0031-6997/09/6102-162–176$20.00
PHARMACOLOGICAL REVIEWS
Vol. 61, No. 2
1321/3499956
Pharmacol Rev 61:162–176, 2009
Printed in U.S.A.

Orexin/Hypocretin: A Neuropeptide at the Interface of Sleep, Energy Homeostasis, and Reward System

NATSUKO TSUJINO AND TAKESHI SAKURAI

Department of Molecular Neuroscience and Integrative Physiology, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

***Abstract*——Recent studies have implicated the orexin system as a critical regulator of sleep/wake states as well as feeding behavior and reward processes. Orexin deficiency results in narcolepsy in humans, dogs, and rodents, suggesting that the orexin system is particularly important for maintenance of wakefulness. In addition, orexin deficiency also cause abnormalities in energy homeostasis and reward systems. Orexin activates waking active monoaminergic and cholinergic neurons in the hypothalamus and brainstem regions to maintain a long, consolidated waking period. Orexin neurons receive abundant input from the limbic system. Orexin neurons also have reciprocal links with the hypothalamic arcuate nucleus, which regulates feeding. Moreover, the responsiveness of orexin neurons to peripheral metabolic cues, such as leptin and glucose, suggest that these neurons have important role as a link between the energy homeostasis and vigilance states. Orexin neurons also have a link with the dopaminergic reward system in the ventral tegmental nucleus. These findings suggest that the orexin system interacts with systems that regulate emotion, reward, and energy homeostasis to maintain proper vigilance states. Therefore, this system may be a potentially important therapeutic target for treatment of sleep disorder, obesity, emotional stress, and addiction.**

Address correspondence to: Dr. Takeshi Sakurai, Department of Molecular Neuroscience and Integrative Physiology, Graduate School of Medical Science, Kanazawa University, 13-1 Takaramachi, Kanazawa-shi, Ishikawa 920-8640, Japan. E-mail: tsakurai@med.kanazawa-u.ac.jp.
This article is available online at http://pharmrev.aspetjournals.org.
doi:10.1124/pr.109.001321.

I. Introduction

The neuropeptides orexin A and orexin B (also known as hypocretin 1 and hypocretin 2, respectively), produced in hypothalamic neurons, were initially identified as endogenous ligands for two orphan G-protein-coupled receptors (Sakurai et al., 1998). They were recognized as regulators of feeding behavior because of 1) their exclusive production in the lateral hypothalamic area (LHA[1]), a region known as the feeding center, and 2) their pharmacological activity (Sakurai et al., 1998; Edwards et al., 1999; Haynes et al., 2000, 2002).

Thereafter, the importance of orexins in the maintenance of consolidated sleep/wake states has been demonstrated by the fact that the sleep disorder narcolepsy is caused by orexin deficiency in human and animals (Chemelli et al., 1999; Lin et al., 1999; Peyron et al., 2000; Thannickal et al., 2000; Hara et al., 2001). Recent findings on input and output systems of orexin neurons, as well as phenotypic characterizations of mice with genetic alterations in the orexin system, indicate that these neurons are involved in sensing the body's external and internal environments as well as regulating the states of sleep and wakefulness, which are beneficial for survival. In particular, these studies have suggested further roles for orexin in the coordination of emotion, energy homeostasis, reward, drug addiction, and arousal (Yamanaka et al., 2003a; Akiyama et al., 2004; Mieda et al., 2004a; Boutrel et al., 2005; Harris et al., 2005; Sakurai et al., 2005; Narita et al., 2006; Yoshida et al., 2006). A new compound has been investigated preclinically in an attempt to control orexin system to improve sleep quality. Several studies suggest that this orexin system modulator might be beneficial for obesity, addiction, and pain as well as sleep disorder.

This review will discuss the mechanisms by which the orexin system maintains sleep and wakefulness, and how this mechanism relates to other systems that regulate emotion, reward, and energy homeostasis.

[1] Abbreviations: 5HT, serotonin; ACT-078573, (2*R*)-2-((1*S*)-6,7-dimethoxy-1-(2-(4-trifluoromethylphenyl)-ethyl)-3,4-dihydro-1*H*-isoquinolin-2-yl)-*N*-methyl-2-phenylacetamide; AgRP, agouti-related peptide; Arc, arcuate nucleus; BF, basal forebrain; BIBO3304, *N*-[(1*R*)-1-[[[[4-[[(aminocarbonyl)amino]methyl]phenyl]methyl]amino]-carbonyl]-4-[(aminoiminomethyl)amino]butyl]-*a*-phenyl-benzeneacetamide; BST, bed nucleus of the stria terminalis; CRF, corticotropin-releasing factor; CSF, cerebrospinal fluid; DMH, dorsomedial hypothalamic nucleus; DR, dorsal raphe; FEO, food entrainable oscillator; GPCR, G-protein-coupled receptor; LC, locus ceruleus; LDT, laterodorsal tegmental nucleus; LHA, lateral hypothalamic area; N/OFQ, nociceptin/orphanin FQ; NAc, nucleus accumbens; NMDA, *N*-methyl-D-aspartate; NMDAR, *N*-methyl-D-aspartate receptor; NPY, neuropeptide; NREM, non–rapid eye movement; OX_1R, orexin receptor-1; OX_2R, orexin receptor-2; PFA, perifornical area; POMC, proopiomelanocortin; PPT, pedunculopontine nucleus; REM, rapid eye movement; SB334867A, 1-(2-methylbenzoxazol-6-yl)-3-(1,5)naphthyridin-4-yl urea; SIA, stress-induced analgesia; TMN, tuberomammillary nucleus; VLPO, ventrolateral preoptic area; VTA, ventral tegmental area.

II. Orexin and Orexin Receptors

A. Identification of Orexin (Hypocretin)

There are more than 600 G-protein-coupled receptor (GPCR) genes in the human genome. Half of these are sensory receptors, most of which are olfactory. The other half are receptors for regulating cell functions, and many small regulatory peptides exert their biological actions by acting on GPCRs. There are almost 100 GPCRs the ligands of which are still unknown and that 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.

Orexin A and B were both identified from rat brain extracts as ligands of an orphan GPCR HFGAN72 (orexin receptor-1; *OX_1R*) (Sakurai et al., 1998). Orexins constitute a novel peptide family with no significant structural similarities to known families of regulatory peptides. Orexin A is a 33-amino acid peptide of 3562 Da with two sets of intrachain disulfide bonds. It has an N-terminal pyroglutamyl residue and C-terminal amidation (Sakurai et al., 1998). 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 that 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), whereas the N-terminal half is variable. Orexin B also has a high degree of sequence similarity among species. Several studies revealed that the structures of fish, *Xenopus laevis,* and chicken orexin A and B have also conserved structures compared with mammalian sequences. (Shibahara et al., 1999; Alvarez and Sutcliffe, 2002; Sakurai, 2005) (Fig. 1).

Orexin A and B are produced from a common precursor polypeptide, prepro-orexin, with usual proteolytic processing presumably by prohormone convertases (Fig. 1). De Lecea et al. (1998) independently isolated an mRNA encoding the same precursor peptide as a hypothalamus-specific transcript. They predicted that this transcript potentially encodes two neuropeptides, hypocretin-1 and -2. The names "hypocretin" and "orexin" are used synonymously in many articles.

B. Orexin Receptors

HFGAN72, now called *orexin receptor-1* (*OX_1R*) was initially identified as an expressed sequence tag from human brain (Soppet et al., 1996; Sakurai et al., 1998). Subsequently, *orexin receptor-2* (*OX_2R*) was identified by searching an expressed sequence tag database by tBLASTn with the *OX_1R* sequence as a query (Sakurai et al., 1998). The amino acid identity between the full-length human OX_1R and OX_2R sequences is 64%. Amino acid identities between the human and rat counterparts

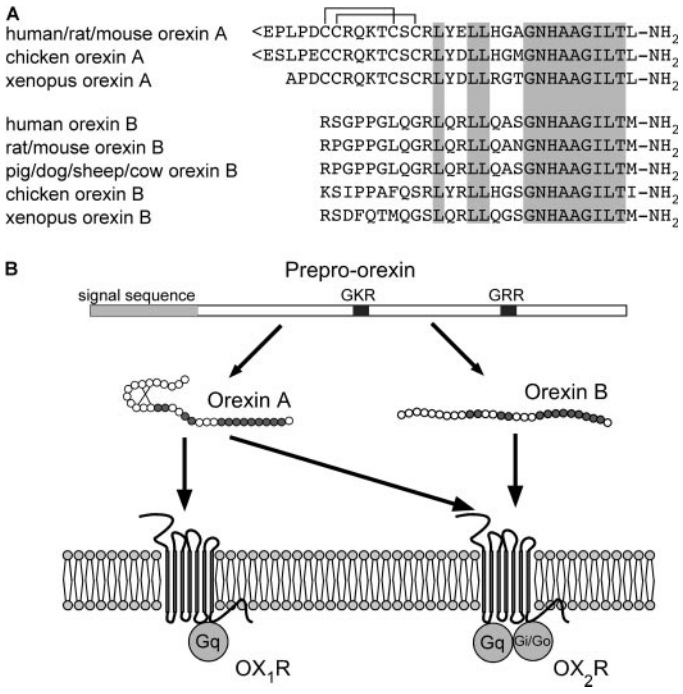

FIG. 1. Overview of orexin system. A, structure of mature orexin A and B peptides. The topology of the two intrachain bonds in orexin A is indicated above the sequence. Shadows indicate amino acid identity. Mammalian orexin A sequences thus far identified (human, rat, mouse, pig, dog, sheep, and cow) are all identical, whereas the sequences of orexin B show some differences among species. B, schematic representation of orexin system. Orexin A and B are derived from a common precursor peptide, prepro-orexin. The actions of orexins are mediated via two G protein-coupled receptors (named OX_1R and OX_2R). OX_1R is selective for orexin A, whereas OX_2R is a nonselective receptor for orexin A and B. OX_1R is coupled exclusively to the G_q subclass of heterotrimeric G proteins, whereas OX_2R couples to $G_{i/o}$ and/or G_q.

of each of these receptors are 94% for OX_1R and 95% for OX_2R, indicating that both receptor genes are highly conserved between the species (Sakurai et al., 1998). OX_1R has greater affinity for orexin A than orexin B by 1 order of magnitude. In contrast, OX_2R has similar affinity for both orexin A and orexin B (Sakurai et al., 1998) (Fig. 1). Furthermore, OX_1R is coupled to the $G_{q/11}$ class of G proteins, which results in activation of phospholipase C with subsequent triggering of the phosphatidylinositol cascade. OX_2R is shown to be coupled to both $G_{q/11}$ and inhibitory G_i proteins when expressed in cell lines (Zhu et al., 2003) (Fig. 1). Studies using non-neuronal cells suggested that G_q, G_s, and G_i proteins are involved in OX_2R-mediated extracellular signal-regulated kinase activation in human embryonic kidney 293 cells (Tang et al., 2008). Moreover, food deprivation exerted a differential effect on coupling between orexin receptors and G proteins (Karteris et al., 2005).

C. Orexin-Producing Neurons

Orexin-producing neurons (orexin neurons) are exclusively localized to the perifornical area and the lateral and posterior hypothalamic area in the rat brain (Peyron et al., 1998; Date et al., 1999; Nambu et al., 1999) (Fig. 2), and this distribution has been confirmed in human tissue (Elias et al., 1998). These cells diffusely project to the entire neuroaxis, excluding the cerebellum (Peyron et al., 1998; Date et al., 1999; Nambu et al., 1999) (Fig. 2). This anatomical structure suggests that the activity of orexin neurons influences multiple brain areas. The heaviest staining of orexin-immunoreactive nerve endings in the brain was found in the paraventricular thalamic nucleus, arcuate nucleus of the hypothalamus, raphe nuclei, tuberomammillary nucleus (TMN), and locus ceruleus (LC). Orexin colocalizes with dynorphin (Chou et al., 2001), galanin (Håkansson et al., 1999), prolactin (Risold et al., 1999), neuronal activity-regulated pentraxin (Reti et al., 2002), and glutamate (Abrahamson et al., 2001). Many orexin neurons express vesicular glutamate transporters, suggesting that many if not all orexin neurons are also glutamatergic (Rosin et al., 2003; Torrealba et al., 2003). In contrast, orexin neurons do not express GAD-67 mRNA, suggesting that orexin neurons are not GABAergic (Rosin et al., 2003).

D. Distribution of Orexin Receptors

In the central nervous system, in situ hybridization studies have demonstrated that both orexin receptor mRNAs are expressed in regions in which contain dense orexin innervations as described above. OX_1R and OX_2R mRNAs show partially overlapping but largely distinct and complementary distribution patterns, suggesting that they play different physiological roles for each receptor subtype. *OX_1R* is expressed in many brain regions, such as the prefrontal and infralimbic cortex, hippocampus (cornu ammonis 2), amygdala, and bed nucleus of the stria terminalis (BST), paraventricular thalamic nucleus, anterior hypothalamus, dorsal raphe (DR), ventral tegmental area (VTA), LC, and laterodorsal tegmental nucleus (LDT)/pedunculopontine nucleus (PPT) (Trivedi et al., 1998; Lu et al., 2000; Marcus et al., 2001). *OX_2R* is expressed in the amygdala and BST, paraventricular thalamic nucleus, DR, VTA, and LDT/PPT (Lu et al., 2000; Marcus et al., 2001). On the other hand, *OX_2R* is abundantly expressed in the arcuate nucleus (Arc), TMN, dorsomedial hypothalamic nucleus (DMH), paraventricular nucleus, LHA in the hypothalamus, cornu ammonis 3 in the hippocampus, and medial septal nucleus (Lu et al., 2000; Marcus et al., 2001). These histological findings suggest that orexins and their receptors are likely to play a broad regulatory role in the central nervous system and could regulate feeding, autonomic control, sleep, memory, and the reward system. OX_1R is also distributed in the peripheral tissues, such as kidney, adrenal, thyroid, testis, ovaries, and jejunum. OX_2R is found in adrenal glands, lung, and pituitary (Jöhren et al., 2001).

III. Input to Orexin Neurons

A. Neuronal Afferents

In mice with a genetically encoded retrograde tracer and in rats with combining antero-and retrograde tracers, upstream neuronal populations that make innervations to orexin neurons were revealed (Sakurai et al., 2005; Yoshida et al., 2006). These studies showed that orexin neurons are innervated by the lateral parabrachial nucleus, ventrolateral preoptic nucleus (VLPO), medial and lateral preoptic areas, basal forebrain (BF), posterior/dorsomedial hypothalamus, VTA, and median raphe nuclei. Many upstream neurons were identified in regions associated with emotion, including the infralimbic cortex, amygdala, shell region of the nucleus accumbens, lateral septum, and BST.

Orexin neurons were also showed to receive innervations from regions associated with energy homeostasis including NPY-, agouti-related peptide, and α-melanin-stimulating hormone-immunoreactive fibers, which presumably come from the Arc (Broberger et al., 1998; Elias et al., 1998).

Hypothalamic regions preferentially innervate orexin neurons in the medial and perifornical parts of the field, but most projections from the brainstem target the lateral part of the field (Yoshida et al., 2006), suggesting functional dichotomy of orexin neurons.

B. Factors that Influence Activity of Orexin Neurons

From the above-mentioned regions, neurons send input to orexin neurons and regulate orexin neuronal activity by secretion of particular neuromodulators. To identify orexin-containing neurons for direct electrophysiological recording, we generated transgenic mice expressing green fluorescent protein exclusively in orexin neurons. Several neurotransmitters and neuromodulators that activate or inhibit the activity of orexin neurons have identified by electrophysiological studies using the transgenic mice (Table 1).

It is noteworthy that both noradrenaline and serotonin (5HT) hyperpolarize and inhibit activity of orexin neurons through activation of G protein-regulated inwardly rectifying K^+ (GIRK or Kir3) channels via α_2-adrenoceptors and $5HT_{1A}$-receptors, respectively (Yamanaka et al., 2003b, 2006; Muraki et al., 2004). In addition, the cholinergic agonist carbachol activates 27% and inhibits 6% of orexin neurons through M3 muscarinic receptors (Yamanaka et al., 2003; Sakurai et al., 2005). However, histamine seems to have no effect on orexin neurons. These observations suggest that serotonin and noradrenaline neurons send inhibitory feedback projections to orexin neurons. These feedback mechanisms might stabilize the activity of both orexin neurons and monoaminergic neurons. Furthermore, although orexin neurons do not express functional dopamine receptors, dopamine can inhibit orexin neurons by acting on α_2-adrenoceptors (Yamanaka et al., 2003b, 2006).

It was also suggested that sleepiness associated with sleep deprivation might be related with modulation of activities of orexin neurons by noradrenaline. A short (2 h) period of total sleep deprivation changed the action of noradrenaline on orexin neurons from excitation to in-

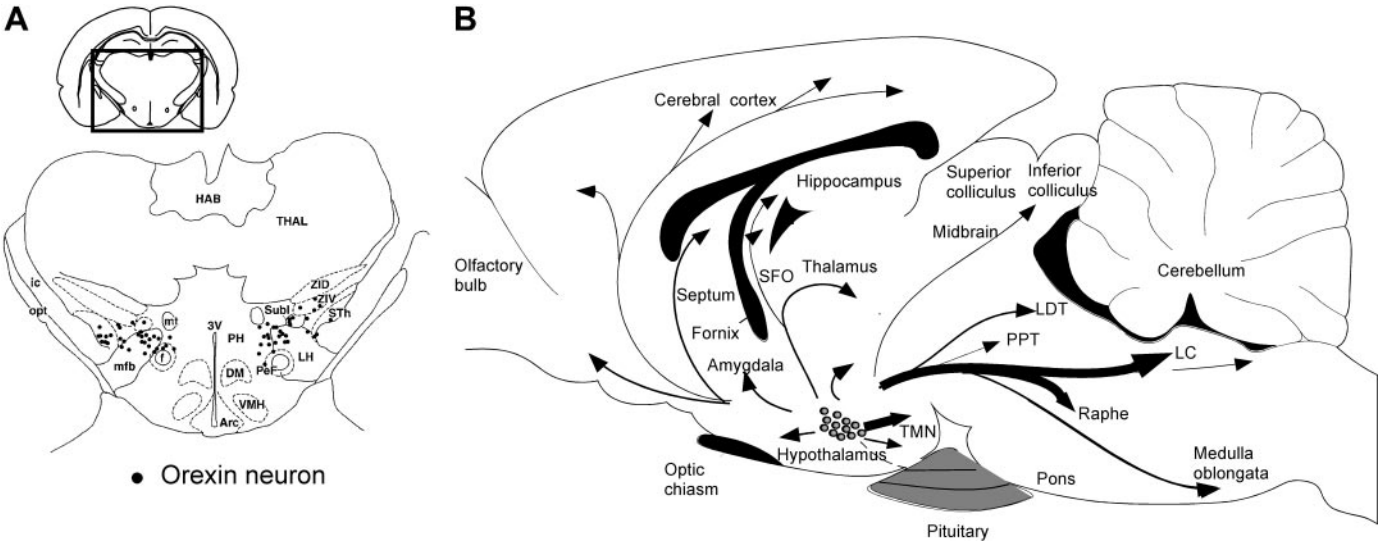

FIG. 2. Schematic drawing of coronal section and sagittal section through the rat brain, summarizing of the orexin neuronal system. A, prepro-orexin mRNA–containing neurons are shown in black superimposed upon anatomical structures of the hypo- and subthalamic areas. The rectangle designates the area schematized in the figure. Abbreviations: LH, lateral hypothalamic area; PeF, perifornical nucleus; PH, posterior hypothalamic area; Sth, subthalamic nucleus; SubI, subincertal nucleus; and ZIV, ventral zona incerta. Additional landmarks include: THAL, thalamus; HAB, habenular complex; ic, internal capsule; opt, optic tract; mt, mammillothalamic tract; f, fornix; mfb, medial forebrain bundle; 3V, third ventricle; DM, dorsomedial hypothalamic nucleus; and VMH, ventromedial hypothalamic nucleus. B, orexin neurons are found only in the lateral hypothalamic area and project to the entire central nerves system. The thickness of arrows represents relative abundance of projection. Abbreviations: 3V, third ventricle; 4V, fourth ventricle.

TABLE 1
Factors that influence the activity of orexin neurons

	Receptor Involved
Excitation	
Glutamate	AMPAR, NMDAR mGluRs
Acetylcholine (muscarinic) (27%)	M3
Ghrelin	GHSR
Cholecystokinin	CCKA
Neurotensin	NTSR2[a]
Vasopressin	V1a
Oxytocin	V1a
Glucagon-like peptide 1	N.D.
CRF	CRFR1
Thyrotropin-releasing hormone	TRH1
ATP	P2X
H^+, CO_2	N.D.
Inhibition	
Glucose	Unknown
GABA	$GABA_A$, $GABA_B$
Serotonin	$5HT_{1A}$
Noradrenaline	α_2
Dopamine	α_2
Acetylcholine (muscarinic) (6%)	N.D.
NPY	Y_1
Enkephalin	μ Opioid receptor
Nociceptin	NOPR
Leptin	OBR
Adenosine	A_1

AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; mGluR, metabotropic glutamate receptor; M3, muscarinic acetylcholine receptor M3; GHSR, ghrelin secretagogue receptor; CCKA, type A cholecystokinin receptor; NTSR2, neurotensin receptor 2; N.D., not determined; CRFR1, CRF-releasing factor 1; TRH1, thyrotropin-releasing hormone receptor 1; NOPR, nociceptin/orphanin FQ receptor; OBR, astrocyte leptin receptor

[a] N. Furutani, T. Abe, M. Hondo, T. Matsuki, N. Tsujino, K. Ichiki, M. Mieda, I. Matsuzaki, H. Takahishi, A. Yamanaka, and T. Sakurai, unpublished observations.

hibition in rats (Grivel et al., 2005), although this phenomenon was not observed in mice (Yamanaka et al., 2006).

It was also shown that agonists of ionotropic glutamate receptors [α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and *N*-methyl-D-aspartate (NMDA)] excite orexin neurons, whereas glutamate antagonists [2-amino-5-phosphonopentanoic acid, 6-cyano-2,3-dihydroxy-7-nitroquinoxaline, or 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(*f*)quinoxaline] reduce their activity (Li et al., 2002; Yamanaka et al., 2003b). These results indicate that orexin neurons are tonically activated by glutamatergic neurons. At the same time, GABAergic input to orexin neurons strongly inhibits activity of orexin neurons (Xie et al., 2006; Matsuki et al., 2009).

Imaging study was also helpful to screen for factors that affect the activity of orexin neurons. Using transgenic mice in which orexin neurons specifically express a genetically encoded intracellular calcium indicator (yellow cameleon, Yc2.1), we identified several factors that influence the activity of orexin neurons: a sulfated octapeptide form of cholecystokinin (CCK-8S) as well as neurotensin, oxytocin, and vasopressin activate orexin neurons (Tsujino et al., 2005; Tsunematsu et al., 2008), whereas GABA, glucose, 5-HT, noradrenaline, and leptin inhibit them (Table 1). Adenosine has been shown to inhibit orexin neurons via the A_1 receptor (Liu and Gao, 2007). This mechanism might relate to the sleep-promoting effect of adenosine (Liu and Gao, 2007). In addition, recent reports suggested that orexin neurons are profoundly affected by physiological fluctuations in acid and CO_2 levels. Acidification increased neural excitability, whereas alkalinization depressed it (Williams et al., 2007). Because orexin affects respiratory function, this mechanism might play an important role on the regulation of respiration (Nakamura et al., 2007).

IV. Orexin Deficiency Causes Narcolepsy

Narcolepsy is a sleep disorder characterized by a primary disorganization of behavioral states. This disorder affects approximately 1 in 2000 persons in the United States (Mignot, 1998). Most cases of human narcolepsy start during adolescence and persist throughout life. Many experiments revealed that human narcolepsy is caused by an orexin deficiency (Chemelli et al., 1999; Lin et al., 1999; Peyron et al., 2000; Thannickal et al., 2000). The symptoms and pathophysiology of the narcolepsy provide insight into the physiological roles of orexin. Narcolepsy is characterized by the inability to maintain vigilance states, pathological intrusion of non-REM and/or REM sleep into wakefulness, and frequent transitions between states of sleep and wakefulness. Human narcolepsy patients feel excessive daytime sleepiness (an insurmountable urge to sleep), manifested particularly as attacks of falling asleep at inappropriate times (sleep attack). They often suffer from an attack called "cataplexy," which is characterized by sudden weakening of postural muscle tone, which can range from jaw dropping and speech slurring to complete bilateral collapse of the postural muscles. These attacks are often triggered by emotional stimuli. These symptoms suggest that orexins have important roles in the maintenance and stabilization of sleep and wakefulness, as well as inhibition of REM sleep or REM sleep-related phenomena.

The first clues toward an involvement of the orexins in narcolepsy came from animal models. *prepro-Orexin* gene knockout mice or dogs with null mutations in the OX_2R gene show phenotypes remarkably similar to humans with narcolepsy (Chemelli et al., 1999; Lin et al., 1999). Some studies elucidated that *prepro-orexin* knockout mice, orexin neuron-ablated (*orexin/ataxin-3*-transgenic) mice, and OX_1R/OX_2R double-knockout mice showed 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 (Chemelli et al., 1999; Hara et al., 2001) (Fig. 3). All of them are important elements of narcolepsy.

A postmortem study of human narcolepsy subjects consistently showed an 80 to 100% reduction in the number of neurons containing detectable prepro-orexin mRNA or orexin-like immunoreactivity in the hypothalamus and undetectable levels of orexin peptides in the

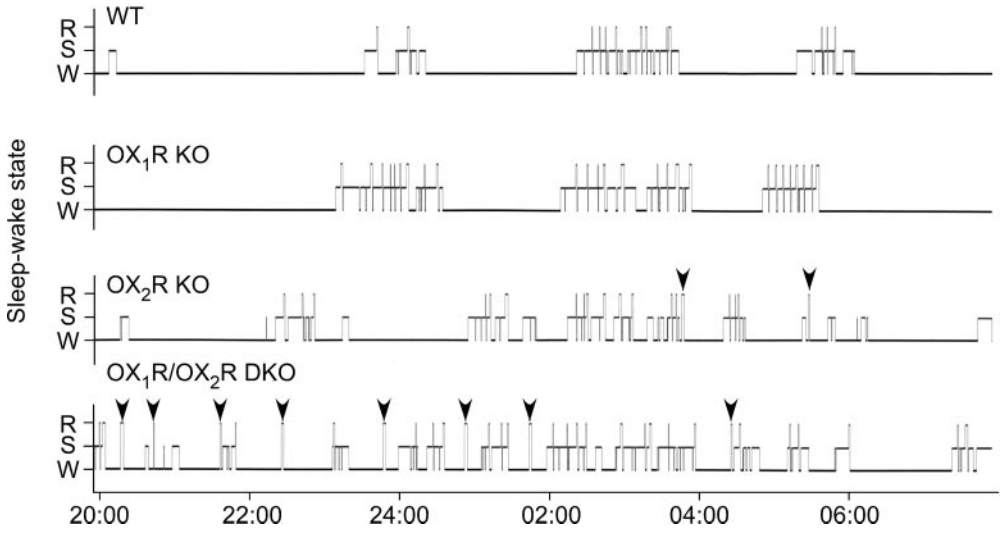

FIG. 3. Narcolepsy in orexin receptor-knockout mice. Typical representative 12-h dark period (8:00 PM–8:00 AM) hypnograms for wild-type (WT), OX_1R knockout (OX_1R KO), OX_2R knockout (OX_2R KO), and double-receptor knockout mice (OX_1R/OX_2R DKO), all on a C57BL/6J background, are shown. The different levels above the baseline indicate state of sleep and wakefulness (R, REM sleep; S, NREM sleep; W, awake) of the mouse at the time. Arrowheads show episodes of direct transition from wakefulness to REM sleep. Note the greater wake/NREM sleep episode fragmentation and reduced duration of wakefulness in the hypnogram of OX_2R-knockout and double-receptor knockout mice compared with wild-type and OX_1R knockout mice. Episodes of direct transition from wakefulness to REM sleep ware not observed in OX_1R knockout mice, and barely in OX_2R knockout mice, whereas they were frequently observed in double-receptor knockout mice. Hypnograms were obtained by simultaneous electroencephalography (EEG) and electromyography (EMG) recording for 4 weeks (n = 18–40).

cortex and pons, in which normally orexinergic projections are found (Fig. 2) (Peyron et al., 2000; Thannickal et al., 2000). In good agreement with this report, undetectable levels of orexin A in the cerebrospinal fluid (CSF) of narcolepsy patients was presented in earlier study (Nishino et al., 2000). In addition, approximately 90% of patients with narcolepsy show decreased orexin A levels in the cerebrospinal fluid (Mignot et al., 2002). In particular, narcolepsy with cataplexy (narcolepsy-cataplexy) shows higher concordance with low CSF orexin A level. A low CSF level of orexin A is now one of the diagnostic criteria for narcolepsy-cataplexy according to the 2nd edition of the *International Classification of Sleep Disorders* (American Academy of Sleep Medicine, 2005).

Furthermore, a recent finding showing concomitant loss of dynorphin, neuronal activity-regulated pentraxin, and orexin, which colocalize in orexin neurons, further suggests a loss of orexin neurons in narcolepsy-cataplexy (Crocker et al., 2005). On the contrary, the number of melanin-concentrating hormone containing neurons, which are intermixed with orexin neuron in the normal brain, was similar in control and narcoleptic brains (Peyron et al., 2000; Thannickal et al., 2000). The cause of the specific loss or degradation of orexin neurons in narcolepsy has been unknown so far, but because of its strong association with certain human leukocyte antigen alleles (Kadotani et al., 1998), it is possible that narcolepsy may result from selective immune-mediated degeneration of orexin neurons, although no specific antibody against orexin neurons has been found in serum of the patients. Although the cause of the neuron loss remains to be clarified, the orexin signaling-deficiency in narcolepsy-cataplexy shows that this orexin system plays an important role in the regulation of sleep and wakefulness, especially in the maintenance of long, consolidated awake periods. Because narcolepsy is a disorder resulting from an absence of orexin, orexin agonists will provide the fundamental cure for narcolepsy as described in detail in section VIII.

V. Roles of Orexins in Regulation of Sleep/Wake States

A. Interactions with Sleep and Waking Centers

Sleep-active neurons in the preoptic area, especially the VLPO, appears to play a critical role in initiation of non–rapid-eye-movement (NREM) sleep and maintenance of both NREM and rapid-eye-movement (REM) sleep (Sherin et al., 1998). The VLPO sends descending inhibitory projections to wake-active neurons producing wake-promoting neurotransmitters, including histamine, noradrenaline, 5-HT, and acetylcholine (Sherin et al., 1998; Lu et al., 2002) (Fig. 4). Neurons in the VLPO fire at a rapid rate during sleep, with attenuation of firing during the waking period. These sleep-promoting neurons in the VLPO mostly contain GABA and/or galanin and are inhibited by wake-active transmitters such as noradrenaline and acetylcholine (Gallopin et al., 2000). These neurons inhibit monoaminergic and cholinergic arousal system during sleep. These reciprocal interactions of inhibition constitute the flip-flop switching of the wake/sleep states. It is noteworthy that GABAergic neurons in the preoptic area also densely innervate orexin neurons (Sakurai et al., 2005; Yoshida et al., 2006). Orexin neurons are strongly inhibited by both a

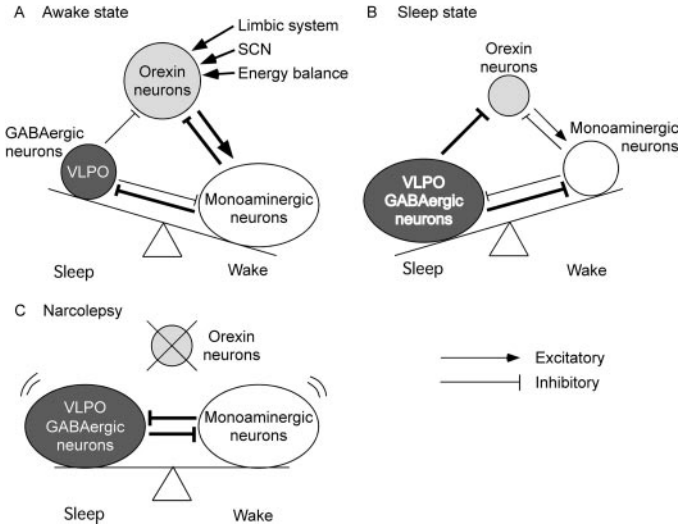

FIG. 4. Mechanisms by which the orexin system maintains consolidated sleep and wakefulness. The figures represent functional interaction between orexin neurons, monoaminergic wake-active centers and VLPO sleep-active centers during various states of sleep and wakefulness. Arrows show excitatory and lines show inhibitory input. The thickness of arrows and lines represent relative strength of input. Circle sizes represent relative activities of each group. A, awake state. Orexin neurons send excitatory input to monoaminergic neurons, which send inhibitory feedback projection to orexin neurons. This system might maintain the activity of monoaminergic neurons. A small decrease in the activity of monoaminergic neurons results in decreased inhibitory influence to orexin neurons. Orexin neurons, therefore, are disinhibited and increase excitatory influence to monoaminergic neurons to maintain their activities. These monoaminergic neurons send inhibitory projection to the VLPO sleep center and send excitatory projections to the thalamus and cerebral cortex. B, sleep state. GABAergic neurons in the VLPO sleep center are activated and send inhibitory projections to monoaminergic neurons and orexin neurons to maintain sleep state. C, model of narcolepsy. Sleep active neurons in the VLPO inhibits monoaminergic neurons and is in turn inhibited by them, thus forming a mutually inhibitory system. This system can cause unnecessary transition between the states. When either side begins to overcome the other, the switch abruptly turns into the alternative state.

$GABA_A$ receptor agonist, muscimol, and a $GABA_B$ receptor agonist, baclofen (Yamanaka et al., 2003a; Xie et al., 2006). These observations suggest that VLPO neurons send GABAergic inhibitory projections to orexin neurons. This pathway might be important to turn off orexin neurons during sleep (Fig. 4). In fact, selective deletion of $GABA_B$ receptor gene in orexin neurons result in highly instable sleep/wake architecture in mice (Matsuki et al., 2009).

As already mentioned, patients with narcolepsy and animals with a defect of orexin system cannot maintain consolidated wakefulness state. To maintain arousal, it is recognized that monoaminergic neurons in the hypothalamus and brain stem, including neurons in the TMN, LC, and DR, play crucial roles (Saper et al., 2005). These neurons are known to be synchronized and strongly associated with sleep/wake states. They fire tonically during the awake state, less during NREM sleep, and are virtually quiescent during REM sleep (Vanni-Mercier et al., 1984). It has been revealed that orexin neurons discharge during active waking and cease firing during sleep, including the NREM and REM periods, in vivo (Lee et al., 2005). Furthermore, the raphe nuclei, LC, and TMN monoaminergic neurons express orexin receptors and are heavily innervated by orexin like immunoreactivity. These results suggest that orexin neurons are activated during the wakeful period and exert an excitatory influence on these wake-active neurons to sustain their activity (Fig. 4). In fact, noradrenergic cells of the LC (Hagan et al., 1999), dopaminergic cells of the VTA (Nakamura et al., 2000), serotonergic cells of the DR (Brown et al., 2002; Liu et al., 2002), and histaminergic cells of the TMN (Yamanaka et al., 2002), are all activated by orexins in vitro. Orexins also have a strong direct excitatory effect on cholinergic neurons in the BF (Eggermann et al., 2001), which also play an important role in regulating arousal (Alam et al., 1999). Orexin neurons also project to LDT/PPT cholinergic neurons. Injection of orexin A into the LDT of cats results in an increase in waking time and a decrease in REM sleep time (Xi et al., 2001). Orexin A induces long-lasting excitation of cholinergic neurons in the LDT (Takahashi et al., 2002). Contrary to above-mentioned reports, orexin A indirectly inhibits cholinergic neurons in the PPT via activation of GABAergic local interneurons and GABAergic neurons in the substantia nigra pars reticulata (Takakusaki et al., 2005). These results suggest that hypothalamic orexin neurons affect the activity of LDT/PPT cholinergic neurons directly and/or indirectly to appropriately regulate the activity of these cells to control wakefulness and REM sleep. On the other hand, orexin neurons are innervated by BF cholinergic neurons (Sakurai et al., 2005). Carbachol, a cholinergic agonist, activates some populations of orexin neurons (Sakurai et al., 2005). This cholinergic input to orexin neurons probably plays a role in stabilization of wakefulness (Sakurai et al., 2005). Furthermore, serotonergic neurons send inhibitory projection to orexin neurons (Muraki et al., 2004; Sakurai et al., 2005). Noradrenergic neurons might also have inhibitory effects on orexin neurons (Yamanaka et al., 2006). These feedback inhibitory mechanisms might also be important for the stability of orexin neuronal activity.

B. Mechanisms that Stabilize Wakefulness

Sleep-active neurons in the VLPO inhibits wake active neurons in the arousal regions and are in turn inhibited by them, thus forming a mutually inhibitory system resembling a flip-flop switch (Saper et al., 2001) (Fig. 4). This switch may sharpen transitions between awake and sleep states, but it is not inevitably stable. This mechanism is vulnerable to sudden unnecessary transition. Orexin neurons localized in the LHA are anatomically well placed between VLPO (sleep promoting) and brain stem (wake promoting). These neurons link the VLPO and brainstem and stabilize behavioral states by activating arousal regions during wakefulness, preventing unwanted transitions between wakefulness and sleep. This neural network ensures and stabilizes

the flip-flop mechanisms. Loss of the link between monoaminergic cells and VLPO sleep-active neurons by orexin neurons might result in behavioral instability, which is a major symptom of narcolepsy (Fig. 4).

Orexins activate the arousal region to maintain wakefulness through activation of OX_1R and/or OX_2R. Which receptor is more important in regulation of wakefulness? Some reports have shown that the effect of orexins during wakefulness is largely mediated by activation of the TMN histaminergic system through OX_2R. In rats, intracerebroventricular injection of orexin during the light period potently increases the awake period, and this effect is markedly attenuated by the H_1 antagonist pyrilamine (Yamanaka et al., 2002). The pharmacological effect of orexin A on waking time in mice is almost completely absent in histamine H_1-receptor-deficient mice (Huang et al., 2001). *OX_2R* is abundantly expressed in the TMN, whereas *OX_1R* is strongly expressed in the LC. These results suggest that the TMN-histaminergic pathway might be an important effector site of orexin for sleep/wake regulation. Furthermore, *OX_2R* knockout mice exhibit characteristics of narcolepsy (Willie et al., 2003), and *OX_1R* knockout mice have no overt behavioral abnormalities and exhibit only very mild fragmentation of the sleep-wake cycle (Willie et al., 2001) (Fig. 3). It is notable, however, that the phenotype of *OX_2R* knockout mice is far less severe than that found in *prepro-orexin* knockout mice and double receptor knockout (*OX_1R*- and *OX_2R*-null) mice, which seem to have the same phenotype as *prepro-orexin* knockout mice (Fig. 3). In particular, *OX_2R* knockout mice are only mildly affected by cataplexy and direct transitions to REM sleep from an awake state, whereas *prepro-orexin* knockout mice and *OX_1R*/*OX_2R*-double knockout mice are severely affected. These observations suggest that *OX_1R* also has additional effects on sleep-wake regulation, especially inhibition and gating of REM sleep. These findings suggest that despite the lack of an overt *OX_1R* phenotype, loss of signaling through both receptor pathways is necessary for emergence of a complete narcoleptic phenotype. It is reasonable to think that the lack of obvious phenotype in *OX_1R* knockout mice might result from compensatory effects of *OX_2R*, whereas lack of *OX_2R* cannot be completely compensated by *OX_1R*. These observations suggest that the profound dysregulation of wakefulness in the narcolepsy syndrome emerges from loss of signaling through both OX_1R-and OX_2R-dependent pathways.

C. Input from the Limbic System

Emotional arousal or fear-related responses increase sympathetic outflow. Intracerebroventricular injection of orexin to rodents elevated sympathetic tone and plasma corticosterone levels (Hagan et al., 1999). This suggests that input coming from regions implicated in emotion might be important for regulation of sleep/wake system and that the orexin system might be involved in this regulation.

Orexin neurons receive input from the limbic system (Winsky-Sommerer et al., 2004; Sakurai et al., 2005; Yoshida et al., 2006), suggesting a role for this system in the regulation of orexin neuron activity. Indeed, the importance of this connection is readily apparent in the defense, or "fight or flight," response. Mice tested in a resident-intruder paradigm show cardiovascular and locomotor responses to the emotional stress evoked by this test, but these responses are diminished in *prepro-orexin* knockout mice (Kayaba et al., 2003). Likewise, air-jet stress-induced elevations of blood pressure and heart rate were attenuated in conscious orexin neurons ablated mice (Zhang et al., 2006). These results indicate that input from the limbic system to orexin neurons might be important for sympathetic responses during emotional events.

One of the limbic inputs to orexin neurons might be corticotropin-releasing factor (CRF) neurons in the amygdala (Winsky-Sommerer et al., 2004). They activate orexin neurons via the CRF-R1 receptor (Winsky-Sommerer et al., 2004). The reciprocal link between the CRF system and orexin neurons might maintain wakefulness during stressful events. Indeed, activation of orexin neurons by foot-shock stress is severely impaired in *CRF-R1 receptor*-deficient mice, suggesting that such activation is mediated by CRF (Winsky-Sommerer et al., 2004). Furthermore, administration of CRF or immobilization stress activates the orexin neurons and increases the production of prepro-orexin mRNA (Ida et al., 2000; Winsky-Sommerer et al., 2004). Excessive activation of orexin neurons during rest period by the limbic input might contribute to sleep disruption under stressful conditions. Patients with insomnia, for example, often show anxiety, stress, and elevations in pulse, blood pressure, and metabolic rate (Bonnet and Arand, 1998; Bonnet and Arand, 2003). The neural input from the limbic system to orexin neurons might also be implicated in pathophysiology of cataplexy, because strong, generally positive emotional stimuli are known to trigger cataplexy in patients with narcolepsy-cataplexy. A local injection of orexin into the PPT strongly inhibited REM-related atonia in cats (Takakusaki et al., 2005). Cholinergic neurons in the LDT/PPT are implicated in REM-related atonia (Shiromani et al., 1988) and the same pathway is implicated in cataplexy. Therefore, emotional stimuli may increase orexin release in the PPT, which indirectly inhibits cholinergic neurons, to prevent muscle atonia in wild-type animals. Projections to orexin neurons from the limbic system might also be important for maintaining orexin neuron activity during the active period by conveying various emotional stimuli to orexin neurons.

The regulation of feeding behavior might be also controlled by input to orexin neuron from the limbic system, because some of the affective content of the perception of

food is thought to be processed in the amygdala and limbic system (Berthoud, 2004), and this information may passed on to orexin neurons. In fact, it is well known that food perception often evokes cataplexy in narcoleptic dogs (Reid et al., 1998), suggesting that orexin signaling is physiologically activated upon perception of food, and that this system is necessary to evoke proper feeding behavior.

VI. Roles of Orexins in Feeding Behavior and Energy Homeostasis

A. Regulation of Orexin Neurons by Humoral Factors

It is well known that the lateral hypothalamus, where orexin neurons are localized, is involved in food intake and energy homeostasis, and intracerebroventricular injection of orexins during the light period induces feeding behavior in rats and mice (Sakurai et al., 1998; Edwards et al., 1999; Haynes et al., 2000, 2002). These observations suggest that orexin neurons might participate in regulation of feeding behavior. In fact, orexin neurons are able to monitor humoral and neural indicators of energy balance. Changes in extracellular glucose concentration produce electrophysiological changes in orexin neurons (Yamanaka et al., 2003a). Increasing extracellular glucose concentration as well as leptin induces marked hyperpolarization and cessation of action potentials in orexin neurons. Conversely, decreasing glucose concentration induces depolarization and increases the frequency of action potentials in these same neurons (Yamanaka et al., 2003a; Burdakov et al., 2005). It has been suggested that the LHA contains glucose-sensitive neurons that are activated by glucopenia and thus implicated in the positive short-term regulation of feeding and energy expenditure (Oomura et al., 1974). These studies suggested that the orexin neurons are glucose-sensitive neurons and might play an important role in feeding and energy expenditure. Orexin neuron-ablated mice cannot respond to fasting by increased locomotor activity and waking time (Yamanaka et al., 2003a). prepro-Orexin mRNA level is also increased in hypoglycemic conditions, suggesting that expression of the gene is also regulated by plasma glucose level (Griffond et al., 1999; Moriguchi et al., 1999). It is noteworthy that this mechanism is sufficiently sensitive to encode variations in glucose levels reflecting those occurring physiologically between normal meals (Burdakov et al., 2005). It is demonstrated that the mechanism for inhibition of orexin neurons by glucose is mediated by tandem-pore K^+ (K_{2P}) channels (Burdakov et al., 2006). The orexigenic peptide ghrelin activated 60% of dispersed orexin neurons when applied in superfused solution, with depolarization and an increase in action potential frequency (Yamanaka et al., 2003a). These findings are consistent with the idea that orexin neurons act as a sensor of the nutritional status of the body (Sakurai et al., 1998). *prepro-Orexin* expression of normal and *ob*/*ob* mice is consistently negatively correlated with changes in blood glucose, leptin, and food intake (Yamanaka et al., 2003a). This suggests that orexin neurons monitor indicators of energy balance and mediate adaptive augmentation of arousal in response to fasting.

B. Mechanism of Orexin-Mediated Feeding

Orexin neurons exclusively localize to the LHA, which has been recognized as a brain region of central importance to feeding. Other hypothalamic regions, including the Arc, ventromedial hypothalamic nucleus, DMH, and paraventricular nucleus, are also involved in energy homeostasis and feeding (Elmquist et al., 1999). Proopiomelanocortin (POMC) neurons and NPY neurons in the Arc have been shown to innervate to orexin neurons (Elias et al., 1998). Injection of agouti-related protein, which is an endogenous antagonist for melanocortin 3 and 4 receptors, resulted in the activation of orexin neurons but not melanin-concentrating hormone and NPY neurons (Zheng et al., 2002). These findings suggest that the orexin system is involved in the hypothalamic neuronal network that regulates feeding behavior and energy homeostasis.

The altered energy homeostasis in patients with narcolepsy also suggests roles for orexin in regulation of energy homeostasis (Honda et al., 1986; Schuld et al., 2000). The finding of decreased caloric intake (Lammers et al., 1996) combined with an increased body mass index (Schuld et al., 2000) suggests that patients with narcolepsy have a feeding abnormality with reduced energy expenditure or low metabolic rate, and orexin neurons have a role in the regulation of energy homeostasis. Orexin neuron-ablated mice consistently show hypophagia and late-onset obesity (Hara et al., 2001).

Moreover, central administration of anti-orexin antibody or an OX_1R-selective antagonist reduced food intake (Haynes et al., 2000; Yamada et al., 2000), and *prepro-orexin* knockout mice and transgenic mice lacking orexin neurons ate less than control wild-type mice (Hara et al., 2001; Willie et al., 2001). Moreover, an OX_1R-selective antagonist reduced food intake and ameliorated obesity of leptin-deficient *ob*/*ob* mice (Haynes et al., 2002), suggesting that leptin deficiency at least partly activates the orexin pathway to increase food intake. This is consistent with electrophysiological findings showing that activity of orexin neurons is inhibited by leptin. The Arc receives projections densely from orexin neurons (Peyron et al., 1998; Date et al., 1999; Yamanaka et al., 2000), and *Fos* expression was induced in NPY neurons of the arcuate nucleus by intracerebroventricular injection of orexin, suggesting that orexin-stimulated feeding may occur at least partly through NPY pathways (Yamanaka et al., 2000). Electrophysiological data showed that orexin directly and indirectly activated NPY neurons (van den Top et al., 2004; Li and

van den Pol, 2006) but inhibited POMC neurons (Muroya et al., 2004; Ma et al., 2007). Furthermore, the orexin A-induced increase in food intake was partly inhibited by prior administration of BIBO3304, an NPY-Y1 receptor antagonist, in a dose-dependent manner (Yamanaka et al., 2000). These experiments suggest that orexin-stimulated food intake is at least partially mediated by activation of NPY neurons.

Recent reports also showed that infusion of orexin A into the shell of the nucleus accumbens (NAc) increased feeding behavior (Thorpe and Kotz, 2005). In addition, infusion of the $GABA_A$ receptor agonist muscimol into the NAc shell strongly induced food intake and simultaneously increased *Fos* expression specifically in orexin neurons (Baldo et al., 2004). These findings indicate that interactions between the orexin and limbic systems have a role in the regulation of feeding.

Orexin-mediated maintenance of consolidated wakefulness states might also be important in supporting motivated behaviors in relation with food intake, such as food seeking, because proper maintenance of arousal during food searching and intake is essential for an animal's survival. For example, when faced with reduced food availability, animals adapt with a longer awake period, which disrupts the normal circadian pattern of activity (Itoh et al., 1990; Challet et al., 1997). During starvation, orexin neurons might be activated by low leptin and glucose levels, along with high ghrelin level. These mechanisms may directly modulate activity of orexin neurons according to appetite and body energy stores to maintain wakefulness. Transgenic mice in which orexin neurons are ablated consistently fail to respond to fasting with increased wakefulness and activity (Yamanaka et al., 2003a). Furthermore, it was reported that after 48-h fast, prepro-orexin mRNA was up-regulated compared with the fed control in rats (Sakurai et al., 1998). These suggest that orexin neurons have a critical role in maintenance of arousal during the period in which the energy balance is negative. These properties might allow the orexin neurons to promote alertness in a hungry animal and maintain long periods of wakefulness throughout the day. These findings indicate that orexin neurons provide a crucial link between energy balance and arousal.

C. Orexin as Effector of Food Entrainable Oscillator

Temporal restriction of feeding can produce an anticipatory locomotor activity rhythm and entrain the peripheral molecular oscillator, which is independent of the central clock located in the suprachiasmatic nucleus. Orexin neurons have been shown to contribute to the promotion and maintenance of this food-anticipatory activity (Akiyama et al., 2004; Mieda et al., 2004a). Restricted feeding was shown to shift the peak of *Fos* expression of orexin neurons from night to the period of restricted feeding (Akiyama et al., 2004; Mieda et al., 2004a). Display of the food-anticipatory activity is severely impaired in orexin neuron-ablated, *orexin/ataxin-3* transgenic mice (Akiyama et al., 2004; Mieda et al., 2004a). Expression of mNpas2 mRNA, a transcription factor thought to be involved in regulation of the food entrainable oscillator (FEO), as well as mPer1 and mBmal1 mRNA, is reduced in *orexin/ataxin-3* mice. These observations suggest that orexin neurons convey an efferent signal from the putative FEO or oscillators to increase wakefulness and locomotor activity. Recently, several reports suggest that the DMH is a candidate for the center of the FEO. The DMH was shown to have marked oscillation of *mPer* expression only under restricted feeding (Mieda et al., 2006). Gooley et al. (2006) also consistently demonstrated that lesions in the DMH in rats blocked food entrainment of wakefulness, locomotor activity, and core body temperature. Furthermore, restoration of the clock gene *Bmal1* gene only in the DMH restored the ability of animals to entrain to food but not to light in mice with targeted disruption of the *Bmal1* (Fuller et al., 2008). In contrast, Landry et al. (2006) reported that complete ablation of the DMH did not affect food-anticipatory activity rhythms in rats. The nature of the discrepancy between these reports remains unknown. However, taken in conjunction with our recent finding that DMH neurons directly project to orexin neurons (Sakurai et al., 2005), these findings indicate a possibility that a link between DMH neurons and orexin neurons might play a key role as a central FEO in the feeding-mediated regulation of circadian behavior.

VII. Roles of Orexins in Reward Systems

A. Orexin Neurons in the Lateral Hypothalamic Area Involved in Reward Systems

Several lines of evidence suggest that orexins are involved in the modulation of the reward function and that there is a functional dichotomy for orexin neurons. The lateral part of orexin neurons is strongly linked to preferences for cues associated with drug and food reward (Harris et al., 2005). Fos expression in orexin neurons of the PFA-DMH show diurnal change consistent with a role in regulating arousal, but LHA orexin neurons did not (Estabrooke et al., 2001). These observations indicate that orexin neurons in the LHA have an important role in reward system. Historically, it is known that an important role of the LHA in reward by both lesion experiments and the intracranial self-stimulation paradigm (Anand and Brobeck, 1951; Olds and Milner, 1954).

Patients with narcolepsy are often treated using highly addictive amphetamine-like drugs such as methylphenidate, amphetamine, and γ-hydroxybutyrate (Nishino and Mignot, 1997), but they rarely become addicted to these drugs (Akimoto et al., 1960; Guilleminault et al., 1974). Orexin knockout mice are consistently less susceptible than wild-type mice to developing morphine dependence as measured by physical with-

drawal response (Georgescu et al., 2003). Moreover, morphine-conditioned animals had greater place preferences and Fos-activated orexin neurons than nonconditioned (Harris et al., 2005). These observations suggest that orexin neurons play important roles in reward processing.

B. Input from Reward Systems

Orexin neurons receive projections from the VTA, NAc, and lateral septum, regions involved in reward systems (Yoshida et al., 2006). Dopamine inhibits orexin neurons by acting on α_2-adrenoceptors (Yamanaka et al., 2003b, 2006) constituting negative feedback regulation. Dopamine has a consistently inhibitory influence on food intake and the reward pathways when injected in the LHA/PFA (Yang et al., 1997). On the contrary, VTA neurons receive a projection from a part of the LHA/PFA including orexin neurons (Marcus et al., 2001; Fadel and Deutch, 2002). These reciprocal interactions might constitute regulatory mechanisms of reward systems (Fig. 4).

C. Output to Reward Systems

Orexin directly activates VTA dopaminergic neurons (Nakamura et al., 2000; Korotkova et al., 2003), and the VTA receives an input from orexin neurons composed of both synaptic terminals (Peyron et al., 1998; Fadel and Deutch, 2002) and en passant fibers (Balcita-Pedicino and Sesack, 2007). The relatively high presence of axonally located orexin-containing dense core vesicles that are capable of exocytosis at extrasynaptic sites suggests that orexins are probably released into the VTA via extrasynaptic mechanisms (Balcita-Pedicino and Sesack, 2007). The dopamine receptor antagonist haloperidol blocks hyperlocomotion and stereotypy induced by intracerebroventricular orexin (Nakamura et al., 2000). Intracerebroventricular or local VTA infusion of orexin reinstates drug- or food-seeking behavior in rodents (Boutrel et al., 2005; Harris et al., 2005). On the other hand, injection of an orexin antagonist into the VTA blocks the development of heroin-conditioned place preferences (Narita et al., 2006).

Recent work has also shown that orexin A input to the VTA potentiates NMDA receptor (NMDAR)-mediated neurotransmission via phospholipase C/Ca^{2+}-phospholipid–dependent protein kinase-dependent recruitment of NMDA receptors in VTA dopamine neuron synapses in slice preparations (Borgland et al., 2006). A recent study demonstrated that orexin B potentiated both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor and NMDAR currents in VTA slices (Borgland et al., 2008). Furthermore, in vivo administration of an OX_1R antagonist blocks locomotor sensitization to cocaine and occludes cocaine-induced potentiation of excitatory currents in VTA dopamine neurons (Borgland et al., 2006). These results suggest a critical role of orexin signaling in neural plastic effects at glutamatergic synapses in the VTA and that orexins play a critical role in cocaine-induced psychomotor sensitization and reward-seeking. These findings suggest roles of orexin in the mechanisms of reward systems and drug addiction.

Similarly to orexin, CRF potentiates NMDAR-mediated synaptic transmission in dopamine neurons of the VTA (Ungless et al., 2003). Changes in synaptic efficacy such as those induced by orexin and CRF are likely to underlie arousal responses to the environment. CRF activates orexin neurons directly (Winsky-Sommerer et al., 2004), and this circuit contributes to activation and maintenance of arousal associated with the stress response. Increased activity of orexin neurons could also lead to a state of hyperarousal and excitement propitious to drug craving or could contribute to the susceptibility to relapse of drug seeking during protracted abstinence (de Lecea et al., 2006).

VIII. Clinical Implications

Current management of narcolepsy relies totally on symptomatic treatment. This includes psychostimulants (including modafinil, methylphenidate, amphetamine, and caffeine) for excessive daytime sleepiness and sleep attacks and tricyclic antidepressants or selective serotonin-reuptake inhibitors for cataplexy and other REM-associated symptoms. Hypnotics are also used for disturbed nighttime sleep. Because narcolepsy-cataplexy is a disorder resulting from an absence of orexin, replacement therapy using orexin receptor agonists may provide an effective treatment for narcolepsy.

Chronic overproduction of orexin peptides from an ectopically expressed transgene prevented cataplectic arrests and other abnormalities of REM sleep in orexin neuron-ablated (*orexin/ataxin-3*) mice (Mieda et al., 2004b). Short-term administration of orexin A to these mice also maintained wakefulness, suppressed sleep, and inhibited cataplectic attacks (Mieda et al., 2004b). Furthermore, after viral vector transferal of the gene for mouse *prepro-orexin* to the LHA of orexin-deficient mice, the incidence of cataplexy declined and the levels of REM sleep during the second half of the night became similar to those in wild-type mice (Liu et al., 2008). These results indicate that orexin neuron-ablated mice retain the ability to respond to orexin neuropeptides and that a temporally regulated and spatially targeted secretion of orexins is not necessary to prevent narcoleptic symptoms. However, chronic overexpression of orexin in an unregulated fashion results in fragmentation of non-REM sleep. Therefore, if orexin agonists were available, a short half-life ($<$12 h) might be desirable. Attempts at using orexin-based treatment after peripheral administration have been disappointing, because the peptides do not cross the blood-brain barrier (Mignot and Nishino, 2005). However, a recent report showed that orexin A delivered via the intravenous and nasal routes signifi-

cantly improved performance in sleep-deprived rhesus monkeys (Deadwyler et al., 2007).

Unfortunately, there are no reported nonpeptide orexin receptor agonists. Orexin-based therapy such as direct use of orexin receptor agonists (Mieda et al., 2004b; Deadwyler et al., 2007) and orexin neuron transplantation is currently under investigation in animal models.

The spinal cord receives projections by orexin-immunoreactive fibers, especially dorsal root ganglion neurons and lamina I and X surrounding the central canal (van den Pol, 1999). OX_1R is localized on C fibers in the spinal cord (Hervieu et al., 2001). These data suggest that the spinal orexin system is involved in transmission of nociceptive information. Several studies have shown that an orexin receptor agonist produces an analgesic effect in a rat model (Bingham et al., 2001; Yamamoto et al., 2002). In addition, the orexin system is involved in stress-induced analgesia (SIA). Orexin knockout mice presented less SIA than wild-type mice (Watanabe et al., 2005). Orexin neurons were hyperpolarized by nociceptin/orphanin FQ (N/OFQ), which is a neuropeptide implicated in blocking SIA (Xie et al., 2008). Coadministration of orexin A overcame N/OFQ inhibition of SIA in mice (Xie et al., 2008). These results suggest that the orexin neurons regulate SIA and its effects might be modulated by N/OFQ. Thus, orexin receptor agonists could possibly also be useful in controlling of pain and dysfunctional conditions resulting from excessive stress. (Kajiyama et al., 2005; Mobarakeh et al., 2005; Xie et al., 2008).

The administration of the OX_2R-selective agonist [Ala^{11},D-Leu^{15}]-orexin B suppressed weight gain on a high-fat diet without altering weight homeostasis on a low-fat diet (Funato et al., 2009). This report suggests that enhanced orexin-OX_2R signaling confers resistance to diet-induced features of the metabolic syndrome (Table 2).

Several pharmaceutical companies have shown interest in the potential therapeutic application of nonpeptide, low-molecular-weight orexin receptor antagonists (for review, see Bingham et al., 2006). Orexin receptor antagonists might be effective for inducing sleep and treating insomnia patients. A new dual-orexin receptor antagonist (ACT-078573) selectively and reversibly blocks both OX_1R and OX_2R at nanomolar concentrations (Brisbare-Roch et al., 2007). The drug is orally active and effectively crosses the blood-brain barrier. Although the drug thoroughly blocks orexin signaling and produces sleepiness, no signs of cataplexy were observed. This might suggest that cataplexy become evident after long-term deficiency of orexins. This antagonist was effective for promoting sleep when given to rodents and dogs during the active period, but it had no effect when given during the rest period in which orexin system might be inactive. Accordingly, it may be very effective in shift workers or people with jet lag trying to sleep when their biological clock is signaling wakefulness. The company indicated that the compound was suitable for use as a sleep quality improver, and the drug has entered phase III clinical trials for insomnia.

It was also shown that OX_1R antagonist (SB334867A) blocked an effect of the orexin-induced increase of food consumption (Bingham et al., 2006). Studies using strains of mice and rats that differ in susceptibility to diet-induced obesity have also demonstrated the anorexic effect of SB334867A. Furthermore, SB334867A blocks 2-deoxy-D-glucose- and orexin A-induced gastric acid secretion in rats. In addition, insomnia is sometimes observed during calorie restriction in humans. The sleep-inducing effect of orexin antagonists might help in treating such a case.

Another possible use of orexin receptor antagonists includes withdrawal from drug addiction. SB334867A significantly suppressed morphine-induced place preference and hyperlocomotion (Harris et al., 2005; Narita et al., 2006) and blocked the reinstatement of previously extinguished cocaine-seeking behavior and locomotor sensitization to cocaine (Boutrel et al., 2005; Borgland et al., 2006) and decreased intravenous nicotine self-administration and the motivation to obtain the drug in rats (Hollander et al., 2008) (Table 2). However, SB334867A has off-target effects that make this selective OX_1R antagonist a less-than-ideal drug for treatment. Although to date there is no report of an OX_1R antagonist in clinical development, OX_1R antagonists may have therapeutic utility in the treatment of obesity and drug addiction. Furthermore, the OX_1R antagonists may provides an opportunity to better understand the functions of orexins in human.

TABLE 2
Disorders and physical conditions potentially ameliorated by pharmacological activation or inhibition of orexin system

Therapeutic Approach	Disorders or Physical Conditions
Activating orexin system	Narcolepsy and other hypersomnia
	Pain
	Diet-induced obesity (OX_2R-selective)
	Inattentiveness, apathy
Inhibiting orexin system	Insomnia
	Jet lag
	Diet-induced obesity (OX_1R-selective)
	Drug addiction (OX_1R-selective)

IX. Conclusion

For more than a decade of research, it was revealed that orexin neurons provide a crucial link between energy balance, emotion, reward systems and arousal (Fig. 5). Symptoms of narcolepsy undoubtedly show that the orexin system plays highly important roles in regulating sleep/awake states and the maintenance of arousal by reciprocal interaction between orexin neuron and monoaminergic/cholinergic nuclei in the brain. This system is also related to the limbic system, which regulates emotional responses, the reward system in the VTA, and

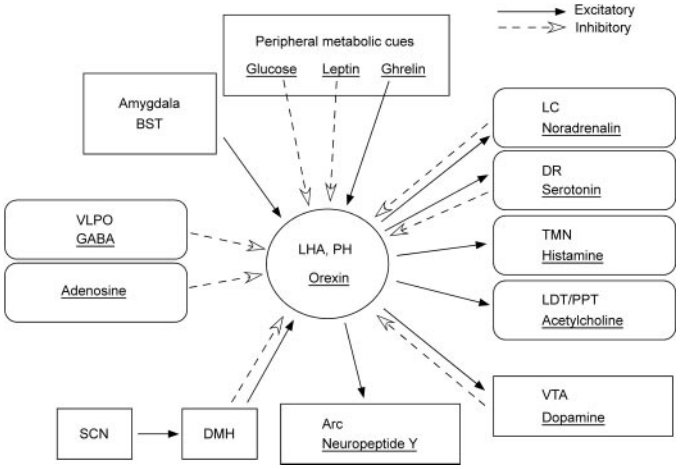

FIG. 5. Input and output of orexin neurons at the interface of sleep, reward systems, and energy homeostasis. Orexin neurons in the LHA and posterior hypothalamus (PH) are placed to provide a link between the limbic system, energy homeostasis, and brainstem monoaminergic and cholinergic neurons. Arrows, excitatory projections; broken arrows, inhibitory projections. Neurotransmitters/modulators are underlined. LC, DR, and TMN are wake-active regions, VLPO is a sleep-active region, and LDT/PPT is a REM-active region. Orexin neurons promote wakefulness through the monoaminergic nuclei that are wake-active. Stimulation of dopaminergic centers by orexins modulates reward systems (VTA). Peripheral metabolic signals, leptin, ghrelin, and glucose influence orexin neuronal activity to coordinate arousal and energy homeostasis. Stimulation of neuropeptide Y neuron by orexin increases food intake. The suprachiasmatic nucleus (SCN), the central body clock, sends input to orexin neurons via the DMH. The DMH acts as a food-entrainable oscillator and influences orexin neural activity. Input from the limbic system (amygdala and BST) might be important to regulate the activity of orexin neurons upon emotional stimuli to evoke emotional arousal or fear-related responses.

hypothalamic mechanisms that regulate feeding behavior. Orexin neurons in the LHA are anatomically well placed to link the limbic system, energy homeostasis, and brainstem monoaminergic/cholinergic neurons. The link between the limbic system and orexin neurons might be important for emotional arousal and sympathetic responses during emotional events such as avoiding danger. The link among the limbic system, VTA, and orexin might be important for reward and motivated behavior such as finding food. On the other hand, the responsiveness of orexin neurons to leptin, glucose, and other peripheral metabolic cues suggests that these cells might act as a sensor for the metabolic status of animals. Orexin systems are involved in sensing the body's external and internal environments as well as regulating the states of sleep and wakefulness, which are beneficial for survival.

Acknowledgments. This study was supported by a grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan.

References

Abrahamson EE, Leak RK, and Moore RY (2001) The suprachiasmatic nucleus projects to posterior hypothalamic arousal systems. *Neuroreport* **12:**435–440.

Akimoto H, Honda Y, and Takahashi Y (1960) Pharmacotherapy in narcolepsy. *Dis Nerv Syst* **21:**704–706.

Akiyama M, Yuasa T, Hayasaka N, Horikawa K, Sakurai T, and Shibata S (2004) Reduced food anticipatory activity in genetically orexin (hypocretin) neuron-ablated mice. *Eur J Neurosci* **20:**3054–3062.

Alam MN, Szymusiak R, Gong H, King J, and McGinty D (1999) Adenosinergic modulation of rat basal forebrain neurons during sleep and waking: neuronal recording with microdialysis. *J Physiol* **521:**679–690.

Alvarez CE and Sutcliffe JG (2002) Hypocretin is an early member of the incretin gene family. *Neurosci Lett* **324:**169–172.

American Academy of Sleep Medicine Diagnostic Classification Steering Committee (2005) *The International Classification of Sleep Disorders*, *2nd Edition Revised* (*ICSD-R2*) American Academy of Sleep Medicine, Westchester, IL.

Anand BK and Brobeck JR (1951) Localization of a "feeding center" in the hypothalamus of the rat. *Proc Soc Exp Biol Med* **77:**323–324.

Balcita-Pedicino JJ and Sesack SR (2007) Orexin axons in the rat ventral tegmental area synapse infrequently onto dopamine and gamma-aminobutyric acid neurons. *J Comp Neurol* **503:**668–684.

Baldo BA, Gual-Bonilla L, Sijapati K, Daniel RA, Landry CF, and Kelley AE (2004) Activation of a subpopulation of orexin/hypocretin-containing hypothalamic neurons by GABAA receptor-mediated inhibition of the nucleus accumbens shell, but not by exposure to a novel environment. *Eur J Neurosci* **19:**376–386.

Berthoud HR (2004) Mind versus metabolism in the control of food intake and energy balance. *Physiol Behav* **81:**781–793.

Bingham MJ, Cai J, and Deehan MR (2006) Eating, sleeping and rewarding: orexin receptors and their antagonists. *Curr Opin Drug Discov Devel* **9:**551–559.

Bingham S, Davey PT, Babbs AJ, Irving EA, Sammons MJ, Wyles M, Jeffrey P, Cutler L, Riba I, Johns A, et al. (2001) Orexin-A, an hypothalamic peptide with analgesic properties. *Pain* **92:**81–90.

Bonnet MH and Arand DL (1998) Heart rate variability in insomniacs and matched normal sleepers. *Psychosom Med* **60:**610–615.

Bonnet MH and Arand DL (2003) Insomnia, metabolic rate and sleep restoration. *J Intern Med* **254:**23–31.

Borgland SL, Storm E, and Bonci A (2008) Orexin B/hypocretin 2 increases glutamatergic transmission to ventral tegmental area neurons. *Eur J Neurosci* **28:**1545–1556.

Borgland SL, Taha SA, Sarti F, Fields HL, and Bonci A (2006) Orexin A in the VTA is critical for the induction of synaptic plasticity and behavioral sensitization to cocaine. *Neuron* **49:**589–601.

Boutrel B, Kenny PJ, Specio SE, Martin-Fardon R, Markou A, Koob GF, and de Lecea L (2005) Role for hypocretin in mediating stress-induced reinstatement of cocaine-seeking behavior. *Proc Natl Acad Sci U S A* **102:**19168–19173.

Brisbare-Roch C, Dingemanse J, Koberstein R, Hoever P, Aissaoui H, Flores S, Mueller C, Nayler O, van Gerven J, de Haas SL, et al. (2007) Promotion of sleep by targeting the orexin system in rats, dogs and humans. *Nat Med* **13:**150–155.

Broberger C, De Lecea L, Sutcliffe JG, and Hökfelt T (1998) Hypocretin/orexin- and melanin-concentrating hormone-expressing cells form distinct populations in the rodent lateral hypothalamus: relationship to the neuropeptide Y and agouti gene-related protein systems. *J Comp Neurol* **402:**460–474.

Brown RE, Sergeeva OA, Eriksson KS, and Haas HL (2002) Convergent excitation of dorsal raphe serotonin neurons by multiple arousal systems (orexin/hypocretin, histamine and noradrenaline). *J Neurosci* **22:**8850–8859.

Burdakov D, Gerasimenko O, and Verkhratsky A (2005) Physiological changes in glucose differentially modulate the excitability of hypothalamic melanin-concentrating hormone and orexin neurons in situ. *J Neurosci* **25:**2429–2433.

Burdakov D, Jensen LT, Alexopoulos H, Williams RH, Fearon IM, O'Kelly I, Gerasimenko O, Fugger L, and Verkhratsky A (2006) Tandem-pore K+ channels mediate inhibition of orexin neurons by glucose. *Neuron* **50:**711–722.

Challet E, Pevet P, and Malan A (1997) Effect of prolonged fasting and subsequent refeeding on free-running rhythms of temperature and locomotor activity in rats. *Behav Brain Res* **84:**275–284.

Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuki Y, et al. (1999) Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* **98:**437–451.

Chou TC, Lee CE, Lu J, Elmquist JK, Hara J, Willie JT, Beuckmann CT, Chemelli RM, Sakurai T, Yanagisawa M, et al. (2001) Orexin (hypocretin) neurons contain dynorphin. *J Neurosci* **21:**RC168.

Crocker A, España RA, Papadopoulou M, Saper CB, Faraco J, Sakurai T, Honda M, Mignot E, and Scammell TE (2005) Concomitant loss of dynorphin, NARP, and orexin in narcolepsy. *Neurology* **65:**1184–1188.

Date Y, Ueta Y, Yamashita H, Yamaguchi H, Matsukura S, Kangawa K, Sakurai T, Yanagisawa M, and Nakazato M (1999) Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. *Proc Natl Acad Sci U S A* **96:**748–753.

Deadwyler SA, Porrino L, Siegel JM, and Hampson RE (2007) Systemic and nasal delivery of orexin-A (Hypocretin-1) reduces the effects of sleep deprivation on cognitive performance in nonhuman primates. *J Neurosci* **27:**14239–14247.

de Lecea L, Jones BE, Boutrel B, Borgland SL, Nishino S, Bubser M, and DiLeone R (2006) Addiction and arousal: alternative roles of hypothalamic peptides. *J Neurosci* **26:**10372–10375.

de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett FS 2nd, et al. (1998) The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A* **95:**322–327.

Edwards CM, Abusnana S, Sunter D, Murphy KG, Ghatei MA, and Bloom SR (1999) The effect of the orexins on food intake: comparison with neuropeptide Y, melanin-concentrating hormone and galanin. *J Endocrinol* **160:**R7–12.

Eggermann E, Serafin M, Bayer L, Machard D, Saint-Mleux B, Jones BE, and Mühlethaler M (2001) Orexins/hypocretins excite basal forebrain cholinergic neurones. *Neuroscience* **108:**177–181.

Elias CF, Saper CB, Maratos-Flier E, Tritos NA, Lee C, Kelly J, Tatro JB, Hoffman GE, Ollmann MM, Barsh GS, et al. (1998) Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area. *J Comp Neurol* **402:**442–459.

Elmquist JK, Elias CF, and Saper CB (1999) From lesions to leptin: hypothalamic control of food intake and body weight. *Neuron* **22:**221–232.

Estabrooke IV, McCarthy MT, Ko E, Chou TC, Chemelli RM, Yanagisawa M, Saper

CB, and Scammell TE (2001) Fos expression in orexin neurons varies with behavioral state. *J Neurosci* **21:**1656–1662.
Fadel J and Deutch AY (2002) Anatomical substrates of orexin-dopamine interactions: lateral hypothalamic projections to the ventral tegmental area. *Neuroscience* **111:**379–387.
Fuller PM, Lu J, and Saper CB (2008) Differential rescue of light- and food-entrainable circadian rhythms. *Science* **320:**1074–1077.
Funato H, Tsai AL, Willie JT, Kisanuki Y, Williams SC, Sakurai T, and Yanagisawa M (2009) Enhanced orexin receptor-2 signaling prevents diet-induced obesity and improves leptin sensitivity. *Cell Metab* **9:**64–76.
Gallopin T, Fort P, Eggermann E, Cauli B, Luppi PH, Rossier J, Audinat E, Mühlethaler M, and Serafin M (2000) Identification of sleep-promoting neurons in vitro. *Nature* **404:**992–995.
Georgescu D, Zachariou V, Barrot M, Mieda M, Willie JT, Eisch AJ, Yanagisawa M, Nestler EJ, and DiLeone RJ (2003) Involvement of the lateral hypothalamic peptide orexin in morphine dependence and withdrawal. *J Neurosci* **23:**3106–3111.
Gooley JJ, Schomer A, and Saper CB (2006) The dorsomedial hypothalamic nucleus is critical for the expression of food-entrainable circadian rhythms. *Nat Neurosci* **9:**398–407.
Griffond B, Risold PY, Jacquemard C, Colard C, and Fellmann D (1999) Insulin-induced hypoglycemia increases preprohypocretin (orexin) mRNA in the rat lateral hypothalamic area. *Neurosci Lett* **262:**77–80.
Grivel J, Cvetkovic V, Bayer L, Machard D, Tobler I, Mühlethaler M, and Serafin M (2005) The wake-promoting hypocretin/orexin neurons change their response to noradrenaline after sleep deprivation. *J Neurosci* **25:**4127–4130.
Guilleminault C, Carskadon M, and Dement WC (1974) On the treatment of rapid eye movement narcolepsy. *Arch Neurol* **30:**90–93.
Hagan JJ, Leslie RA, Patel S, Evans ML, Wattam TA, Holmes S, Benham CD, Taylor SG, Routledge C, Hemmati P, 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.
Håkansson M, de Lecea L, Sutcliffe JG, Yanagisawa M, and Meister B (1999) Leptin receptor- and STAT3-immunoreactivities in hypocretin/orexin neurones of the lateral hypothalamus. *J Neuroendocrinol* **11:**653–663.
Hara J, Beuckmann CT, Nambu T, Willie JT, Chemelli RM, Sinton CM, Sugiyama F, Yagami K, Goto K, Yanagisawa M, et al. (2001) Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron* **30:**345–354.
Harris GC, Wimmer M, and Aston-Jones G (2005) A role for lateral hypothalamic orexin neurons in reward seeking. *Nature* **437:**556–559.
Haynes AC, Chapman H, Taylor C, Moore GB, Cawthorne MA, Tadayyon M, Clapham JC, and Arch JR (2002) Anorectic, thermogenic and anti-obesity activity of a selective orexin-1 receptor antagonist in ob/ob mice. *Regul Pept* **104:**153–159.
Haynes AC, Jackson B, Chapman H, Tadayyon M, Johns A, Porter RA, and Arch JR (2000) A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. *Regul Pept* **96:**45–51.
Hervieu GJ, Cluderay JE, Harrison DC, Roberts JC, and Leslie RA (2001) Gene expression and protein distribution of the orexin-1 receptor in the rat brain and spinal cord. *Neuroscience* **103:**777–797.
Hollander JA, Lu Q, Cameron MD, Kamenecka TM, and Kenny PJ (2008) Insular hypocretin transmission regulates nicotine reward. *Proc Natl Acad Sci U S A* **105:**19480–19485.
Honda Y, Doi Y, Ninomiya R, and Ninomiya C (1986) Increased frequency of non-insulin-dependent diabetes mellitus among narcoleptic patients. *Sleep* **9:**254–259.
Huang ZL, Qu WM, Li WD, Mochizuki T, Eguchi N, Watanabe T, Urade Y, and Hayaishi O (2001) Arousal effect of orexin A depends on activation of the histaminergic system. *Proc Natl Acad Sci U S A* **98:**9965–9970.
Ida T, Nakahara K, Murakami T, Hanada R, Nakazato M, and Murakami N (2000) Possible involvement of orexin in the stress reaction in rats. *Biochem Biophys Res Commun* **270:**318–323.
Itoh T, Murai S, Nagahama H, Miyate H, Abe E, Fujiwara H, and Saito Y (1990) Effects of 24-hr fasting on methamphetamine- and apomorphine-induced locomotor activities, and on monoamine metabolism in mouse corpus striatum and nucleus accumbens. *Pharmacol Biochem Behav* **35:**391–396.
Jöhren O, Neidert SJ, Kummer M, Dendorfer A, and Dominiak P (2001) Prepro-orexin and orexin receptor mRNAs are differentially expressed in peripheral tissues of male and female rats. *Endocrinology* **142:**3324–3331.
Kadotani H, Faraco J, and Mignot E (1998) Genetic studies in the sleep disorder narcolepsy. *Genome Res* **8:**427–434.
Kajiyama S, Kawamoto M, Shiraishi S, Gaus S, Matsunaga A, Suyama H, and Yuge O (2005) Spinal orexin-1 receptors mediate anti-hyperalgesic effects of intrathecally-administered orexins in diabetic neuropathic pain model rats. *Brain Res* **1044:**76–86.
Karteris E, Machado RJ, Chen J, Zervou S, Hillhouse EW, and Randeva HS (2005) Food deprivation differentially modulates orexin receptor expression and signaling in rat hypothalamus and adrenal cortex. *Am J Physiol Endocrinol Metab* **288:**E1089–100.
Kayaba Y, Nakamura A, Kasuya Y, Ohuchi T, Yanagisawa M, Komuro I, Fukuda Y, and Kuwaki T (2003) Attenuated defense response and low basal blood pressure in orexin knockout mice. *Am J Physiol Regul Integr Comp Physiol* **285:**R581–93.
Korotkova TM, Sergeeva OA, Eriksson KS, Haas HL, and Brown RE (2003) Excitation of ventral tegmental area dopaminergic and nondopaminergic neurons by orexins/hypocretins. *J Neurosci* **23:**7–11.
Lammers GJ, Pijl H, Iestra J, Langius JA, Buunk G, and Meinders AE (1996) Spontaneous food choice in narcolepsy. *Sleep* **19:**75–76.
Landry GJ, Simon MM, Webb IC, and Mistlberger RE (2006) Persistence of a behavioral food-anticipatory circadian rhythm following dorsomedial hypothalamic ablation in rats. *Am J Physiol Regul Integr Comp Physiol* **290:**R1527–34.
Lee MG, Hassani OK, and Jones BE (2005) Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. *J Neurosci* **25:**6716–6720.
Li Y, Gao XB, Sakurai T, and van den Pol AN (2002) Hypocretin/Orexin excites hypocretin neurons via a local glutamate neuron-A potential mechanism for orchestrating the hypothalamic arousal system. *Neuron* **36:**1169–1181.
Li Y and van den Pol AN (2006) Differential target-dependent actions of coexpressed inhibitory dynorphin and excitatory hypocretin/orexin neuropeptides. *J Neurosci* **26:**13037–13047.
Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qiu X, de Jong PJ, Nishino S, and Mignot E (1999) The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* **98:**365–376.
Liu M, Thankachan S, Kaur S, Begum S, Blanco-Centurion C, Sakurai T, Yanagisawa M, Neve R, and Shiromani PJ (2008) Orexin (hypocretin) gene transfer diminishes narcoleptic sleep behavior in mice. *Eur J Neurosci* **28:**1382–1393.
Liu RJ, van den Pol AN, and Aghajanian GK (2002) Hypocretins (orexins) regulate serotonin neurons in the dorsal raphe nucleus by excitatory direct and inhibitory indirect actions. *J Neurosci* **22:**9453–9464.
Liu ZW and Gao XB (2007) Adenosine inhibits activity of hypocretin/orexin neurons by the A1 receptor in the lateral hypothalamus: a possible sleep-promoting effect. *J Neurophysiol* **97:**837–848.
Lu J, Bjorkum AA, Xu M, Gaus SE, Shiromani PJ, and Saper CB (2002) Selective activation of the extended ventrolateral preoptic nucleus during rapid eye movement sleep. *J Neurosci* **22:**4568–4576.
Lu XY, Bagnol D, Burke S, Akil H, and Watson SJ (2000) Differential distribution and regulation of OX1 and OX2 orexin/hypocretin receptor messenger RNA in the brain upon fasting. *Horm Behav* **37:**335–344.
Ma X, Zubcevic L, Brüning JC, Ashcroft FM, and Burdakov D (2007) Electrical inhibition of identified anorexigenic POMC neurons by orexin/hypocretin. *J Neurosci* **27:**1529–1533.
Marcus JN, Aschkenasi CJ, Lee CE, Chemelli RM, Saper CB, Yanagisawa M, and Elmquist JK (2001) Differential expression of orexin receptors 1 and 2 in the rat brain. *J Comp Neurol* **435:**6–25.
Matsuki T, Nomiyama M, Takahira H, Hirashima N, Kunita S, Takahashi S, Yagami K, Kilduff TS, Bettler B, Yanagisawa M, 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.
Mieda M, Williams SC, Richardson JA, Tanaka K, and Yanagisawa M (2006) The dorsomedial hypothalamic nucleus as a putative food-entrainable circadian pacemaker. *Proc Natl Acad Sci U S A* **103:**12150–12155.
Mieda M, Williams SC, Sinton CM, Richardson JA, Sakurai T, and Yanagisawa M (2004a) 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.
Mieda M, Willie JT, Hara J, Sinton CM, Sakurai T, and Yanagisawa M (2004b) 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.
Mignot E (1998) Genetic and familial aspects of narcolepsy. *Neurology* **50 (2 Suppl 1):**S16–S22.
Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, Vankova J, Black J, Harsh J, Bassetti C, et al. (2002) The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. *Arch Neurol* **59:**1553–1562.
Mignot E and Nishino S (2005) Emerging therapies in narcolepsy-cataplexy. *Sleep* **28:**754–763.
Mobarakeh JI, Takahashi K, Sakurada S, Nishino S, Watanabe H, Kato M, and Yanai K (2005) Enhanced antinociception by intracerebroventricularly and intrathecally-administered orexin A and B (hypocretin-1 and -2) in mice. *Peptides* **26:**767–777.
Moriguchi T, Sakurai T, Nambu T, Yanagisawa M, and Goto K (1999) Neurons containing orexin in the lateral hypothalamic area of the adult rat brain are activated by insulin-induced acute hypoglycemia. *Neurosci Lett* **264:**101–104.
Muraki Y, Yamanaka A, Tsujino N, Kilduff TS, Goto K, and Sakurai T (2004) Serotonergic regulation of the orexin/hypocretin neurons through the 5-HT1A receptor. *J Neurosci* **24:**7159–7166.
Muroya S, Funahashi H, Yamanaka A, Kohno D, Uramura K, Nambu T, Shibahara M, Kuramochi M, Takigawa M, Yanagisawa M, 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.
Nakamura A, Zhang W, Yanagisawa M, Fukuda Y, and Kuwaki T (2007) Vigilance state-dependent attenuation of hypercapnic chemoreflex and exaggerated sleep apnea in orexin knockout mice. *J Appl Physiol* **102:**241–248.
Nakamura T, Uramura K, Nambu T, Yada T, Goto K, Yanagisawa M, and Sakurai T (2000) Orexin-induced hyperlocomotion and stereotypy are mediated by the dopaminergic system. *Brain Res* **873:**181–187.
Nambu T, Sakurai T, Mizukami K, Hosoya Y, Yanagisawa M, and Goto K (1999) Distribution of orexin neurons in the adult rat brain. *Brain Res* **827:**243–260.
Narita M, Nagumo Y, Hashimoto S, Narita M, Khotib J, Miyatake M, Sakurai T, Yanagisawa M, Nakamachi T, Shioda S, 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.
Nishino S and Mignot E (1997) Pharmacological aspects of human and canine narcolepsy. *Prog Neurobiol* **52:**27–78.
Nishino S, Ripley B, Overeem S, Lammers GJ, and Mignot E (2000) Hypocretin (orexin) deficiency in human narcolepsy. *Lancet* **355:**39–40.
Olds J and Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J Comp Physiol Psychol* **47:**419–427.
Oomura Y, Ooyama H, Sugimori M, Nakamura T, and Yamada Y (1974) Glucose inhibition of the glucose-sensitive neurone in the rat lateral hypothalamus. *Nature* **247:**284–286.
Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, 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* **6:**991–997.

Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, and Kilduff TS (1998) Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci* **18:**9996–10015.

Reid MS, Nishino S, Tafti M, Siegel JM, Dement WC, and Mignot E (1998) Neuropharmacological characterization of basal forebrain cholinergic stimulated cataplexy in narcoleptic canines. *Exp Neurol* **151:**89–104.

Reti IM, Reddy R, Worley PF, and Baraban JM (2002) Selective expression of Narp, a secreted neuronal pentraxin, in orexin neurons. *J Neurochem* **82:**1561–1565.

Risold PY, Griffond B, Kilduff TS, Sutcliffe JG, and Fellmann D (1999) Preprohypocretin (orexin) and prolactin-like immunoreactivity are coexpressed by neurons of the rat lateral hypothalamic area. *Neurosci Lett* **259:**153–156.

Rosin DL, Weston MC, Sevigny CP, Stornetta RL, and Guyenet PG (2003) Hypothalamic orexin (hypocretin) neurons express vesicular glutamate transporters VGLUT1 or VGLUT2. *J Comp Neurol* **465:**593–603.

Sakurai T (2005) Reverse pharmacology of orexin: from an orphan GPCR to integrative physiology. *Regul Pept* **126:**3–10.

Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, 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.

Sakurai T, Nagata R, Yamanaka A, Kawamura H, Tsujino N, Muraki Y, Kageyama H, Kunita S, Takahashi S, Goto K, et al. (2005) Input of orexin/hypocretin neurons revealed by a genetically encoded tracer in mice. *Neuron* **46:**297–308.

Saper CB, Chou TC, and Scammell TE (2001) The sleep switch: hypothalamic control of sleep and wakefulness. *Trends Neurosci* **24:**726–731.

Saper CB, Scammell TE, and Lu J (2005) Hypothalamic regulation of sleep and circadian rhythms. *Nature* **437:**1257–1263.

Schuld A, Hebebrand J, Geller F, and Pollmacher T (2000) Increased body-mass index in patients with narcolepsy. *Lancet* **355:**1274–1275.

Sherin JE, Elmquist JK, Torrealba F, and Saper CB (1998) Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. *J Neurosci* **18:**4705–4721.

Shibahara M, Sakurai T, Nambu T, Takenouchi T, Iwaasa H, Egashira SI, Ihara M, and Goto K (1999) Structure, tissue distribution, and pharmacological characterization of Xenopus orexins. *Peptides* **20:**1169–1176.

Shiromani PJ, Armstrong DM, Berkowitz A, Jeste DV, and Gillin JC (1988) Distribution of choline acetyltransferase immunoreactive somata in the feline brainstem: implications for REM sleep generation. *Sleep* **11:**1–16.

Soppet DR, Li Y, and Rosen CA (1996), inventors; Human Genome Sciences Inc., Soppet DR, Li Y, and Rosen CA, assignees. Human neuropeptide receptor. World Patent no. WO9634877. 1996 Nov 7.

Takahashi K, Koyama Y, Kayama Y, and Yamamoto M (2002) Effects of orexin on the laterodorsal tegmental neurones. *Psychiatry Clin Neurosci* **56:**335–336.

Takakusaki K, Takahashi K, Saitoh K, Harada H, Okumura T, Kayama Y, and Koyama Y (2005) Orexinergic projections to the cat midbrain mediate alternation of emotional behavioural states from locomotion to cataplexy. *J Physiol* **568:**1003–1020.

Tang J, Chen J, Ramanjaneya M, Punn A, Conner AC, and Randeva HS (2008) The signalling profile of recombinant human orexin-2 receptor. *Cell Signal* **20:**1651–1661,.

Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, and Siegel JM (2000) Reduced number of hypocretin neurons in human narcolepsy. *Neuron* **27:**469–474.

Thorpe AJ and Kotz CM (2005) Orexin A in the nucleus accumbens stimulates feeding and locomotor activity. *Brain Res* **1050:**156–162.

Torrealba F, Yanagisawa M, and Saper CB (2003) Colocalization of orexin a and glutamate immunoreactivity in axon terminals in the tuberomammillary nucleus in rats. *Neuroscience* **119:**1033–1044.

Trivedi P, Yu H, MacNeil DJ, Van der Ploeg LH, and Guan XM (1998) Distribution of orexin receptor mRNA in the rat brain. *FEBS Lett* **438:**71–75.

Tsujino N, Yamanaka A, Ichiki K, Muraki Y, Kilduff TS, Yagami K, Takahashi S, Goto K, and Sakurai T (2005) Cholecystokinin activates orexin/hypocretin neurons through the cholecystokinin A receptor. *J Neurosci* **25:**7459–7469.

Tsunematsu T, Fu LY, Yamanaka A, Ichiki K, Tanoue A, Sakurai T, and van den Pol AN (2008) Vasopressin increases locomotion through a V1a receptor in orexin/hypocretin neurons: implications for water homeostasis. *J Neurosci* **28:**228–238.

Ungless MA, Singh V, Crowder TL, Yaka R, Ron D, and Bonci A (2003) Corticotropin-releasing factor requires CRF binding protein to potentiate NMDA receptors via CRF receptor 2 in dopamine neurons. *Neuron* **39:**401–407.

van den Pol AN (1999) Hypothalamic hypocretin (orexin): robust innervation of the spinal cord. *J Neurosci* **19:**3171–3182.

van den Top M, Lee K, Whyment AD, Blanks AM, and Spanswick D (2004) Orexigen-sensitive NPY/AgRP pacemaker neurons in the hypothalamic arcuate nucleus. *Nat Neurosci* **7:**493–494.

Vanni-Mercier G, Sakai K, and Jouvet M (1984) [Specific neurons for wakefulness in the posterior hypothalamus in the cat.] *C R Acad Sci III* **298:**195–200.

Watanabe S, Kuwaki T, Yanagisawa M, Fukuda Y, and Shimoyama M (2005) Persistent pain and stress activate pain-inhibitory orexin pathways. *Neuroreport* **16:**5–8.

Williams RH, Jensen LT, Verkhratsky A, Fugger L, and Burdakov D. (2007) Control of hypothalamic orexin neurons by acid and CO2. *Proc Natl Acad Sci U S A* **104:**10685–10690.

Willie JT, Chemelli RM, Sinton CM, Tokita S, Williams SC, Kisanuki YY, Marcus JN, Lee C, Elmquist JK, Kohlmeier KA, 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.

Willie JT, Chemelli RM, Sinton CM, and Yanagisawa M (2001) To eat or to sleep? Orexin in the regulation of feeding and wakefulness. *Annu Rev Neurosci* **24:**429–458.

Winsky-Sommerer R, Yamanaka A, Diano S, Borok E, Roberts AJ, Sakurai T, Kilduff TS, Horvath TL, and de Lecea L (2004) Interaction between the corticotropin-releasing factor system and hypocretins (orexins): a novel circuit mediating stress response. *J Neurosci* **24:**11439–11448.

Xi MC, Morales FR, and Chase MH (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.

Xie X, Crowder TL, Yamanaka A, Morairty SR, Lewinter RD, Sakurai T, and Kilduff TS (2006) GABA(B) receptor-mediated modulation of hypocretin/orexin neurones in mouse hypothalamus. *J Physiol* **574:**399–414.

Xie X, Wisor JP, Hara J, Crowder TL, LeWinter R, Khroyan TV, Yamanaka A, Diano S, Horvath TL, Sakurai T, et al. (2008) Hypocretin/orexin and nociceptin/orphanin FQ coordinately regulate analgesia in a mouse model of stress-induced analgesia. *J Clin Invest* **118:**2471–2481.

Yamada H, Okumura T, Motomura W, Kobayashi Y, and Kohgo Y (2000) Inhibition of food intake by central injection of anti-orexin antibody in fasted rats. *Biochem Biophys Res Commun* **267:**527–531.

Yamamoto T, Nozaki-Taguchi N, and Chiba T (2002) Analgesic effect of intrathecally administered orexin-A in the rat formalin test and in the rat hot plate test. *Br J Pharmacol* **137:**170–176.

Yamanaka A, Beuckmann CT, Willie JT, Hara J, Tsujino N, Mieda M, Tominaga M, Yagami K, Sugiyama F, Goto K, et al. (2003a) Hypothalamic orexin neurons regulate arousal according to energy balance in mice. *Neuron* **38:**701–713.

Yamanaka A, Kunii K, Nambu T, Tsujino N, Sakai A, Matsuzaki I, Miwa Y, Goto K, and Sakurai T (2000) Orexin-induced food intake involves neuropeptide Y pathway. *Brain Res* **859:**404–409.

Yamanaka A, Muraki Y, Ichiki K, Tsujino N, Kilduff TS, Goto K, and Sakurai T (2006) Orexin neurons are directly and indirectly regulated by catecholamines in a complex manner. *J Neurophysiol* **96:**284–298.

Yamanaka A, Muraki Y, Tsujino N, Goto K, and Sakurai T (2003b) Regulation of orexin neurons by the monoaminergic and cholinergic systems. *Biochem Biophys Res Commun* **303:**120–129.

Yamanaka A, Tsujino N, Funahashi H, Honda K, Guan JL, Wang QP, Tominaga M, Goto K, Shioda S, and Sakurai T (2002) Orexins activate histaminergic neurons via the orexin 2 receptor. *Biochem Biophys Res Commun* **290:**1237–1245.

Yang ZJ, Meguid MM, Chai JK, Chen C, and Oler A (1997) Bilateral hypothalamic dopamine infusion in male Zucker rat suppresses feeding due to reduced meal size. *Pharmacol Biochem Behav* **58:**631–635.

Yoshida K, McCormack S, España RA, Crocker A, and Scammell TE (2006) Afferents to the orexin neurons of the rat brain. *J Comp Neurol* **494:**845–861.

Zhang W, Sakurai T, Fukuda Y, and Kuwaki T (2006) Orexin neuron-mediated skeletal muscle vasodilation and shift of baroreflex during defense response in mice. *Am J Physiol Regul Integr Comp Physiol* **290:**R1654–63.

Zheng H, Corkern MM, Crousillac SM, Patterson LM, Phifer CB, and Berthoud HR (2002) Neurochemical phenotype of hypothalamic neurons showing Fos expression 23 h after intracranial AgRP. *Am J Physiol Regul Integr Comp Physiol* **282:**R1773–R1781.

Zhu Y, Miwa Y, Yamanaka A, Yada T, Shibahara M, Abe Y, Sakurai T, and Goto K (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.