Review

Cell PRESS

Feature review

Connectomics of orexin-producing neurons: interface of systems of emotion, energy homeostasis and arousal

Takeshi Sakurai and Michihiro Mieda

Department of Molecular Neuroscience and Integrative Physiology, Faculty of Medicine, Kanazawa University, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8640, Japan

Avoiding danger and finding food, which are life-sustaining activities that are regulated by emotion, reward and energy balance, require proper wakefulness. The orexin system controls sleep and wakefulness through interactions with systems that regulate emotion, reward and energy homeostasis. Recent findings have brought about the possibility of novel therapies targeting the orexin system for sleep disorders, including insomnia and narcolepsy–cataplexy, as well as other pathological conditions such as obesity and drug addiction [1]. In this review, we will discuss the current understanding of the integrative physiology and clinical perspectives of the orexin system. We will briefly review signaling through orexin A and B receptors and discuss the role of orexins in the pathophysiology of narcolepsy. We will also examine connections between orexin neurons and other brain areas involved in feeding behavior, reward and emotion. Finally, we will consider the therapeutic potential of drugs that target orexin receptors.

Orexin neurons as a link between arousal center and other systems

Feeding behavior is greatly affected by emotion, reward and energy balance. Simultaneously, it requires proper wakefulness and vigilance. Regulatory centers of feeding and energy homeostasis are believed to be localized within the hypothalamus, whereas the most crucial neuronal network for wakefulness is the ascending reticular activating system (ARAS) in the brainstem that sends projections to multiple areas of the forebrain (see Glossary). These observations suggest the existence of a neuronal system that acts as a link between the feeding centers in the hypothalamus and the brainstem 'waking center'. Recent evidence clearly suggests that orexin-producing neurons in the hypothalamus are crucial for such linkage.

Orexin A and orexin B (also known as hypocretin 1 and hypocretin 2) are hypothalamic neuropeptides that were discovered 13 years ago. Initially, these peptides were recognized as regulators of feeding behavior [2]. Subsequently, several studies [3] suggested that orexin deficiency causes narcolepsy in humans and other mammalian species, highlighting roles of this hypothalamic neuropeptide in the regulation of sleep and wakefulness [4]. Studies of

Glossary

Ascending reticular activating system (ARAS): the ascending reticular activating system is a concept originally proposed by Moruzzi and Magoun in 1949. They found that stimulation at the core of the reticular formation in the brainstem is effective in inducing desynchronized electroencephalogram (EEG) patterns in the cat, suggesting that this stimulation causes cortical arousal. Lesion of the reticular formation produced chronic EEG inactivation and somnolence. These studies introduced the concept that cortical activation was actively maintained by the tonic activation of brainstem core neurons. Currently, it is commonly considered that the reticular activating system is composed of several ascending pathways including monoaminergic and cholinergic neurons in the brainstem.

Cataplexy: episodic condition featuring loss of muscle function, ranging from slight weakness (such as limpness at the neck or knees, sagging facial muscles or inability to speak clearly) to complete body collapse. Cataplexy is often observed in narcolepsy patients.

Lateral hypothalamic area (LHA): the lateral hypothalamus or lateral hypothalamic area is a region of the hypothalamus, generally including the lateral hypothalamic area and lateral preoptic nucleus. Classically, this region is considered as a feeding center. Stimulation of this area causes increased food intake, whereas bilateral lesion of this area causes cessation of feeding. This region receives input from the amygdala. This area contains glucose sensitive neurons, which are inhibited by glucose This region is also crucially involved in the regulation of sympathetic nerve function and arousal.

Narcolepsy: neurological condition mostly characterized by excessive daytime sleepiness, episodes of sleep attacks and disorder of REM or rapid eye movement sleep.

Non-rapid eye movement (NREM) sleep: sleep is a cyclic process. Under normal conditions, sleep is characterized by regular alternation between rapid eye movement (REM) sleep and NREM sleep. NREM sleep is divided into four stages. Stages 1 and 2 are characterized by low arousal thresholds and are considered light sleep. Stages 3 and 4 are characterized by high arousal thresholds and are considered deep or 'slow wave' sleep. All four of these stages can be differentiated by electroencephalography (EEG). Stage 1 is marked by low voltage, mixed frequency EEG. Stage 2 is marked by the presence of K complexes and sleep spindles on EEG recordings. Both stages 3 and 4 are marked by delta waves, which have a voltage of 75 μV or more and a frequency range of 0.5 to 4 Hz, but differ in composition. Stage 3 is defined as sleep consisting of 20–50% delta waves and stage 4 as sleep consisting of more than 50% delta waves.

Rapid eye movement (REM) sleep: the stage of sleep characterized by rapid movements of the eyes. It was identified and defined by Nathaniel Kleitman and Eugene Aserinsky in 1953. Criteria for REM sleep include low muscle tone and a rapid, low-voltage EEG. REM sleep in adult humans typically occupies 20–25% of total sleep, approximately 90–120 min of a night's sleep.

Ventrolateral preoptic nucleus (VLPO): the ventrolateral preoptic nucleus (VLPO) is a region of the anterior hypothalamus, which includes sleep-active neurons that inhibit the major ascending monoaminergic arousal systems during sleep.

Corresponding author: Sakurai, T. (tsakurai@med.kanazawa-u.ac.jp).

0165-6147/$ – see front matter doi:10.1016/j.tips.2011.03.007 Trends in Pharmacological Sciences, August 2011, Vol. 32, No. 8

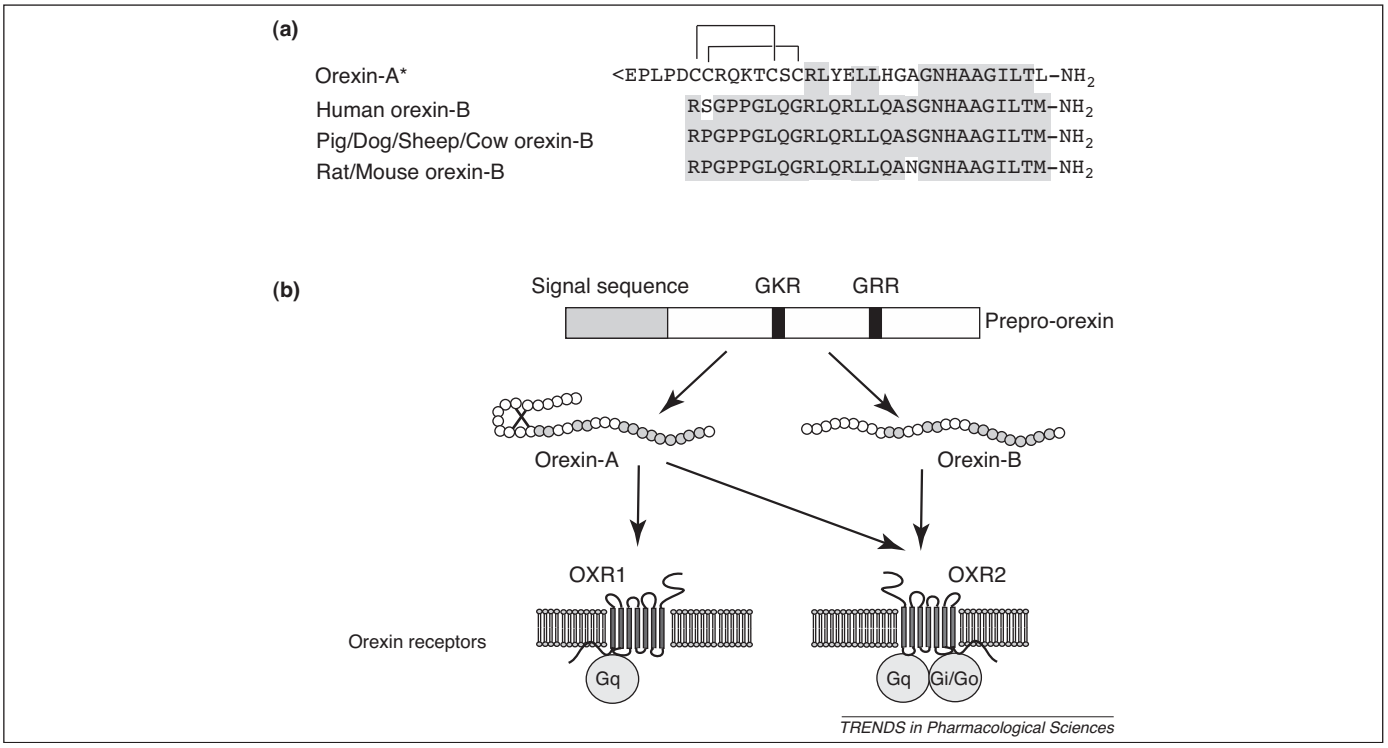

Figure 1. Orexin and orexin receptors. **(a)** Structures of various species of orexin A and orexin B. The topology of the two intrachain disulfide bonds of orexin A is indicated above the sequence. Shadows indicate amino acid identity. Mammalian orexin A sequences thus far identified (human, rat, mouse, pig, dog, sheep, cow) are all identical. **(b)** 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 OXR-1 and OXR-2 receptors. OXR-1 is selective for orexin A, whereas OXR-2 shows similar affinities for both orexin A and orexin B. OXR-1 is coupled to the G_q subclass of heterotrimeric G proteins, whereas OXR-2 couples to $G_{i/o}$ and/or G_q in cell lines.

efferent and afferent systems of orexin-producing neurons have shown that the orexin neuronal system has close interactions with systems that regulate emotion, energy homeostasis, reward and arousal [5–12]. These observations suggest that orexin neurons are involved in sensing the external and internal environments of the body, and regulate vigilance states accordingly. In this review, we will discuss the mechanisms by which the orexin system regulates wakefulness in relation to energy homeostasis and other systems. We will also discuss the therapeutic potential of drugs that target orexin receptors.

Orexin and orexin receptors

In 1998, our research team identified novel neuropeptides, orexin A and orexin B, from rat brain extracts as two endogenous ligands for two orphan G protein-coupled receptors (GPCRs) by a method called 'reverse pharmacology' [2]. Molecular cloning studies showed that both orexin A and orexin B are derived from a common precursor peptide, *prepro-orexin* (Figure 1). An mRNA encoding the same precursor peptide was independently identified by de Lecea *et al.* as a hypothalamus-specific transcript [13]. The authors predicted that the transcript encoded a polypeptide precursor that is cleaved to form two neuropeptides, termed hypocretin 1 and hypocretin 2 (corresponding to orexin A and orexin B, respectively). Orexin A and orexin B constitute a novel distinct peptide family, showing no significant homology with any previously described peptides [14]. Structural analysis of purified peptide showed that orexin A is a 33-amino acid peptide with an N-terminal pyroglutamyl residue, two intrachain disulfide bonds and C-terminal amidation. This structure is completely conserved among several mammalian species (human, rat, mouse, cow, sheep, dog and pig). Orexin B is a 28-amino acid, C-terminally amidated linear peptide. Amino acid sequences of various species of orexin B show that there are several species differences, although highly conserved. The C-terminal half of orexin B is very similar to that of orexin A, whereas the N-terminal half is more variable.

The actions of orexins are mediated via two GPCRs, orexin 1 (OXR-1) and orexin 2 (OXR-2) receptors (also known as Hcrtr1 and Hcrtr2). OXR-1 has one order of magnitude greater affinity for orexin A over orexin B. By contrast, OXR-2 accepts both ligands with similar affinities [2]. OXR-1 couples to $G_{q/11}$ class of G protein, which results in activation of phospholipase C to trigger the phosphatidylinositol cascade and influx of extracellular Na^+ and Ca^{2+}, presumably through TRP channels to cause depolarization of neurons. OXR-2 couples to both $G_{q/11}$ and G_i classes of G proteins in a neuronal cell line [15]. *OXR-1* and *OXR-2* mRNAs exhibit a markedly different and basically complementary distribution, suggesting that these receptors have distinct physiological roles through different neuronal pathways [16].

Orexin neurons, which have been assumed to number around 3000 in rat brains, or 70 000 in human brains [17,18], are localized exclusively in the hypothalamus, including the lateral hypothalamic area (LHA), perifornical area and posterior hypothalamus (PH) [17–19].

Implication of orexins in the pathophysiology of narcolepsy

What is narcolepsy?

An understanding of narcolepsy, a sleep disorder that results from loss of orexin neurons, provides insight into

the physiological functions of orexins. Narcolepsy is a debilitating neurological disorder that affects approximately 1 in 2000 individuals in the United States [20]. A cardinal symptom of the disorder is excessive daytime sleepiness (an insurmountable urge to sleep), which often results in falling asleep at inappropriate times and situations ('sleep attack'). The latency for rapid eye movement (REM) sleep is notably reduced in narcolepsy patients and the existence of 'sleep-onset REM periods' (i.e. REM sleep is directly preceded by an awake period) is one of the diagnostic criteria for narcolepsy. Nocturnal sleep is often disturbed by sleep fragmentation combined with the occurrence of hypnagogic hallucinations, vivid dreaming and sleep paralysis, which usually occur immediately after patients fall asleep. Narcolepsy patients often suffer from a condition called 'cataplexy', which is a sudden weakening of muscle tone, ranging from jaw dropping and speech slurring to complete bilateral collapse of the postural muscles. Usually, cataplexy is triggered by emotional stimuli (see Glossary). Unlike the sleep attack, consciousness is preserved during cataplexy. Narcolepsy with cataplexy is sometimes referred as 'narcolepsy–cataplexy'.

Thus, narcolepsy is characterized by the inability to maintain wakefulness states, pathological intrusion of REM sleep into wakefulness and frequent transitions between states of sleep and wakefulness, which suggests that orexins have important roles in the maintenance and stabilization of sleep and wakefulness and REM sleep regulation.

Roles of orexins in pathophysiology of narcolepsy

The first indications that orexins are involved in narcolepsy came from animal models. Mice lacking the *orexin* gene or dogs with null mutations in *OXR-2* show phenotypes remarkably similar to human narcoleptic patients [21,22]. *Prepro-orexin* knockout (KO) mice, orexin neuron-ablated (*orexin/ataxin-3* transgenic) mice and *OXR-1/OXR-2* double KO mice show similar phenotypes that have strong parallels to the human condition, characterized by behavioral arrests that are similar to cataplexy, occasional direct transitions to REM sleep from wakefulness and highly fragmented sleep–wake cycles [21,23], all of which are important elements of narcolepsy.

The link between orexin dysfunction and narcolepsy (especially if narcolepsy–cataplexy) has since been supported by studies with human patients. A postmortem study of human narcolepsy subjects showed no detectable levels of orexin peptides in the cortex and pons, in which normally orexinergic projections are found (Figure 2a), and an 80–100% reduction in the number of neurons containing detectable *prepro-orexin* mRNA or orexin-like immunoreactivity in the hypothalamus [24,25]. Approximately 90% of patients with narcolepsy are shown to have decreased orexin A levels in the cerebrospinal fluid (CSF) [26]. Therefore, a low CSF level of orexin A (<110 pg/ml) is now one of the diagnostic criteria for narcolepsy–cataplexy according to the second edition of the *International Classification of Sleep Disorders* [27].

Because of its strong association with certain human leukocyte antigen alleles [28], it has been speculated that narcolepsy results from selective immune-mediated degeneration of orexin neurons. Recently, Tribbles homolog 2 (Trib2) was reported as a possible antigen involved in the autoimmune destruction of orexin neurons [29]. Trib2 was abundantly expressed in orexin neurons and levels of Trib2-specific antibodies were much greater in people with narcolepsy. Recent studies also reported that susceptibility to narcolepsy is associated with single nucleotide polymorphisms in the T cell receptor α locus [30] and those located between the *carnitine palmitoyl transferase 1B* and *choline kinase β* [31].

Regardless of the cause of the neuronal 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.

Pathological contribution of each receptor subtype

Narcolepsy can be divided into two pathological phenomena, suggesting that pathophysiology of narcolepsy is caused by two independent mechanisms. One is a difficulty in maintaining long awake periods, characterized by abrupt transitions from wakefulness to non-REM (NREM) sleep (dysregulation of NREM sleep onset). This phenomenon manifests clinically as excessive daytime sleepiness, which sometimes results in sleep attacks. Mouse studies suggested that it largely results from a lack of OXR-2 signaling [32]. Psychostimulant drugs, such as modafinil, methyl phenidate, amphetamine and caffeine, are used to treat these symptoms.

The other key phenomenon is the pathological intrusions of REM sleep into wakefulness (dysregulation of REM sleep onset); it is during these periods that the patient might experience cataplexy, hypnagogic hallucinations and sleep paralysis. Available therapy for this symptom consists of tricyclic antidepressants such as imipramine and serotonin-specific reuptake inhibitors [33], suggesting existence of abnormal monoaminergic neurotransmission in cataplexy. Lack of signaling from both receptors is crucially associated with this symptom [32,34,35].

Input to orexin neurons

Recent studies using anterograde and retrograde tracers suggest that orexin neurons receive abundant projections from the lateral septum, the preoptic area, the amygdala, the bed nucleus of the stria terminalis (BNST), the posterior/dorsomedial hypothalamus and the serotonergic neurons in the raphe nuclei [9,10].

Input from limbic system

Abundant input from the limbic system [9,10,36] (which is implicated in emotion) suggests it plays a part in the regulation of the firing rate of orexin neurons. Indeed, the importance of this connection is apparent in the defense (or 'fight or flight') response. Mice tested in a resident–intruder paradigm show cardiovascular and locomotor responses to emotional stress, but these responses are diminished in *prepro-orexin* KO mice [37]. Similarly, air-jet stress-induced elevations of blood pressure and heart rate were attenuated in conscious *orexin/ataxin-3* transgenic mice, in which orexin neurons are genetically ablated [38]. Furthermore, in urethane-anesthetized mice, microinjection of the $GABA_A$ receptor antagonist bicuculline into

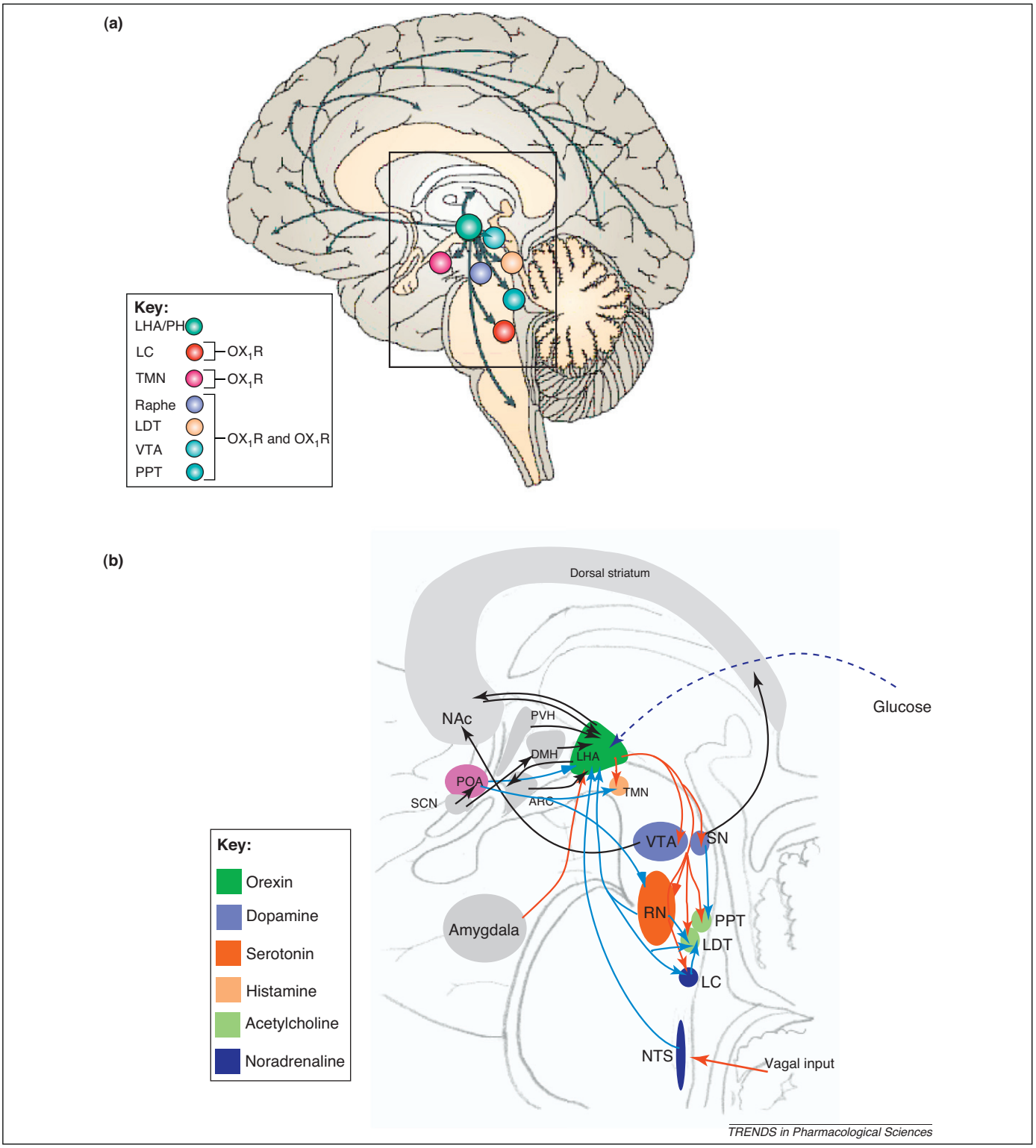

Figure 2. Connections of orexin neurons with other regions. Orexin neurons in the LHA provide a link between the limbic system, energy homeostasis and the brain stem nuclei. **(a)** Major projections of orexin neurons. Modified from Sakurai [4]. Circles show major target sites for orexins. Included in these are the LC (containing noradrenaline, NA), TMN (containing histamine, HA), raphe nuclei (Raphe, containing 5HT), VTA (containing dopamine, DA) and PPT/LDT (containing Ach). Orexin neurons promote wakefulness through the monoaminergic/cholinergic nuclei that are wake-active. **(b)** Schematic presentations of output and input of orexin neurons shown in regions of the rectangle in **(a)**. Connection between dopaminergic centers and orexin neurons modulates the reward systems. Input from the limbic system might be important to regulate the activity of orexin neurons upon emotional stimuli to evoke emotional arousal or fear-related responses. Sleep-active neurons in the POA send inhibitory influences to monoaminergic/cholinergic neurons and orexin neurons. Orexin neurons send both direct excitatory input to cholinergic neurons in the LDT/PPT and indirect inhibitory input to these cells through GABAergic local interneurons and GABAergic neurons in the substantia nigra pars reticulata [41]. Noradrenergic neurons in the LC and serotonergic neurons in the RN also send inhibitory influences to these cholinergic neurons. Blood glucose levels also affect the activity of orexin neurons through fluctuations of glucose levels in the CSF and vagal afferent. NAc, nucleus accumbens; PVH, paraventricular hypothalamic nucleus; TMN, tuberomammillary nucleus; LHA, lateral hypothalamic area; DMH, dorsomedial hypothalamus; ARC, arcuate nucleus; VTA, ventral tegmental area; SN, substantia nigra; SCN, suprachiasmatic nucleus; RN, raphe nucleus; LC, locus coeruleus; PPT, pedunculopontine tegmental nucleus; LDT, laterodorsal tegmental nucleus.

the amygdala or BNST increased Fos immunoreactivity in orexin neurons and induced long-lasting and dose-dependent cardiorespiratory excitation in wild-type mice. By contrast, in *orexin-ataxin 3* mice, cardiorespiratory responses after disinhibition of the amygdala were not observed and those after activation of the BNST were attenuated. These observations suggest that orexin-containing neurons mediate at least a part of amygdala- and BNST-induced increases in the sympathetic outflow [39].

The lower level of activation of the sympathetic outflow by emotional stimuli might affect the establishment of emotional memory, because emotional reaction has been known to be strongly affected by sympathetic responses. Abnormality of amygdala function was recently reported in human narcolepsy patients, using functional magnetic resonance imaging [40]. The study reported no enhancement of amygdala response to conditioned stimuli and no increase in functional coupling between the amygdala and medial prefrontal cortex. These findings suggest that human narcolepsy is accompanied by abnormal emotional learning and that, in line with animal data, the orexin system is crucial for the function of the amygdala in this process.

The neural input from the limbic system to orexin neurons is also implicated in the pathophysiology of cataplexy, because strong, generally positive emotional stimuli are well known to trigger cataplexy in narcolepsy–cataplexy patients. Local injection of orexin into the pedunculopontine tegmental nucleus (PPT) strongly inhibited REM-related atonia in cats [41]. These cholinergic neurons are implicated in REM-related atonia [42] and the same pathway is implicated in cataplexy. Therefore, through excitatory influences from the limbic system, emotional stimuli might increase orexin release in the PPT to prevent muscle atonia in wild-type animals. Mechanisms of action of orexin in the PPT/laterodorsal tegmental nucleus (LDT) will be discussed further below. In addition to the suppression of muscle atonia, activation of orexin neurons by the limbic system is likely to maintain wakefulness during emotional arousal by conveying various emotional stimuli to orexin neurons.

The limbic input to orexin neurons might also be involved in the regulation of feeding behavior. Some of the affective content of the perception of food is thought to be processed in the amygdala and limbic system [43], and this information might be passed on to orexin neurons. Food perception often evokes cataplexy in narcoleptic dogs [44], suggesting that orexin signaling is physiologically activated upon perception of food, and that this system is necessary to evoke proper feeding behavior including the maintenance of motor activity.

Input from preoptic areas

The preoptic area, especially the ventrolateral preoptic nucleus (VLPO) and median preoptic nucleus, appears to play a crucial role in NREM sleep initiation and maintenance. Some neurons in the VLPO are sleep-active neurons, which fire at a rapid rate during sleep and show attenuation of firing during wakefulness. GABA and galanin are the primary inhibitory neurotransmitters of the VLPO [45], which sends out multiple inhibitory projections onto the locus coeruleous (LC), tuberomammillary nucleus (TMN) and dorsal raphe (DR) [45,46].

Orexin neurons, which are strongly inhibited by both the $GABA_A$ agonist muscimol and the $GABA_B$ receptor agonist bacrofen [6,47], are innervated by GABA-containing cells in the VLPO [9,10]. This pathway might be important for turning off orexin neurons during sleep (Figure 2b). Selective disruption of $GABA_B$ receptor in orexin neurons resulted in severe fragmentation of sleep–wake states during both light and dark periods, suggesting that $GABA_B$ receptor-mediated regulation of orexin neurons play an important role in sleep–wake maintenance [48].

Input from the brainstem regions

Orexin neurons receive inhibitory projections from the raphe nuclei [49,50]. An electrophysiological study showed that orexin neurons are also inhibited by noradrenalin [51]. As described later, serotonergic neurons in the raphe nuclei and noradrenergic neurons in the LC have been considered crucial for wakefulness and receive dense excitatory projections of orexin neurons. Thus, these observations suggest that orexin neurons and monoaminergic neurons constitute negative feedback loop circuitry, which might play important parts in the maintenance of firing rates of each component within appropriate ranges (Figure 2b).

Factors that regulate orexin neurons

In addition to the classical neurotransmitters, glutamate and GABA, several neurotransmitters/neuromodulators have been shown to influence the firing rate of orexin neurons. Both noradrenaline and serotonin (5HT) hyperpolarize and inhibit GFP-expressing orexin neurons through the activation of G protein regulated inwardly rectifying K^+ (GIRK or Kir3) channels via α_2-adrenoceptors and $5HT_{1A}$ receptors, respectively [51–53]. The cholinergic agonist carbachol activates 27% and inhibits 6% of orexin neurons [9,53], whereas histamine does not have any effect on orexin neurons. Although orexin neurons do not express functional dopamine receptors, dopamine can inhibit orexin neurons by acting on α_2-adrenoceptors [51,53]. Several neuropeptides, including cholecystokinin (CCK-8S), neurotensin, oxytocin and vasopressin, induce depolarization and excitation of orexin neurons [54]. A synthetic surrogate ligand for an orphan receptor, BRS-3, also activates these neurons [55]. Recently, orexin itself was shown to activate orexin neurons through OXR-2, suggesting a positive feedback mechanism that maintains orexin neuronal activity [56].

Interestingly, metabolic signals also contribute to the regulation of firing rate of orexin neurons: decreasing the extracellular glucose concentration produced depolarization and increased the frequency of firing of orexin neurons, whereas increasing glucose concentration induced hyperpolarization and cessation of firing [6,57]. Importantly, this mechanism is sufficiently sensitive to encode variations in glucose levels in CSF reflecting those occurring physiologically between normal meals [6,57]. These responses were shown to be mediated by tandem-pore K^+ (K_{2P}) channels [58]. In addition, hypoglycemic signals transmitted via vagal afferents and the nucleus of the solitary tract were also shown to inhibit orexin neurons [59,60].

Ghrelin, a stomach-derived orexigenic peptide, activated 60% of dispersed orexin neurons with depolarization and an increase in firing frequency [6]. By contrast, bath application of leptin, an anorexigenic protein hormone secreted by adipocytes, was found to robustly inhibit most of the orexin neurons examined, causing hyperpolarization and a decrease in firing rate [6].

These findings show that peripheral humoral factors that are related to energy metabolism influence the activity of orexin neurons. In addition, orexin expressions in wild-type and *ob*/*ob* mice are negatively correlated with changes in blood glucose, leptin and food intake [6]. These observations support the idea that orexin neurons act as a sensor of the nutritional status [2,6,14].

This suggests that nutritional depletion-induced metabolic cues activate orexin neurons to increase arousal, thereby reinforcing food-seeking/feeding pathways. Consistently, orexin neuron-ablated mice fail to exhibit this fasting-induced arousal [6], suggesting that orexin neurons are necessary for evoking adaptive behavioral arousal during fasting. The mechanism that helps to ensure survival in nature clearly involves orexins and might hinder attempts to treat obesity by food restriction. This might also explain why orexin receptor antagonists decrease food intake (see below) [61].

Output of orexin neurons

Orexin-immunoreactive fibers were observed in the almost entire neuroaxis, excluding the cerebellum [17–19]. The densest staining of fibers is found in the paraventricular nucleus of the thalamus, arcuate nucleus (Arc) of the hypothalamus and, most notably, monoaminergic nuclei in the brain stem regions, including the LC (containing noradrenergic neurons), raphe nuclei (containing serotonergic neurons), TMN (containing histaminergic neurons) and LDT/PPT (containing cholinergic neurons) [17–19]. The distribution of the orexin receptor mRNA is consistent with these projection sites; within the brain, *OXR-1* is most abundantly expressed in the LC, whereas *OXR-2* is highly expressed in the TMN [16]. Both regions are important for the maintenance of arousal [16]. The raphe nuclei, LDT/PPT and ventral tegmental area (VTA) contain both *OXR-1* and *OXR-2* [16], although they are expressed in distinct neuronal populations in each region [35] (discussed further below). These observations suggest that these monoaminergic/cholinergic regions, implicated in the regulation of wakefulness, are major effector sites of orexins (Figure 2).

These monoaminergic neurons diffusely innervate the forebrain. Their projections are thought to be involved in the ARAS and have been shown to play important roles in promoting arousal. Pontine neurons that produce acetylcholine (Ach) in PPT/LDT are also important for the regulation of wakefulness as well as REM sleep.

These observations suggest that the main output regions of orexin neurons are monoaminergic/cholinergic neurons in the brain stem. Through these connections, orexins exert their physiological functions.

Differential expression of two subtypes of orexin receptors

Although it is clear that monoaminergic/cholinergic nuclei are important regions for orexin action, our recent obser-

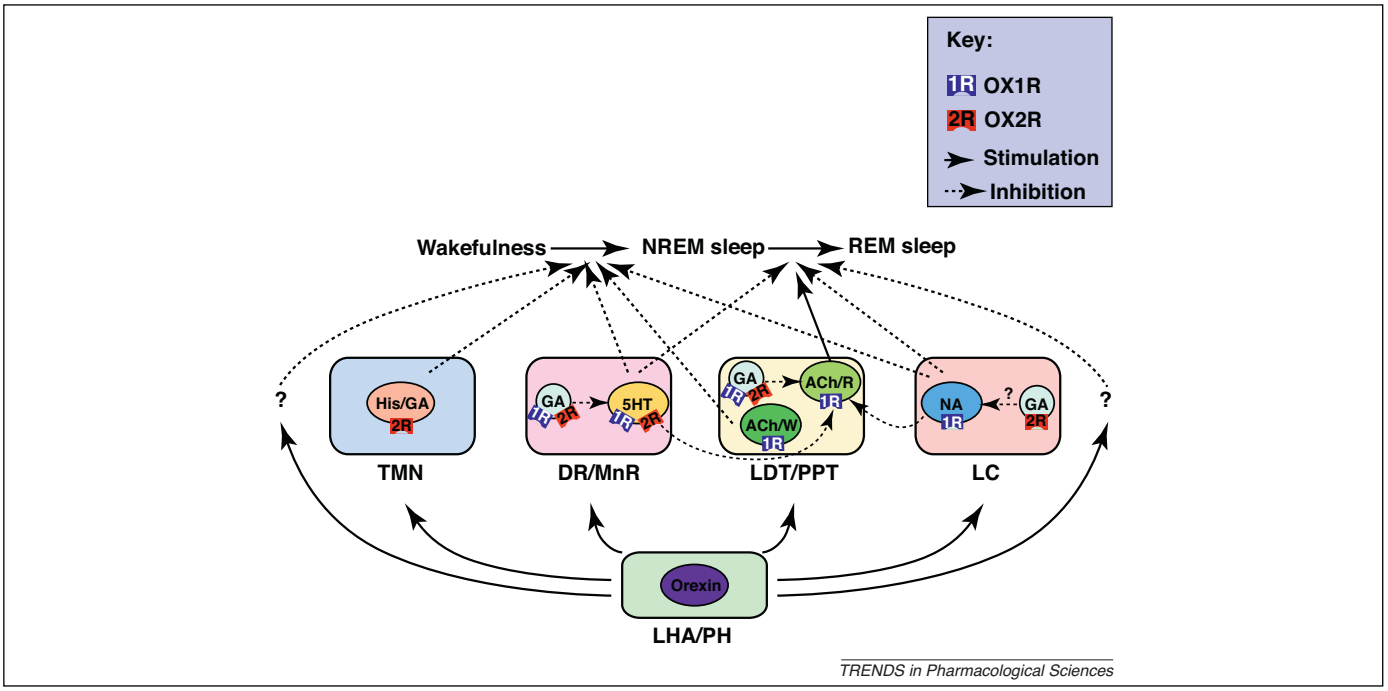

Figure 3. Schematic illustration of presumed pathways underlying orexin actions on NREM and REM sleep [35]. Orexins activate histaminergic (His)/GABAergic (GA), serotonergic (5HT), noradrenergic (NA) and cholinergic (ACh) neurons, as well as GABAergic putative interneurons, in wake-promoting nuclei, including the TMN, DR/MnR, LDT/PPT and LC. These neurons differentially express OXR-1 and/or OXR-2, and regulate wakefulness/NREM sleep and NREM/REM sleep transitions. OXR-1 and OXR-2 can be expressed in the same populations of GABAergic neurons, as shown in the figure, or can be expressed in distinct populations of these neurons in each area. Wake/REM-on cholinergic neurons (ACh/W) are likely to suppress NREM sleep but REM-on cholinergic neurons (ACh/R) are likely to induce REM sleep. Wake-active serotonergic and noradrenergic neurons in the DR/MnR and LC, respectively, counteract activation of REM-on cholinergic neurons in the LDT/PPT, as well as REM-on neurons in the brainstem reticular formation [4,75]. Previous reports have suggested contributions of GABAergic interneurons inhibiting PPT cholinergic and raphe serotonergic neurons [41,67]. LHA, lateral hypothalamic area; PH, posterior hypothalamus.

vations suggest that regulation of these nuclei by two subtypes of orexin receptors seems to be more complicated. As discussed earlier, the TMN abundantly expressed *OXR-2* and the LC predominantly expressed *OXR-1*. In the LDT and PPT, expressions of both *OXR-1* and *OXR-2* were observed. Likewise, in the dorsal and median raphe nuclei (DR and MnR), both *OXR-1* and *OXR*-2 mRNA are expressed.

Notably, in the TMN, we found that most of *OXR-2*-positive histaminergic neurons also express *Gad1* mRNA, suggesting that *OXR-2*-positive histaminergic neurons are also GABAergic. The majority of serotonergic neurons in the DR and MnR express both *OXR-1* and *OXR-2*. We also observed expressions of *OXR-1* or *OXR-2* mRNA in GABAergic neurons in the raphe nuclei. In the LC, all *OXR-1*-positive neurons are noradrenergic neurons.

In the LDT and PPT, *OXR-1* mRNA is expressed in all cholinergic neurons. Both *OXR-1* and *OXR-2* mRNA were found to be expressed in GABAergic neurons [35].

These observations suggest that in the monoaminergic/cholinergic nuclei, two orexin receptors play distinct roles in regulating activity of neurons that constitutes the ARAS (Figure 3).

Sleep–wake regulation and orexins

Physiological roles of orexins on sleep–wake states

Intracerebroventricular (ICV) injection of orexin A or orexin B in rats or mice during the light (rest) period potently increases awake time and decreases REM and NREM sleep time [62]. Optogenetic excitation of orexin neurons results in increases in the probability of an awakening event during both NREM and REM sleep [63,64]. As discussed above, the projection pattern of orexin neurons and distributions of orexin receptor mRNAs suggest that main effector sites for orexin are monoaminergic/cholinergic neurons in the brainstem. Indeed, electrophysiological experiments showed that firing rates of monoaminergic cells in these nuclei are increased by orexins. Noradrenergic cells of the LC [62,65], dopaminergic cells of the VTA [66], serotonergic cells of the DR [67,68] and histaminergic cells of the TMN [69] have all been shown to increase their firing rates by orexins. The firing rates of these monoaminergic neurons are strongly associated with sleep–wakefulness states: they fire tonically during awake period, less during NREM sleep and cease firing during REM sleep [70]. These observations suggest that increased firing of these wake-active monoaminergic neurons mediates arousal induced by orexins.

Orexins also have strong direct excitatory effects on cholinergic neurons in the basal forebrain, which are important for maintaining arousal and attention [71,72]. In addition, orexin neurons project directly to the LDT/PPT cholinergic neurons. Some populations of these cholinergic neurons are implicated in the maintenance of wakefulness and REM sleep (W/REM-on neurons) [73], whereas other populations are implicated in desynchronization of cerebral cortex and muscle atonia during REM sleep (REM-on neurons) [73]. Direct injection of orexin A into the LDT of cats results in an increased awake time and a decreased REM sleep time [74]. In addition, several reports have shown that orexin induces long-lasting excitation of cholinergic neurons in the LDT [75]. However, recent research also showed that orexin A inhibits cholinergic neurons in the PPT via activation of GABAergic local interneurons and GABAergic neurons in the substantia nigra pars reticulata, which send inhibitory projections to the PPT [41]. In fact, as discussed earlier, we found robust expression of *OXR-1* in the cholinergic neurons and expression of both receptors in GABAergic neurons in these regions [35]. Consistently, orexin A excites both cholinergic and non-cholinergic neurons of the LDT in slice preparations [76]. Taken together, these results indicate that in the LDT/PPT, orexin might activate W/REM-on cholinergic neurons through OX_1R to facilitate wakefulness. Simultaneously, orexin might activate GABAergic interneurons to inhibit REM-on cholinergic neurons through activation of both receptors in these nuclei. Additionally, orexinergic activations of wake-active noradrenergic and serotonergic neurons in the LC and raphe nuclei through OXR-1 and both receptors, respectively, are likely to counteract activation of REM-on cholinergic neurons in the LDT/PPT during wakefulness [4,77]. This is consistent with the fact that tricyclic antidepressants and serotonin-specific reuptake inhibitors are effective for treating cataplexy in narcoleptic patients.

Orexin receptors in sleep and wakefulness

It is believed that the effect of orexin on wakefulness is largely mediated by activation of the TMN histaminergic neurons that express OXR-2 [69,78]. *OXR-2* KO mice and *prepro-orexin* KO mice are similarly affected by behaviorally abnormal attacks of NREM sleep ('sleep attacks') [32], but *OXR-2* KO mice show a lower degree of disrupted wakefulness compared with double receptor KO (*OXR-1*- and *OXR-2*-null) mice [4,32,79]. In particular, *OXR-2* KO mice are only mildly affected by cataplexy and direct transitions to REM sleep from awake states [32], as compared with orexin KO mice and double receptor KO mice [14,21,32]. *Orexin* KO mice and double receptor KO mice have very similar phenotypes [14,32]. Thus, these observations suggest that *OXR-2* plays a pivotal role, but *OXR-1* has additional effects on sleep–wake regulation. The gating of REM sleep is likely to crucially involve both receptors.

Orexinergic activity in the sleep–wake cycle

Transgenic mice in which orexin is constitutively expressed in a diffuse, ectopic pattern in the brain exhibit 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 [80]. These results suggest that firing rate of orexin neurons should be decreased or ceased to maintain consolidated NREM sleep and the muscle atonia that accompanies REM sleep.

Consistent with this idea, Fos expression (a marker of neuronal activity) in orexin neurons in rats is increased during the dark, active period in which the awake state is dominant [81]. Orexin levels in CSF peak during the dark period and decrease during the light period in which the sleep state is dominant [82]. Moreover, *in vivo* recording studies revealed changes of orexin neuronal activity across the sleep–wake cycle in rats or mice [83–85]. These studies

showed that orexin neurons fire during active waking, decrease discharge during quiet waking and virtually cease firing during both REM and NREM sleep.

Mechanisms that underlie orexin-mediated stabilization of sleep–wakefulness states

A reciprocal feedback loop between orexin neurons and monoaminergic neurons in the brainstem (including the LC and DR [49,51,53]) might maintain the activity of monoaminergic neurons to stabilize wakefulness (Figure 2b). Wake-active monoaminergic centers are also influenced by the inhibitory projections from the preoptic area (POA), especially VLPO (see Glossary) [46]. During sleep, VLPO sleep-active neurons are thought to be activated by sleep substances such as adenosine [86–88]. As discussed above, the sleep-active neurons also send inhibitory projections to hypothalamic orexin neurons [10,89]. These neural circuits might be important for stability of wakefulness.

Without orexin neurons, the balance between these waking-active neurons and sleep-active neurons in the POA is likely to be delicate, and sleep and wakefulness will turn into the alternative mode abruptly, as observed in narcolepsy.

Dual center model of sleep–wake regulation

The dual center hypothesis of feeding regulation, which was proposed around 50 years ago, states that the ventromedial hypothalamus (VMH) (see Glossary) is the satiety center, whereas the LHA is the feeding center. Although later studies have shown that the regulatory mechanisms of feeding are more complex (involving several hypothalamic regions including the Arc, paraventricular nucleus and dorsomedial hypothalamus), satiety and feeding is a balancing process between two major groups of neurochemical pathways.

Similarly, in sleep–wakefulness regulation, there seems to be two major groups of neural pathways that balance sleep and wakefulness. The effector mechanism that regulates cortical function is the monoaminergic/cholinergic neurons in the brainstem (ARAS), whereby diffuse innervations to cerebral cortex play an important role in desynchronization of cortical neurons during wakefulness. Orexin neurons in the LHA appear to be the accelerator, whereas sleep-active neurons in the preoptic area are likely to be the decelerator of the ARAS to regulate cortical function and sleep–wake states.

Roles of orexins in feeding behavior and energy homeostasis

Initially, orexins were recognized as regulators of feeding behavior because of their exclusive production in the LHA and their actions on food intake. ICV injection of orexins during the light period induces feeding behavior in rats and mice [2,61,90,91].

In addition, orexin mRNA expression was shown to increase during fasting [2]. A transcription factor Foxa2 is likely to mediate this phenomenon by translocating into the nucleus, binding to orexin promoter and subsequently stimulating transcription of orexin gene in response to fasting [92].

Genetic studies have also shown that orexins play a role in the regulation of energy homeostasis. For example, orexin neuron-ablated mice display hypophagia and late-onset obesity, although the extent of abnormality crucially depends on the genetic backgrounds of the mice [23,93]. Furthermore, narcolepsy patients have a decreased caloric intake but an increased body mass index [94,95]. The orexin system is likely to positively regulate feeding as well as arousal, activity and basal energy expenditure, and this might explain why narcoleptic mice and humans show increased body weight despite their hypophagia.

Supporting the physiological relevance of orexin in the control of feeding, ICV administration of an anti-orexin antibody or an OXR-1-selective antagonist reduced food intake [61,96].

OXR-2-mediated pathways might also be important for energy homeostasis [97]. Transgenic mice with ubiquitous orexin overexpression are resistant to high-fat diet-induced obesity and insulin insensitivity by promoting energy expenditure and reducing consumption. Genetic and pharmacological studies indicated that OXR-2 (rather than OXR-1) signaling predominantly mediates this phenotype through negative energy homeostasis and improved leptin sensitivity [97].

Consistent with the dense projection of orexin neurons to the Arc [17,19,98], several studies have suggested that the increased food intake following orexin A administration is at least partly mediated by activation of neuropeptide Y neurons in the Arc [98,99]. Other events involved in orexin-induced feeding behavior include inhibition of proopiomelanocortin neurons in the Arc, which are thought to play an important role in leptin-mediated inhibition of food intake [99]. Recent reports also showed that infusions of orexin A into the shell of the nucleus accumbens (NAc) increase feeding behavior [100]. In addition, infusions of the $GABA_A$ receptor agonist muscimol into the NAc shell strongly induced food intake and simultaneously increased Fos expression specifically in orexin neurons [101]. These findings suggest that reciprocal interactions between the orexin and limbic systems play a role in the regulation of feeding. In addition, the orexin system stimulates feeding-associated glucose utilization in skeletal muscle by activation of sympathetic nervous system through acting on the VMH [102].

As discussed above, orexin-mediated maintenance of consolidated wakefulness might also be important in feeding behavior, because maintenance of arousal during food searching and intake is essential for the survival of an animal [6]. In other words, if energy stores are low, firing rate of orexin neurons might be increased to maintain wakefulness, allowing more time to search for food.

Other functions

The orexin system has been also implicated in many other systems, such as reward system, autonomic nervous system and stress response. In this review, we will just briefly mention these functions. Please refer to another review for detailed descriptions [4].

Orexin and the autonomic nervous system

Several studies clearly showed that orexins play a role in mobilizing sympathetic responses. It has been demonstrated that ICV orexin injections increase blood pressure and

Table 1. List of available orexin receptor antagonists

	Compound	Affinity		Units	Ref.
		OXR-1	OXR-2		
DORA	ACT078573 (almorexant)	7.9 (human), 7.8 (rat)	8.1 (human), 7.8 (rat)	pIC_{50}	[106]
DORA	MK4305	9.26	9.46	pK_i	[115]
OXR-1 SORA	SB410220	7.7	n.d.	pK_i	[116]
OXR-1 SORA	SB334867	7.2	n.d.	pK_i	[116]
OXR-1 SORA	SB408124	7	n.d.	pK_i	[116]
OXR-1 SORA	[^{3}H]SB674042	8.3	n.d.	pK_d	[116]
OXR-1 SORA	SB410220	8.1	6.3	pK_b	[116]
OXR-1 SORA	SB334867	7.4	5.7	pK_b	[117]
OXR-1 SORA	SB408124	7.7	5.9	pK_b	[116]
OXR-1 SORA	SB674042	9	6.9	pK_b	[116]
OXR-2 SORA	1-(2-Bromo-phenyl)-3-((4*S*, 5*S*)-2,2-dimethyl-4-phenyl-[1,3] dioxan-5-yl)-urea	5.3–6.1	6.8–7.1	pK_i	[118]
OXR-2 SORA	1-(2,4-Dibromo-phenyl)-3-((4*S*, 5*S*)-2,2-dimethyl-4-phenyl-[1,3] dioxan-5-yl)-urea (JNJ10397049)	5.3–5.8	8.0–8.6	pK_i	[114]

SORA, single orexin receptor antagonist; DORA, dual orexin receptor antagonist.
n.d., not determined.

heart rate, and that these effects are abolished by administration of drugs that block α- or β-adrenoceptors [103]. Moreover, blood pressure in orexin-deficient mice is 10–15 mm Hg lower than in wild-type littermates [38]. As discussed earlier, the orexin-mediated increase in sympathetic tone might also be involved in the mechanisms by which the limbic system modulates the sympathetic outflow responding to emotional stimuli [37,38].

Orexin and the reward system

Activation of orexin neurons was shown to be strongly linked to preferences for cues associated with drug and food rewards [11]. ICV or local VTA infusions of orexins have been shown to reinstate drug-seeking or food-seeking behavior in rodents [5,11]. Conversely, the subcutaneous morphine (μ opioid receptor agonist)-induced place preference and hyperlocomotion observed in wild-type mice were abolished in mice that lacked the *prepro-orexin* gene [12], and injections of an OXR-1 antagonist into the VTA block the development of morphine-conditioned place preference [12]. These observations suggest the strong functional interaction between orexinergic pathways and the dopaminergic system. *In vivo* administration of an OXR-1 antagonist blocks locomotor sensitization to cocaine and occludes cocaine-induced potentiation of excitatory currents in VTA dopamine neurons [104]. These results suggest an important role for orexin signaling in the VTA in the neural plasticity associated with reward and indicate that orexins also contribute to cocaine-induced psychomotor sensitization and reward-seeking. Interestingly, some narcolepsy patients with daytime sleepiness who had been treated with amphetamine-like stimulants and/or sodium oxybate (γ hydroxybutyrate, also known as GHB) for a long time rarely developed drug abuse [105].

Therapeutic potential of drugs that target orexin receptors

Orexin receptor antagonists

Orexin initially drew attention as a regulator of food intake. Therefore, several pharmaceutical companies developed orexin receptor antagonists to control appetite for obesity treatment. Indeed, an OXR-1-selective antagonist reduced food intake and ameliorated obesity in leptin-deficient *ob*/*ob* mice [90].

Subsequently, because orexins have been implicated in the maintenance of arousal, several companies have been exploring the possibility of using orexin receptor antagonists as drugs for insomnia treatment. To date, several orexin receptor antagonists with different pharmacological characteristics have been developed (Table 1). A first-in-class dual orexin receptor antagonist almorexant (ACT078573, Actelion Pharmaceuticals Ltd) was recently developed. It blocks both OXR-1 and OXR-2 with almost equimolar potency (IC_{50} of 16 and 15 nM, respectively). It reduces time spent in wake states and enables and maintains sleep in rats, dogs and humans [106,107]. Recently, the results of a study on almorexant in 147 patients with primary insomnia were reported [107]. The drug significantly improved the primary parameter of sleep efficiency (time spent sleeping while confined to bed during an 8-h period at night) in a dose-dependent manner. Almorexant decreased latency to sleep onset and number of wake bouts after sleep onset. Importantly, Actelion Pharmaceuticals Ltd reports that almorexant not only changed these physiological sleep parameters but also significantly improved subjective sleep qualities. Effective doses of almorexant did not cause any relevant negative effects on next-day performance (assessed by fine motor testing and mean reaction time), as is commonly observed with current insomnia treatments such as benzodiazepines which are GABA receptor modulators. In addition, it was reported that rats treated with administration of high doses of almorexant (300 mg/kg, p.o.) are fully capable of spatial and avoidance learning [108]. Importantly, almorexant increases time spent in REM sleep, which is thought to be of key importance for our ability to store and process information. However, at the same the effect of REM period elongation might cause unwanted complications such as vivid dreaming, nightmare and sleep paralysis. Notably, almorexant was well tolerated with no signs of cataplexy, suggesting

that acute, short-lived, intermittent temporary blockage of orexin receptors will not result in a narcolepsy-like phenotype [109].

MK4305 is a compound 10 with a 7-methyl substitution on the diazepane core with a potent dual orexin receptor antagonistic activity. This compound is also currently under Phase III clinical trials for the treatment of primary insomnia.

Recently, administrations of an OXR-2 selective antagonist JNJ10397049 were shown to decrease the latency for persistent sleep and increased NREM sleep time more potently than the dual antagonist almorexant [110]. Administrations of an OXR-1 selective antagonist SB408124 had no effect on sleep parameters. Rather, the OXR-1 antagonist attenuated the sleep-promoting effects of OXR-2 antagonist when simultaneously administered, possibly by increasing dopamine release in the prefrontal cortex. Thus, selective OXR-2 antagonists can offer an advantage for the treatment of insomnia. As discussed earlier, OXR-1 and OXR-2 are differentially distributed in the monoaminergic/cholinergic regions, suggesting distinct physiological roles of these receptors. Further research using selective antagonists and/or each receptor KO mouse would be required to understand effectiveness, advantages and disadvantages of these compounds.

It has been shown that orexin mediates many behaviors associated with drug addiction in rodents owing to its effects on the VTA [111]. A recent report showed that orexin-1 receptor antagonist SB334867 reduces the acquisition and expression of cocaine-conditioned reinforcement and the expression of amphetamine-conditioned reward, suggesting that OXR1-selective antagonists have the potential to be a treatment method for individuals struggling with drug relapse and dependency [112]. Although whether the effects of orexin are the same in humans is still unknown, it is reasonable to speculate that orexin receptor antagonists might be effective for treating drug addiction. This notion is supported by the fact that drug addiction is seldom found in narcolepsy patients who are treated with psychostimulants.

OXR-1 antagonists might also be effective for panic disorders. They inhibit elevations of mean arterial pressure, heart rates and freezing responses in rat models of panic disorder [113].

Orexin receptor agonists

Because narcolepsy–cataplexy results from the absence of orexin, replacement therapy using orexin receptor agonists could be valuable for treating narcolepsy. These drugs might also be effective in other conditions of excessive daytime sleepiness. Indications that this might be successful come from a study demonstrating that chronic overproduction of orexin peptides from an ectopically expressed transgene prevented the development of a narcolepsy syndrome in orexin neuron-ablated (*orexin/ataxin-3* transgenic) mice [114]. Acute ICV administration of orexin A also maintained wakefulness, suppressed sleep and inhibited cataplectic attacks in *orexin/ataxin-3* mice [114]. ICV administration of orexin A has stronger arousal effects in *orexin/ataxin-3* transgenic mice than in wild-type controls [114]. The greater effectiveness might not result from increased expression of orexin receptors [114]. Rather, in the *orexin/ataxin-3* mice, monoaminergic neurons in the brainstem become more sensitive to various stimuli (our unpublished results). This mechanism might explain why narcoleptics cannot maintain long consolidated NREM sleep periods.

Effectiveness of ICV orexin to narcoleptic phenotype suggests that orexin receptor agonists would be of potential value for treating narcolepsy. However, as mentioned before, chronic overexpression of orexin in an unregulated manner results in disruption of NREM sleep and, therefore, it will be beneficial for therapeutically relevant orexin agonists to have a short half-life (<12 h).

Concluding remarks and perspectives

The symptoms and the cellular and systems-level bases of narcolepsy–cataplexy unequivocally show that orexins and orexin receptors are important regulators of sleep and wakefulness and of arousal maintenance by regulating monoaminergic and cholinergic nuclei in the brain. Orexin neurons receive afferents from multiple neuronal systems and send excitatory signals to monoaminergic and cholinergic nuclei in the brainstem. Understanding connectomics of orexin neurons more precisely might provide further insights into how the systems regulating emotion, energy homeostasis and reward interact with the mechanism that regulates sleep and wakefulness. Further studies dissecting roles of each receptor, in particular brain regions, by means of spatially restricted deletion of each receptor gene, as well as specific regional rescue of each receptor to receptor KO mice, might shed light on the mechanisms of action of these peptides.

References

1 Moruzzi, G. and Magoun, H.W. (1949) Brain stem reticular formation and activation of the EEG. *Electroencephalogr Clin Neurophysiol* 1, 455–473

2 Sakurai, T. *et al.* (1998) Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92, 573–585

3 Aserinsky, E. and Kleitman, N. (1953) Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. *Science* 118, 273–274

4 Sakurai, T. (2007) The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness. *Nat. Rev. Neurosci.* 8, 171–181

5 Boutrel, B. *et al.* (2005) Role for hypocretin in mediating stress-induced reinstatement of cocaine-seeking behavior. *Proc. Natl. Acad. Sci. U.S.A.* 102, 19168–19173

6 Yamanaka, A. *et al.* (2003) Hypothalamic orexin neurons regulate arousal according to energy balance in mice. *Neuron* 38, 701–713

7 Akiyama, M. *et al.* (2004) Reduced food anticipatory activity in genetically orexin (hypocretin) neuron-ablated mice. *Eur. J. Neurosci.* 20, 3054–3062

8 Mieda, M. *et al.* (2004) Orexin neurons function in an efferent pathway of a food-entrainable circadian oscillator in eliciting food-anticipatory activity and wakefulness. *J. Neurosci.* 24, 10493–10501

9 Sakurai, T. *et al.* (2005) Input of orexin/hypocretin neurons revealed by a genetically encoded tracer in mice. *Neuron* 46, 297–308

10 Yoshida, K. *et al.* (2006) Afferents to the orexin neurons of the rat brain. *J. Comp. Neurol.* 494, 845–861

11 Harris, G.C. *et al.* (2005) A role for lateral hypothalamic orexin neurons in reward seeking. *Nature* 437, 556–559

12 Narita, M. *et al.* (2006) Direct involvement of orexinergic systems in the activation of the mesolimbic dopamine pathway and related behaviors induced by morphine. *J. Neurosci.* 26, 398–405

13 de Lecea, L. *et al.* (1998) The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc. Natl. Acad. Sci. U.S.A.* 95, 322–327
14 Willie, J.T. *et al.* (2001) To eat or to sleep? Orexin in the regulation of feeding and wakefulness. *Annu. Rev. Neurosci.* 24, 429–458
15 Zhu, Y. *et al.* (2003) Orexin receptor type-1 couples exclusively to pertussis toxin-insensitive G-proteins, while orexin receptor type-2 couples to both pertussis toxin-sensitive and -insensitive G-proteins. *J. Pharmacol. Sci.* 92, 259–266
16 Marcus, J.N. *et al.* (2001) Differential expression of orexin receptors 1 and 2 in the rat brain. *J. Comp. Neurol.* 435, 6–25
17 Peyron, C. *et al.* (1998) Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J. Neurosci.* 18, 9996–10015
18 Nambu, T. *et al.* (1999) Distribution of orexin neurons in the adult rat brain. *Brain Res.* 827, 243–260
19 Date, Y. *et al.* (1999) Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. *Proc. Natl. Acad. Sci. U.S.A.* 96, 748–753
20 Mignot, E. (1998) Genetic and familial aspects of narcolepsy. *Neurology* 50, S16–S22
21 Chemelli, R.M. *et al.* (1999) Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* 98, 437–451
22 Lin, L. *et al.* (1999) The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* 98, 365–376
23 Hara, J. *et al.* (2001) Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron* 30, 345–354
24 Peyron, C. *et al.* (2000) A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat. Med.* 9, 991–997
25 Thannickal, T.C. *et al.* (2000) Reduced number of hypocretin neurons in human narcolepsy. *Neuron* 27, 469–474
26 Mignot, E. *et al.* (2002) The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. *Arch. Neurol.* 59, 1553–1562
27 ICSD2 (2005) *The International Classification of Sleep Disorders: Diagnostic and Coding Manual*, (second ed.), Amer Academy of Sleep Medicine
28 Kadotani, H. *et al.* (1998) Genetic studies in the sleep disorder narcolepsy. *Genome Res.* 8, 427–434
29 Cvetkovic-Lopes, V. *et al.* (2010) Elevated Tribbles homolog 2-specific antibody levels in narcolepsy patients. *J. Clin. Invest.* 120, 713–719
30 Hallmayer, J. *et al.* (2009) Narcolepsy is strongly associated with the T-cell receptor alpha locus. *Nat. Genet.* 41, 708–711
31 Miyagawa, T. *et al.* (2008) Variant between CPT1B and CHKB associated with susceptibility to narcolepsy. *Nat. Genet.* 40, 1324–1328
32 Willie, J.T. *et al.* (2003) Distinct narcolepsy syndromes in Orexin receptor-2 and Orexin null mice: molecular genetic dissection of non-REM and REM sleep regulatory processes. *Neuron* 38, 715–730
33 Zeitzer, J.M. *et al.* (2006) The neurobiology of hypocretins (orexins), narcolepsy and related therapeutic interventions. *Trends Pharmacol. Sci.* 27, 368–374
34 Mieda, M. and Sakurai, T. (2009) Integrative physiology of orexins and orexin receptors. *CNS Neurol Disord Drug Targets* 8, 281–295
35 Mieda, M. *et al.* Differential roles of orexin receptor-1 and -2 in the regulation of non-REM and REM sleep. *J. Neurosci.* in press
36 Winsky-Sommerer, R. *et al.* (2004) Interaction between the corticotropin-releasing factor system and hypocretins (orexins): a novel circuit mediating stress response. *J. Neurosci.* 24, 11439–11448
37 Kayaba, Y. *et al.* (2003) Attenuated defense response and low basal blood pressure in orexin knockout mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 285, R581–R593
38 Zhang, W. *et al.* (2006) Orexin neuron-mediated skeletal muscle vasodilation and shift of baroreflex during defense response in mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 290, R1654–R1663
39 Zhang, W. *et al.* (2009) Orexin neurons in the hypothalamus mediate cardiorespiratory responses induced by disinhibition of the amygdala and bed nucleus of the stria terminalis. *Brain Res.* 1262, 25–37
40 Ponz, A. *et al.* (2010) Reduced amygdala activity during aversive conditioning in human narcolepsy. *Ann. Neurol.* 67, 394–398
41 Takakusaki, K. *et al.* (2005) Orexinergic projections to the midbrain mediate alternation of emotional behavioral states from locomotion to cataplexy. *J. Physiol.* 568, 1003–1020
42 Shiromani, P.J. *et al.* (1988) Distribution of choline acetyltransferase immunoreactive somata in the feline brainstem: implications for REM sleep generation. *Sleep* 11, 1–16
43 Berthoud, H.R. (2004) Mind versus metabolism in the control of food intake and energy balance. *Physiol. Behav.* 81, 781–793
44 Reid, M.S. *et al.* (1998) Neuropharmacological characterization of basal forebrain cholinergic stimulated cataplexy in narcoleptic canines. *Exp. Neurol.* 151, 89–104
45 Sherin, J.E. *et al.* (1998) Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. *J. Neurosci.* 18, 4705–4721
46 Lu, J. *et al.* (2002) Selective activation of the extended ventrolateral preoptic nucleus during rapid eye movement sleep. *J. Neurosci.* 22, 4568–4576
47 Xie, X. *et al.* (2006) GABA(B) receptor-mediated modulation of hypocretin/orexin neurones in mouse hypothalamus. *J. Physiol.* 574, 399–414
48 Matsuki, T. *et al.* (2009) Selective loss of GABA(B) receptors in orexin-producing neurons results in disrupted sleep/wakefulness architecture. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4459–4464
49 Muraki, Y. *et al.* (2004) Serotonergic regulation of the orexin/hypocretin neurons through the 5-HT1A receptor. *J. Neurosci.* 24, 7159–7166
50 Sakurai, T. (2005) Reverse pharmacology of orexin: from an orphan GPCR to integrative physiology. *Regul. Pept.* 126, 3–10
51 Yamanaka, A. *et al.* (2006) Orexin neurons are directly and indirectly regulated by catecholamines in a complex manner. *J. Neurophysiol.* 96, 284–298
52 Li, Y. *et al.* (2002) Hypocretin/Orexin excites hypocretin neurons via a local glutamate neuron-A potential mechanism for orchestrating the hypothalamic arousal system. *Neuron* 36, 1169–1181
53 Yamanaka, A. *et al.* (2003) Regulation of orexin neurons by the monoaminergic and cholinergic systems. *Biochem. Biophys. Res. Commun.* 303, 120–129
54 Tsujino, N. *et al.* (2005) Cholecystokinin activates orexin/hypocretin neurons through the cholecystokinin A receptor. *J. Neurosci.* 25, 7459–7469
55 Furutani, N. *et al.* (2010) Activation of bombesin receptor subtype-3 influences activity of orexin neurons by both direct and indirect pathways. *J. Mol. Neurosci.* 42, 106–111
56 Yamanaka, A. *et al.* (2010) Orexin directly excites orexin neurons through orexin 2 receptor. *J. Neurosci.* 30, 12642–12652
57 Burdakov, D. *et al.* (2005) Physiological changes in glucose differentially modulate the excitability of hypothalamic melanin-concentrating hormone and orexin neurons in situ. *J. Neurosci.* 25, 2429–2433
58 Burdakov, D. *et al.* (2006) Tandem-pore K+ channels mediate inhibition of orexin neurons by glucose. *Neuron* 50, 711–722
59 Cai, X.J. *et al.* (2001) Hypoglycemia activates orexin neurons and selectively increases hypothalamic orexin-B levels: responses inhibited by feeding and possibly mediated by the nucleus of the solitary tract. *Diabetes* 50, 105–112
60 Williams, G. *et al.* (2001) The hypothalamus and the control of energy homeostasis: different circuits, different purposes. *Physiol. Behav.* 74, 683–701
61 Haynes, A.C. *et al.* (2000) A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. *Regul. Pept.* 96, 45–51
62 Hagan, J.J. *et al.* (1999) Orexin A activates locus coeruleus cell firing and increases arousal in the rat. *Proc. Natl. Acad. Sci. U.S.A.* 96, 10911–10916
63 Carter, M.E. *et al.* (2009) Sleep homeostasis modulates hypocretin-mediated sleep-to-wake transitions. *J Neurosci* 29, 10939–10949
64 Adamantidis, A.R. *et al.* (2007) Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature* 450, 420–424
65 Horvath, T.L. *et al.* (1999) Hypocretin (orexin) activation and synaptic innervation of the locus coeruleus noradrenergic system. *J. Comp. Neurol.* 415, 145–159
66 Nakamura, T. *et al.* (2000) Orexin-induced hyperlocomotion and stereotypy are mediated by the dopaminergic system. *Brain Res.* 873, 181–187

67 Liu, R.J. *et al.* (2002) Hypocretins (orexins) regulate serotonin neurons in the dorsal raphe nucleus by excitatory direct and inhibitory indirect actions. *J. Neurosci.* 22, 9453–9464
68 Brown, R.E. *et al.* (2002) Convergent excitation of dorsal raphe serotonin neurons by multiple arousal systems (orexin/hypocretin, histamine and noradrenaline). *J. Neurosci.* 22, 8850–8859
69 Yamanaka, A. *et al.* (2002) Orexins activate histaminergic neurons via the orexin 2 receptor. *Biochem. Biophys. Res. Commun.* 290, 1237–1245
70 Vanni-Mercier, G. *et al.* (1984) Neurons specifiques de l'eveil dans l'hypothalamus posterieur du chat. *C.R. Acad. Sci. III* 298, 195–200
71 Eggermann, E. *et al.* (2001) Orexins/hypocretins excite basal forebrain cholinergic neurones. *Neuroscience* 108, 177–181
72 Alam, M.N. *et al.* (1999) Adenosinergic modulation of rat basal forebrain neurons during sleep and waking: neuronal recording with microdialysis. *J Physiol.* 521, 679–690
73 Shouse, M.N. and Siegel, J.M. (1992) Pontine regulation of REM sleep components in cats: integrity of the pedunculopontine tegmentum (PPT) is important for phasic events but unnecessary for atonia during REM sleep. *Brain Res.* 571, 50–63
74 Xi, M. *et al.* (2001) Effects on sleep and wakefulness of the injection of hypocretin-1 (orexin-A) into the laterodorsal tegmental nucleus of the cat. *Brain Res.* 901, 259–264
75 Takahashi, K. *et al.* (2002) Effects of orexin on the laterodorsal tegmental neurones. *Psychiatry Clin. Neurosci.* 56, 335–336
76 Burlet, S. *et al.* (2002) Direct and indirect excitation of laterodorsal tegmental neurons by hypocretin/orexin peptides: implication for wakefulness and narcolepsy. *J. Neurosci.* 22, 2862–2872
77 Pace-Schott, E.F. and Hobson, J.A. (2002) The neurobiology of sleep: genetics, cellular physiology and subcortical networks. *Nat. Rev. Neurosci.* 3, 591–605
78 Huang, Z.L. *et al.* (2001) Arousal effect of orexin A depends on activation of the histaminergic system. *Proc. Natl. Acad. Sci. U.S.A.* 98, 9965–9970
79 Hondo, M. *et al.* (2010) Histamine-1 receptor is not required as a downstream effector of orexin-2 receptor in maintenance of basal sleep/wake states. *Acta Physiol. (Oxf.)* 198, 287–294
80 Willie, J.T. *et al.* (2011) Ectopic overexpression of orexin alters sleep/wakefulness states and muscle tone regulation during REM sleep in mice. *J. Mol. Neurosci.* 43, 155–161
81 Estabrooke, I.V. *et al.* (2001) Fos expression in orexin neurons varies with behavioral state. *J. Neurosci.* 21, 1656–1662
82 Yoshida, Y. *et al.* (2001) Fluctuation of extracellular hypocretin-1 (orexin A) levels in the rat in relation to the light-dark cycle and sleep-wake activities. *Eur. J. Neurosci.* 14, 1075–1081
83 Mileykovskiy, B.Y. *et al.* (2005) Behavioral correlates of activity in identified hypocretin/orexin neurons. *Neuron* 46, 787–798
84 Lee, M.G. *et al.* (2005) Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. *J. Neurosci.* 25, 6716–6720
85 Takahashi, K. *et al.* (2008) Neuronal activity of orexin and non-orexin waking-active neurons during wake-sleep states in the mouse. *Neuroscience* 153, 860–870
86 Morairty, S. *et al.* (2004) Disinhibition of ventrolateral preoptic area sleep-active neurons by adenosine: a new mechanism for sleep promotion. *Neuroscience* 123, 451–457
87 Arrigoni, E. *et al.* (2006) Adenosine inhibits basal forebrain cholinergic and noncholinergic neurons in vitro. *Neuroscience* 140, 403–413
88 Huang, Z.L. *et al.* (2005) Adenosine A2A, but not A1, receptors mediate the arousal effect of caffeine. *Nat. Neurosci.* 8, 858–859
89 Sakurai, T. (2006) Roles of orexins and orexin receptors in central regulation of feeding behavior and energy homeostasis. *CNS Neurol. Disord. Drug Targets* 5, 313–325
90 Haynes, A.C. *et al.* (2002) Anorectic, thermogenic and anti-obesity activity of a selective orexin-1 receptor antagonist in ob/ob mice. *Regul. Pept.* 104, 153–159
91 Edwards, C.M. *et al.* (1999) The effect of the orexins on food intake: comparison with neuropeptide Y, melanin-concentrating hormone and galanin. *J. Endocrinol.* 160, R7–R12
92 Silva, J.P. *et al.* (2009) Regulation of adaptive behaviour during fasting by hypothalamic Foxa2. *Nature* 462, 646–650
93 Hara, J. *et al.* (2005) Difference in obesity phenotype between orexin-knockout mice and orexin neuron-deficient mice with same genetic background and environmental conditions. *Neurosci. Lett.* 380, 239–242
94 Schuld, A. *et al.* (2000) Increased body-mass index in patients with narcolepsy. *Lancet* 355, 1274–1275
95 Lammers, G.J. *et al.* (1996) Spontaneous food choice in narcolepsy. *Sleep* 19, 75–76
96 Yamada, H. *et al.* (2000) Inhibition of food intake by central injection of anti-orexin antibody in fasted rats. *Biochem. Biophys. Res. Commun.* 267, 527–531
97 Funato, H. *et al.* (2009) Enhanced orexin receptor-2 signaling prevents diet-induced obesity and improves leptin sensitivity. *Cell Metab.* 9, 64–76
98 Yamanaka, A. *et al.* (2000) Orexin-induced food intake involves neuropeptide Y pathway. *Brain Res.* 24, 404–409
99 Muroya, S. *et al.* (2004) Orexins (hypocretins) directly interact with neuropeptide Y, POMC and glucose-responsive neurons to regulate Ca^{2+} signaling in a reciprocal manner to leptin: orexigenic neuronal pathways in the mediobasal hypothalamus. *Eur. J. Neurosci.* 19, 1524–1534
100 Thorpe, A.J. and Kotz, C.M. (2005) Orexin A in the nucleus accumbens stimulates feeding and locomotor activity. *Brain Res.* 1050, 156–162
101 Baldo, B.A. *et al.* (2004) Activation of a subpopulation of orexin/hypocretin-containing hypothalamic neurons by $GABA_A$ receptor-mediated inhibition of the nucleus accumbens shell, but not by exposure to a novel environment. *Eur. J. Neurosci.* 19, 376–386
102 Shiuchi, T. *et al.* (2009) Hypothalamic orexin stimulates feeding-associated glucose utilization in skeletal muscle via sympathetic nervous system. *Cell Metab.* 10, 466–480
103 Shirasaka, T. *et al.* (1999) Sympathetic and cardiovascular actions of orexins in conscious rats. *Am. J. Physiol.* 277, R1780–R1785
104 Borgland, S.L. *et al.* (2006) Orexin A in the VTA is critical for the induction of synaptic plasticity and behavioral sensitization to cocaine. *Neuron* 49, 589–601
105 Guilleminault, C. *et al.* (1974) On the treatment of rapid eye movement narcolepsy. *Arch. Neurol.* 30, 90–93
106 Brisbare-Roch, C. *et al.* (2007) Promotion of sleep by targeting the orexin system in rats, dogs and humans. *Nat. Med.* 13, 150–155
107 Hoever, P. *et al.* (2010) Orexin receptor antagonism, a new sleep-promoting paradigm: an ascending single-dose study with almorexant. *Clin. Pharmacol. Ther.* 87, 593–600
108 Dietrich, H. and Jenck, F. (2010) Intact learning and memory in rats following treatment with the dual orexin receptor antagonist almorexant. *Psychopharmacology (Berl.)* 212, 145–154
109 Neubauer, D.N. (2010) Almorexant, a dual orexin receptor antagonist for the treatment of insomnia. *Curr. Opin. Investig. Drugs* 11, 101–110
110 Dugovic, C. *et al.* (2009) Blockade of orexin-1 receptors attenuates orexin-2 receptor antagonism-induced sleep promotion in the rat. *J. Pharmacol. Exp. Ther.* 330, 142–151
111 Harris, G.C. *et al.* (2007) Lateral hypothalamic orexin neurons are critically involved in learning to associate an environment with morphine reward. *Behav. Brain Res.* 183, 43–51
112 Hutcheson, D.M. *et al.* (2011) Orexin-1 receptor antagonist SB-334867 reduces the acquisition and expression of cocaine-conditioned reinforcement and the expression of amphetamine-conditioned reward. *Behav. Pharmacol.* 22, 173–181
113 Johnson, P.L. *et al.* (2010) A key role for orexin in panic anxiety. *Nat. Med.* 16, 111–115
114 Mieda, M. *et al.* (2004) Orexin peptides prevent cataplexy and improve wakefulness in an orexin neuron-ablated model of narcolepsy in mice. *Proc. Natl. Acad. Sci. U.S.A.* 101, 4649–4654
115 Cox, C.D. *et al.* (2010) Discovery of the dual orexin receptor antagonist [(7R)-4-(5-chloro-1,3-benzoxazol-2-yl)-7-methyl-1,4-diazepan-1-yl][5-methy l-2-(2H-1,2,3-triazol-2-yl)phenyl]methanone (MK-4305) for the treatment of insomnia. *J. Med. Chem.* 53, 5320–5332
116 Langmead, C.J. *et al.* (2004) Characterisation of the binding of [3H]-SB-674042, a novel nonpeptide antagonist, to the human orexin-1 receptor. *Br. J. Pharmacol.* 141, 340–346
117 Porter, R.A. *et al.* (2001) 1,3-Biarylureas as selective non-peptide antagonists of the orexin-1 receptor. *Bioorg. Med. Chem. Lett.* 11, 1907–1910
118 McAtee, L.C. *et al.* (2004) Novel substituted 4-phenyl-[1,3]dioxanes: potent and selective orexin receptor 2 (OX(2)R) antagonists. *Bioorg. Med. Chem. Lett.* 14, 4225–4229