protein coupled receptors in 1998<sup>1</sup>. They were initially recognized as regulators of feeding behavior, firstly because of their exclusive production in the lateral hypothalamic area (LHA), a region known as the feeding center, and secondly owing to their pharmacological activity.<sup>1-4</sup> Subsequently, this peptide system was shown to be a critical regulator of sleep/wake states. Since then, this and other groups have been using a multidisciplinary approach to understand various aspects of the physiological roles of orexin peptides, and have uncovered crucial roles of the orexin system in the regulatory mechanisms in sleep/wake states, energy homeostasis, and the reward systems. Especially, the finding that an orexin deficiency causes narcolepsy in humans and other animals has had a huge impact on the studies of sleep and wakefulness and other areas.<sup>5-9</sup> Recent studies of orexin-producing neurons' efferent and afferent systems have suggested further roles for orexin in the coordination of emotion, energy homeostasis, reward and arousal.<sup>10-17</sup> These findings suggest that orexin neurons are involved in sensing the body's external and internal environments, and regulate sleep/wake states accordingly, which is beneficial for survival. This review will discuss the mechanisms by which the orexin system regulates sleep/wake states, and how this mechanism relates to other systems that regulate emotion, reward, and energy homeostasis.

## **Orexin and orexin receptors**

Orexins were identified by a strategy called reverse pharmacology. There are over 100 of G-protein coupled receptors (GPCRs) whose ligands are still unknown and are therefore, referred to as orphan GPCRs. Many of these orphan GPCRs are likely to be receptors for heretofore unidentified signaling molecules, including new peptide hormones and neuropeptides. In 1998, during searching for endogenous ligands for various orphan GPCRs, our group identified orexin A and orexin B as endogenous ligands for two related orphan GPCRs.<sup>1</sup> Because these peptides were localized in the LHA, and intracerebroventricular (ICV) injection of them in rats or mice acutely increased food consumption, they were named orexin A and B after the Greek word 'orexis', meaning appetite. Orexin A and -B are produced by cleavage of a common precursor polypeptide, prepro-orexin. The primary structure of orexin A predicted from the cDNA sequences is completely conserved among several mammalian species (human, rat, mouse, cow, sheep, dog and pig). On the other hand, rat orexin B is a 28-amino-acid, C-terminally amidated linear peptide of 2937 Da, which is 46% (13/28) identical in sequence to orexin A. The C-terminal half of orexin B is very similar to that of orexin A (73%; 11/15), while the N-terminal half is variable. Orexin B also has a high degree of sequence similarity among species. Several study revealed that the structures of fish, xenopus, and chicken orexin A and orexin B have also conserved structures as compared with mammalian sequences(Figure 1).<sup>18-20</sup> Furthermore,

several studies have shown that orexins play roles in regulation of food intake and sleep/wake behavior in goldfishes and zebrafishes.<sup>21-24</sup> These observations suggest that structures and functions of orexins are phylogenetically well conserved.

As an independent work, de Lecea et al. identified mRNA expressed specifically within the hypothalamus and identified a cDNA encoding a polypeptide identical to *prepro-orexin*, and named the putative mature peptides hypocretin-1 and -2 (Hcrt-1 and Hcrt-2).<sup>25</sup> The terms 'orexin' and 'hypocretin' are used as synonyms in many papers currently.

In mammals, the actions of orexins are mediated by two GPCRs, named orexin 1 (OX<sub>1</sub>R) and orexin 2 (OX<sub>2</sub>R) receptors (also named as HCRT1 and HCRT2). OX<sub>1</sub>R has one-order higher affinity for orexin A than orexin B, while OX<sub>2</sub>R binds orexin A and orexin B with similar affinities. OX<sub>1</sub>R couples to the G<sub>q/11</sub> subclass of heterotrimeric G proteins, while OX<sub>2</sub>R couples to G<sub>q/11</sub> or G<sub>i/o</sub> in a neuronal cell line in culture.<sup>26</sup>

Orexin-expressing neurons (orexin neurons) are distributed within the lateral hypothalamic area (LHA) and posterior hypothalamus (PH).<sup>1, 27-29</sup> The number of these neurons has been estimated around 3000 in rat and 50000 in human brains. Although their cell bodies are exclusively localized in the hypothalamus, orexin neurons send their axonal projections diffusely throughout the central nervous system (CNS), excluding the cerebellum. Especially dense projections are found in the hypothalamus (such as the arcuate nucleus (ARC) and tuberomammillary nucleus (TMN)), and the brain stem (such as the central gray, locus coeruleus (LC), and raphe nuclei).<sup>27-29</sup> Consistent with the broad projections of orexin neurons, OX<sub>1</sub>R and OX<sub>2</sub>R mRNAs show wide distributions in the CNS with partly overlapping but distinct patterns.<sup>30</sup> For instance, nuclei such as the LC and ventral tegmental area (VTA) mainly express OX<sub>1</sub>R mRNA, while those including the TMN, nucleus accumbens (NAc), and septal nuclei mainly express OX<sub>2</sub>R mRNA. Both mRNAs are observed in the raphe nuclei, laterodorsal tegmental nucleus (LDT), and pedunculopontine tegmental nucleus (PPT). These distributions of both receptor mRNAs suggest partly overlapping and partly distinct roles of orexin receptors.

## **Narcolepsy**

In 1999, two independent studies utilizing dog forward genetics and mouse reverse genetics clearly showed a causal linkage between disruption of the orexin system and narcolepsy-cataplexy. Subsequently, several studies established that loss of orexin neurons is accompanied by human narcolepsy patients. The symptoms and pathophysiology of the sleep disorder narcolepsy, caused by an orexin deficiency, provide insight into the physiological roles of orexin.<sup>5-8</sup> Therefore, we will briefly mention about this disorder in this section.

Human narcolepsy is a debilitating neurological disorder that affects approximately 1 in 2000 individuals in the U.S.<sup>31</sup> Onset of the condition is usually during adolescence. A cardinal symptom of the disorder is excessive daytime

sleepiness (an insurmountable urge to sleep), which manifests itself primarily when the subject falls asleep at inappropriate times (“sleep attacks”). When normal individuals fall asleep, a certain period of non-REM (NREM) sleep (60-90 minutes) usually precedes REM sleep. However, the latency of rapid eye movement (REM) sleep is markedly reduced in narcolepsy patients, and REM sleeps are sometimes observed immediately after wakefulness (sleep-onset REM). The existence of “sleep-onset REM” periods is one of the important diagnostic criteria for narcolepsy. In patients, nocturnal sleep is also disturbed and is often accompanied by hypnagogic hallucinations, vivid dreaming, and sleep paralysis, which usually occur when they fall asleep.

Narcolepsy patients often suffer from attacks called “cataplexy”, which is a sudden weakening of bilateral muscle tone (muscle atonia), ranging from jaw dropping and speech slurring to complete bilateral collapse of the postural muscles. These attacks are often triggered by emotional stimuli, such as laughter, excitement, and pleasure. Narcolepsy which is accompanied with cataplexy is sometimes referred as “narcolepsy-cataplexy”.

Symptoms of narcolepsy-cataplexy can be divided into two distinct pathological phenomena. One is an inability to maintain a consolidated waking period, characterized by abrupt transitions from wakefulness to NREM sleep (i.e. dysregulation of NREM sleep onset). This phenomenon manifests as excessive daytime sleepiness or a sleep attack. This symptom is treated by psychostimulants, such as methylphenidate, methamphetamine, and modafinil. The other key phenomenon is the pathological intrusion of REM sleep into wakefulness (i.e. dysregulation of REM sleep onset): it is during these periods that patients may experience cataplexy, hypnagogic hallucinations and sleep paralysis. These symptoms are treated by tricyclic depressants and selective serotonin re-uptake inhibitors.

Collectively, this disorder is characterized by the inability to maintain each vigilance state, pathological intrusion of non-REM and/or REM sleep into wakefulness, and frequent transitions between states of sleep and wakefulness. This suggests that orexins have important physiological roles in the maintenance and stabilization of sleep and wakefulness.

### **Orexin or orexin receptor-2 deficiencies cause narcoleptic phenotype**

The first clues towards an involvement of the orexins in narcolepsy came from animal models; mice lacking the *orexin* gene or dogs with null mutations in the *orexin receptor-2* (*OX<sub>2</sub>R*) gene show phenotypes remarkably similar to humans with narcolepsy.<sup>7, 8</sup> Orexin knockout mice (*orexin*<sup>-/-</sup> mice) exhibit frequent sudden collapses that resemble human cataplexy attacks during the dark phase, when mice spend the most time awake and active.<sup>7</sup> These attacks are thought to be homologous to cataplexy.<sup>32</sup> Quantitative sleep state parameters of *orexin*<sup>-/-</sup> mice revealed slightly decreased waking time, increased REM sleep time during dark period, decreased REM sleep latency, and, most importantly, a markedly decreased duration of wake

time during the dark phase (i.e. inability to maintain a long awake period). Dogs with null mutations in the *orexin receptor-2 (OX<sub>2</sub>R)* gene show phenotypes remarkably similar to humans with narcolepsy.<sup>7, 8</sup>

The link between orexin dysfunction and narcolepsy, especially which accompanied with cataplexy (narcolepsy-cataplexy), has since been supported by studies with human patients. In contrast to normal control individuals, the vast majority of narcoleptic individuals have low or undetectable levels of orexin A in the cerebrospinal fluid (CSF).<sup>33</sup> (In CSF, orexin B is not detectable even in healthy individuals.) A postmortem study of human narcolepsy subjects showed undetectable levels of orexin peptides in the cortex and pons, and an 80-100% reduction in the number of neurons containing detectable *prepro-orexin* mRNA or orexin-like immunoreactivity in the hypothalamus.<sup>5, 6</sup> No mutation has been found either in the *prepro-orexin* or *orexin receptor* genes of human narcolepsy-cataplexy patients, except for an unusually severe, early onset case associated with a mutation in the signal peptide of *prepro-orexin* that impairs peptide trafficking and processing.<sup>5</sup> In this case, abnormal signal sequence cleavage results in accumulation of abnormal orexin peptide in cells leading cell death. A recent finding showing concomitant loss of dynorphin, neuronal activity-regulated pentraxin, and orexin, which colocalize in orexin neurons, further suggests a loss of functional orexin neurons, rather than the selective inhibition of orexin expression, in narcolepsy-cataplexy.<sup>34</sup> Based on these observations, as well as a strong association of human narcolepsy-cataplexy with certain HLA alleles,<sup>35</sup> it has been speculated that narcolepsy-cataplexy may result from selective autoimmune degeneration of orexin neurons. Regardless of the cause of the neuron loss, the orexin signaling deficiency in narcolepsy-cataplexy shows that this neuropeptide system plays an important role in the regulation of sleep and wakefulness, especially in the maintenance of long, consolidated awake periods.

We produced transgenic mice in which orexin neurons are ablated by expression of a N-terminally truncated ataxin-3, which induces post-natal apoptotic death of all orexin neurons by adulthood (orexin neuron-ablated mice).<sup>9</sup> Adult mice show essentially the same phenotype of sleep/wake regulation as *orexin*<sup>-/-</sup> mice. Therefore, although orexin neurons produce other neurotransmitters such as glutamate and dynorphin,<sup>36-38</sup> orexin is the most important factor among them for regulation of sleep/wake states by these neurons.

Regarding the contribution of each receptor, *OX<sub>2</sub>R*<sup>-/-</sup> mice have characteristics of narcolepsy-cataplexy, although their narcoleptic phenotype is less severe than that found in *orexin*<sup>-/-</sup> mice.<sup>39, 40</sup> They show behavioral arrests that are less frequent and severe than those of *orexin*<sup>-/-</sup> mice. *OX<sub>1</sub>R*<sup>-/-</sup> mice do not have overt behavioral abnormalities. *OX<sub>1</sub>R*<sup>-/-</sup>; *OX<sub>2</sub>R*<sup>-/-</sup> mice appear to be a phenocopy of orexin knockout mice,<sup>41</sup> implying that these two receptors are sufficient to mediate regulation of sleep/wake by orexins. These observations also suggest that despite the lack of an overt *OX<sub>1</sub>R*<sup>-/-</sup> phenotype, loss of signaling through both receptor pathways appears to

be necessary for the emergence of a complete narcoleptic phenotype, suggesting that both receptors are involved in the regulation of sleep and wakefulness.

### **Therapeutic potentials of orexin agonists and antagonists**

The finding of low orexin A levels in CSF in patients with narcolepsy-cataplexy led to the development of a novel, definitive diagnostic test for this disease.<sup>33</sup> A low orexin A levels in CSF has been used as one of the diagnostic criteria for narcolepsy-cataplexy according to the 2nd edition of the international classification of sleep disorders.<sup>42</sup>

Moreover, the discovery of causal link between loss of orexin signaling and human narcolepsy-cataplexy has brought about a possibility of novel therapies for this disease. Currently, excessive sleepiness in narcolepsy is treated using psychostimulants, while cataplexy is treated with tricyclic antidepressants.  $\gamma$ -hydroxybutyrate (sodium oxybate) is also used to consolidate nocturnal sleep and reduce cataplexy.<sup>43</sup> Treatment with these compounds are problematic due to limited effectiveness, undesirable side effects such as insomnia or symptom rebounds, and the potential for abuse. Since orexin neuron-ablated mice (*orexin/ataxin-3* mice) have an etiology and course of disease similar to those of human narcolepsy-cataplexy, these mice may represent the most accurate pathophysiological model of narcolepsy-cataplexy available.<sup>9</sup> We demonstrated rescue of the narcoleptic phenotype of these mice by genetic and pharmacological means.<sup>44</sup> Chronic overproduction of orexin peptides from an ectopically expressed transgene prevented development of narcolepsy syndrome in orexin neuron-ablated mice. Acute ICV administration of orexin A also maintained wakefulness, suppressed sleep, and inhibited cataplectic attacks in these mice. Intriguingly, ICV administration of orexin A had stronger arousal effects in orexin neuron-ablated mice than in wild-type controls, suggesting that responsiveness of effector sites for orexins remains intact, or even more responsible for orexins in narcoleptics. These results also indicate that a spatially targeted secretion of orexin is not necessary to prevent narcoleptic symptoms. Unfortunately, however, constitutive production of orexin peptides from a *prepro-orexin* transgene in mice per se caused fragmentation of NREM sleep episodes in the light period; when mice spend the most time asleep (our unpublished observations). These results indicate that orexin neurons should be turned on and switched off to maintain consolidated wakefulness and NREM sleep, respectively. These also suggest that orexin receptor agonists with relatively short half-lives (several hours) would be of potential value for treating human narcolepsy-cataplexy. Such agonists might also be useful in the treatment of other conditions of excessive daytime sleepiness in humans.

Conversely, orexin receptor antagonists might be useful for treatment of insomnia patients or as a sleep-inducer. Indeed, Almorexant, an orally available antagonist for OX<sub>1</sub>R and OX<sub>2</sub>R, has been reported to cause subjective and objective



electrophysiological signs of sleep in humans,<sup>45</sup> and now its Phase III studies are going on.

### **Neural mechanisms of sleep/wake regulation by orexins**

Wakefulness is maintained by multiple neurotransmitters and neuronal pathways. Included in this system are monoaminergic and cholinergic neurons reside in the brain stem. Monoaminergic neurons, including LC noradrenergic, raphe serotonergic, and TMN histaminergic neurons, project diffusely to the forebrain promoting arousal. Other important wake-inducing signals are cholinergic neurons in the brain stem and project to key forebrain targets such as the thalamus, an area critical to regulating cortical activity.<sup>46</sup> Monoaminergic neurons are firing at rapid rates during wakefulness, while they reduce their activities during NREM sleep and almost cease discharge during REM sleep. A subset of PPT/LDT neurons is active during both wakefulness and REM sleep (W-REM on), regulating activity of thalamo-cortical projections to generate EEG desynchronization characteristics of wakefulness and REM sleep. Others are active exclusively during REM sleep (REM-on) and thought to induce REM sleep and REM atonia. Conversely, GABA/galaninergic neurons in the ventrolateral preoptic nucleus (VLPO) of the hypothalamus are active during sleep, especially non-REM sleep,<sup>45</sup> and thought to initiate and maintain NREM sleep. VLPO neurons and monoaminergic neurons reciprocally inhibit each other. This reciprocal interaction of wake center and sleep center maintains states of sleep and wakefulness.

As mentioned above, orexin neurons send dense projections to the monoaminergic/cholinergic nuclei involved in sleep/wake regulation (Fig. 2, 3). The distribution of the orexin receptors mRNA is consistent with these projection sites; within the brain, *OX<sub>1</sub>R* is most abundantly expressed in the LC, while *OX<sub>2</sub>R* is highly expressed in the TMN,<sup>30</sup> and both mRNAs are detectable in the raphe nuclei, PPT/LDT and BF.

ICV administration of orexin A in rodents potently reduces REM and non-REM sleep, and increases wakefulness.<sup>47</sup> Application of orexin directly into the LC, TMN, LDT, and the lateral preoptic area has effects similar to ICV injection on sleep/wake states.<sup>48-52</sup> In vitro slice electrophysiology studies has shown that orexin increases firing rates of monoaminergic neurons in the LC,<sup>53, 54</sup> raphe,<sup>55, 56</sup> TMN,<sup>57-59</sup> and cholinergic neurons in the BF and LDT,<sup>60, 61</sup> but have no effect on the GABAergic neurons in the VLPO.<sup>60</sup> A work using cats showed that orexin A inhibits cholinergic neurons in the PPT in vivo through activation of GABAergic interneurons and GABAergic neurons in the substantia nigra pars reticulata.<sup>62</sup> These results indicate that orexin neurons affect the activity of PPT/LDT cholinergic neurons both directly and indirectly to regulate arousal and REM sleep.

More recently, Adamantidis et al. succeeded to demonstrate that direct and selective photostimulation of orexin neurons in freely moving mice, in which orexin

neurons were genetically targeted to express a photo-activatable cation channel (channelrhodopsin-2), increased the probability of transition to wakefulness from either NREM or REM sleep.<sup>63</sup>

### **Regulatory mechanisms and input systems of orexin neurons**

Considering symptoms of narcolepsy-cataplexy, orexin neurons are expected to be active during wakefulness and to be silent during sleep. In fact, transgenic mice with constitutive activation of orexinergic tone (*CAG/orexin* mice), in which orexin is expressed in a diffuse, ectopic pattern in the brain in unregulated fashion,<sup>44</sup> exhibited abnormal sleep and wakefulness patterns, including fragmented NREM sleep in the light period and incomplete REM sleep atonia with abnormal myoclonic activity during REM sleep (our unpublished results). These suggest that orexin neurons need to be switched off to maintain consolidated NREM sleep and the muscle atonia that accompanies REM sleep.

Fos expression in orexin neurons in rats is higher during the dark phase (active period) than during light phase (rest period).<sup>64</sup> Consistently, orexin levels in CSF peak during the dark period and decrease during the light period.<sup>65</sup> In vivo extracellular recordings further confirmed activity patterns of orexin neurons across sleep/wake cycles with high temporal resolutions.<sup>66-68</sup> Essentially, orexin neurons fired most actively during active waking, decreased discharge during quiet waking, were virtually silent during NREM sleep, and almost silent but exhibited occasional firing during REM sleep.

How these regulations could be achieved? Orexin neurons receive projections from nuclei involved in sleep/wake regulation. GABAergic neurons in the preoptic area, including the VLPO, densely innervate orexin neurons (Fig. 3).<sup>14, 15</sup> Orexin neurons are strongly inhibited by both GABA<sub>A</sub> receptor agonist, muscimol, and GABA<sub>B</sub> receptor agonist, baclofen.<sup>11, 69, 70</sup> Orexin neurons are also innervated by BF cholinergic neurons.<sup>14</sup> Carbachol, a cholinergic agonist, activates a subset of orexin neurons.<sup>14, 71</sup> Thus, orexin neurons are likely to be inhibited by sleep-promoting neurons and activated by wake-promoting BF neurons: these regulations of orexin neurons are in consistent with their proposed function to stabilize wakefulness.

In contrast, wake-active serotonergic neurons and noradrenergic neurons in the brain stem send inhibitory projection to orexin neurons.<sup>14, 72, 73</sup> Serotonergic and noradrenergic inputs hyperpolarize orexin neurons through activation of G-protein-regulated inwardly rectifying K<sup>+</sup> (GIRK or Kir3) channels mediated by 5-HT<sub>1A</sub> receptors and  $\alpha_2$ -adrenoceptors, respectively.<sup>72, 73</sup> These negative feedback mechanisms may also be important for fine adjustment of orexin neuronal activity to stabilize wakefulness. Notably, histamine has little effect on orexin neurons.<sup>74</sup>

Local interneurons may also play important roles in the regulation of orexin neurons. In slice preparations, orexin A and orexin B were reported to depolarize orexin neurons.<sup>69</sup> This effect appears to be mediated by orexin-mediated excitation of

local glutamatergic neurons that regulate orexin neuronal activity, in part by presynaptic facilitation of glutamate release. On the other hand, GABAergic input from local interneurons to orexin neurons is also important for organization of orexin neuronal activity.<sup>75</sup> Genetic disruption of GABA<sub>B</sub> input resulted in marked sleep/wake abnormality.<sup>75</sup>

Horvath and Gao reported an unusual synaptic organization on orexin neurons in which excitatory synaptic currents and asymmetric synapses exert control on the perikarya of these long-projective neurons with minimal inhibitory input, which is sharp contrast to the fact that neuronal cell bodies in the central nervous system are either dominated by inhibitory inputs (long-projection neurons), or have an approximate ratio of excitatory to inhibitory inputs of 1:1.<sup>76</sup> This unique input organization of orexin neurons may be a necessary element for the maintenance of a low threshold for arousal and alertness. On the other hand, this circuitry, along with abundant input from the limbic system, may also be an underlying cause of insomnia. Consistent with these results, an electrophysiological study with slice preparations indicated that orexin neurons are tonically activated by glutamatergic neurons,<sup>69</sup> while basal tone of GABAergic input seems to be low, because GABA antagonists do not influence basal activity of orexin neurons. However, in slice from mice with selective deletion of GABA<sub>B</sub> receptor in orexin neurons (oxGKO mice), orexin neurons responded with depolarization to bicuculline application. This indicates that GABA<sub>A</sub> receptors are tonically activated in orexin neurons of these mice. As a consequence, orexin neurons in these mice showed decreased responsiveness to both excitatory and inhibitory inputs as compared with wild type mice.<sup>75</sup> With the observation showing that oxGKO mice had highly fragmented sleep/wake state, these observations suggest that proper local GABAergic regulation and normal synaptic organization of orexin neurons are highly important for sleep/wake regulation.

### **Orexin neurons as a stabilizer of sleep/wake states**

How do the orexins physiologically regulate sleep and wakefulness, and why does a lack of orexin signaling result in narcolepsy, a disorder characterized by instability of wakefulness? As mentioned above, orexin neurons, monoaminergic/cholinergic centers in the brain stem, and the sleep-active VLPO neurons constitute a triangular interaction; orexin neurons send excitatory projections to monoaminergic neurons, and these monoaminergic neurons send inhibitory projections back to orexin neurons. VLPO sleep-active neurons send inhibitory projections to both monoaminergic neurons and orexin neurons. Monoaminergic neurons send inhibitory projections to VLPO sleep-active neurons. This triangular organization seems to be highly important for stability of vigilance states. VLPO sleep-active neurons and monoaminergic neurons inhibit each other. This reciprocal inhibition is well suited to avoid intermediate states. Orexin neurons can stabilize waking state by enhancing activity of monoaminergic neurons during wakefulness, avoiding state instability caused by

small perturbations. At the same time, negative feedback input to orexin neurons from serotonergic and noradrenergic neurons work to maintain the activities of orexin and monoaminergic neurons within appropriate ranges. During sleep, orexin neurons are turned off together with monoaminergic neurons by VLPO GABA/galaninergic neurons.

In narcoleptics, the orexin-target neurons, such as monoaminergic neurons in the brain stem, might undergo changes in their synaptic organization through plasticity or synaptic scaling mechanisms due to chronic deficiency of orexins. These changes might help the monoaminergic cells to be activated without orexins. Simultaneously, this mechanism may set up a “flip-flop” circuit between Sleep-active VLPO neurons and wake-active monoaminergic neurons;<sup>46</sup> these two components reciprocally inhibit each other. In this type of mutually-inhibitory circuits, when activity on either side begins to overcome the other, the system will flip into one of two possible extremes. A small perturbation on the activity of one side can easily cause abrupt switching between two states, resulting in frequent state transitions, fundamental problems seen in narcolepsy.

The fact described earlier that orexin neurons are much more active during active waking than during quiet waking *in vivo* clearly suggest roles of orexin go beyond mere global arousal. Recently, Deadwyler et al. reported that systemic and nasal delivery of orexin A reduced the effects of sleep deprivation on cognitive performance in nonhuman primates; interestingly, orexin A did not produce facilitative effects if the animals were not sleep deprived,<sup>77</sup> although it is not clear whether orexin A penetrates the blood-brain barrier, or orexin A can influence vigilance states by acting on peripheral receptors. In slice preparations from rat prefrontal cortex, orexin was reported to induce calcium transients in single spines postsynaptic to identified thalamocortical boutons.<sup>78, 79</sup> By this cellular mechanism, orexinergic projections to the prefrontal cortex may play a role in prefrontal or “executive” aspects of alertness and attention.

### **Roles of orexin in other functions**

Orexins are thought to be also involved in many other functions, including feeding behavior, autonomic regulation, endocrine functions, nociception, reward system and emotion. All these systems should be well coordinated. Sleep and wakefulness are regulated to occur at appropriate times that are in accordance with our internal and external environments. Avoiding danger and finding food, which are life-essential activities that are regulated by emotion, reward and energy balance, require vigilance and thus, by definition, wakefulness. The orexin system regulates sleep and wakefulness through interactions with systems that regulate emotion, reward and energy homeostasis.

Reciprocal connections between the orexin system and multiple neuronal systems indicate that orexin neurons provide crucial links between multiple brain functions, such as energy homeostasis, reward system, emotion, and arousal. Future studies with full use of mouse molecular genetics, such as selective deletions of genes for particular receptor or signaling molecule in orexin neurons, would lead to further understanding of integrative physiology orchestrated by the orexin system.

From clinical perspective, discovery of the linkage between the orexin system and human narcolepsy-cataplexy led to the development of a novel diagnosis of this disease and to the expectation for development of novel drugs for treatment of narcolepsy-cataplexy. Moreover, future studies may let us understand why orexin neurons degenerate in narcolepsy-cataplexy patients, which would lead to more fundamental therapies for narcolepsy-cataplexy.

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Figure legends

**Fig. 1. An overview of orexin system.**

(A) Structure of mature orexin A and orexin B peptides. The topology of the two-intrachain bonds in orexin A is indicated above the sequence. Shadows indicate amino acid identity. Mammalian orexin A sequences thus far identified (human, rat, mouse, pig, dog, sheep, and cow) are all identical, while the sequences of orexin B show some differences among species.

(B) Orexins and their receptors. Orexin A and orexin B are derived from a common precursor peptide, prepro-orexin. The actions of orexins are mediated via two G protein-coupled receptors named orexin-1 (OX<sub>1</sub>R) and orexin-2 (OX<sub>2</sub>R) receptors. OX<sub>1</sub>R is selective for orexin A, whereas OX<sub>2</sub>R is a nonselective receptor for both orexin A and orexin B. OX<sub>1</sub>R is coupled exclusively to the G<sub>q</sub> subclass of heterotrimeric G proteins, whereas OX<sub>2</sub>R couples to G<sub>i/o</sub> and /or G<sub>q</sub>.

Fig. 2. Schematic drawing of main axonal projections of orexin neurons and distribution of orexin receptors in brain.

This figure summarizes predicted orexinergic projections in the human brain. Please note that distributions of orexin fibres and receptors (OX<sub>1</sub>R, OX<sub>2</sub>R) are predicted from the results of studies on rodent brains, because it is on rats or mice that most histological studies on the orexin system have been carried out. Circles show regions with strong receptor expression and dense orexinergic projections. Orexin neurons originating in the lateral hypothalamic area (LHA) and posterior hypothalamus (PH) regulate sleep and wakefulness and the maintenance of wakefulness by sending excitatory projections to the entire CNS, excluding the cerebellum, with particularly dense projections to monoaminergic and cholinergic nuclei in the brain stem and hypothalamic regions, including the locus coeruleus (LC, containing noradrenaline), tuberomammillary nucleus (TMN, containing histamine), raphe nuclei (Raphe, containing serotonin) and laterodorsal/pedunclopontine tegmental nuclei (LDT/PPT), containing acetylcholine). Orexin neurons also have links with the reward system through the ventral tegmental area (VTA, containing dopamine) and with the hypothalamic nuclei that stimulate feeding behaviour. This figure is adapted with modification from<sub>80</sub>.

Fig.3. Interactions between orexin neurons with other brain regions.

Orexin neurons in the lateral hypothalamic area (LHA) and posterior hypothalamus (PH) are anatomically well placed to provide a link between the limbic system, systems involved in energy homeostasis and monoaminergic and cholinergic neurons in the brain stem. Solid arrows show excitatory projections and broken lines inhibitory ones. Wake-active regions, sleep-active regions and REM-active regions are shown by red, blue and green boxes, respectively. Orexin neurons promote wakefulness through the monoaminergic nuclei that are wake-active. Stimulation of dopaminergic centres

by orexins can modulate reward systems (purple). Peripheral metabolic signals such as leptin, ghrelin and glucose influence orexin neuronal activity to coordinate arousal and energy homeostasis. The nucleus suprachiasmaticus (SCN), the central body clock, may send signals to orexin neurons via the dorsomedial hypothalamus (DMH). Input from the limbic system (amygdala and bed nucleus of the stria terminalis (BST)) might regulate the activity of orexin neurons upon emotional stimuli to evoke emotional arousal or fear-related responses. VLPO, ventrolateral preoptic area; DR, dorsal raphe; GABA,  $\gamma$ -aminobutyric acid; LC, locus coeruleus; LDT, laterodorsal tegmental nucleus; PPT, pedunculopontine tegmental nucleus; SNr, substantia nigra pars reticulata; TMN, tuberomammillary nucleus.

This figure is adapted with modification from<sub>80</sub>.

Fig. 4. Mechanisms by which the orexin system stabilizes sleep /wake states.

The figures represent functional interactions between orexin neurons, monoaminergic wake-active centres and the ventrolateral preoptic area (VLPO) sleep-active centre. Solid arrows show excitatory input, and broken lines inhibitory input. The thickness of arrows and lines represents the relative strength of excitatory and inhibitory input, respectively. Circle sizes represent relative activities of each region. (a) Awake state. Orexin neurons send excitatory influences to monoaminergic neurons, which send inhibitory feedback projections to orexin neurons. This feedback mechanism might maintain the activity of monoaminergic neurons. A slight decrease in input to the monoaminergic neurons results in decreased inhibitory influence to orexin neurons. Orexin neurons, therefore, are disinhibited and increase excitatory influence to monoaminergic cells to maintain their activity. These monoaminergic cells send excitatory projections to the thalamus and cerebral cortex, and send inhibitory projections to the VLPO sleep centre. These mechanisms maintain wakefulness states. (b) Sleep state. VLPO sleep-active neurons are activated and send inhibitory projections to monoaminergic neurons and orexin neurons to maintain sleep. (c) Narcolepsy. If orexin neurons are removed, monoaminergic neurons and VLPO neurons set up a mutually inhibitory circuit, which can cause unwanted and abrupt transitions between the states. Activity in one of the competing sides shuts down inhibitory inputs from the other side, and therefore disinhibits its own action. So, when either side begins to overcome the other, the switch abruptly turns into the alternative state. This figure is adapted with modification from<sub>80</sub>.

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