Results Probl Cell Differ (46)
O. Civelli, Q.-Y. Zhou: Orphan G Protein-Coupled Receptors and Novel Neuropeptides
DOI 10.1007/400_2007_047/Published online: 19 January 2008

Orexins and Orexin Receptors: From Molecules to Integrative Physiology

Taizo Matsuki[1,2,3] · Takeshi Sakurai[1,2,3] (✉)

[1]Department of Pharmacology, Institute of Basic Medical Science,
University of Tsukuba, Tsukuba, 305-8575 Ibaraki, Japan
stakeshi@md.tsukuba.ac.jp

[2]Yanagisawa Orphan Receptor Project, ERATO,
Japan Science and Technology Agency, 135-0064 Tokyo, Japan

[3]*Present address:*
Department of Molecular Neuroscience and Integrative Physiology,
Graduate School of Medical Science, Kanazawa University, 920 8640 Kanazawa, Japan

Abstract Recent studies have implicated the orexin system as a critical regulator of sleep/wake states, feeding behavior, and reward processes. Orexin deficiency results in narcolepsy-cataplexy in humans, dogs, and rodents, suggesting that the orexin system is particularly important for maintenance of wakefulness. Orexin agonists and antagonists are thought to be promising avenues toward the treatment of sleep disorders, eating disorders, and drug addiction. In this chapter, we discuss the current understanding of the physiological roles of orexins in regulation of arousal, sleep/wake states, energy homeostasis, and reward systems.

1 Introduction

Identification of a transmitter that naturally activates an orphan GPCR is crucial for understanding the physiological roles of a particular ligand-receptor system. Identification of ligands has the potential to define unknown functions and therapeutic targets in novel fields. One of the most compelling cases is the orexin system, in which classical and modern molecular pharmacological approaches have crossed paths, producing spectacular results. Starting from the discovery of molecules (orexins and orexin receptors), identification of the distribution of ligands and receptors predicted the role of this neuropeptide system. Investigations of gene-modified animals led to the finding of previously unknown functions of the orexins (sleep/arousal regulation), as well as the understanding of the pathophysiology of narcolepsy-cataplexy in humans. Anatomical, physiological, and pharmacological approaches to investigating the orexin system resulted in the appreciated knowledge of brain functions and regional networks. Referring to the orexin system is indispensable for research in the field of sleep, feeding, and reward control. Moreover, the possibility of clinical treatment for sleep disorders using antagonists or agonists of orexin receptors has been examined.

Since there are many comprehensive reviews on the orexin system (for a recent review, see Sakurai 2007), in this chapter, we will discuss an overview of its physiological significance, in relation to the possibility of clinical utility of orexin antagonists and agonists in the treatment of eating disorders, sleep disorders, and drug addiction.

2 Orexin and Orexin Receptors

2.1 Identification of Orexin (Hypocretin) by Deorphaning of Two GPCRs

Both orexin A and B were identified by the orphan GPCR strategy using stably expressing HFGAN72 *orexin-1 receptor* (*OX_1R*)-transfected HEK293 cells from rat brain extracts by detecting intracellular Ca^{2+} mobilization (Sakurai et al. 1998). Orexins constitute a novel peptide family with no significant homology with any previously described peptides. Orexin A is a 33-amino-acid peptide of 3562 Da. It has an N-terminal pyroglutamyl residue and C-terminal amida-

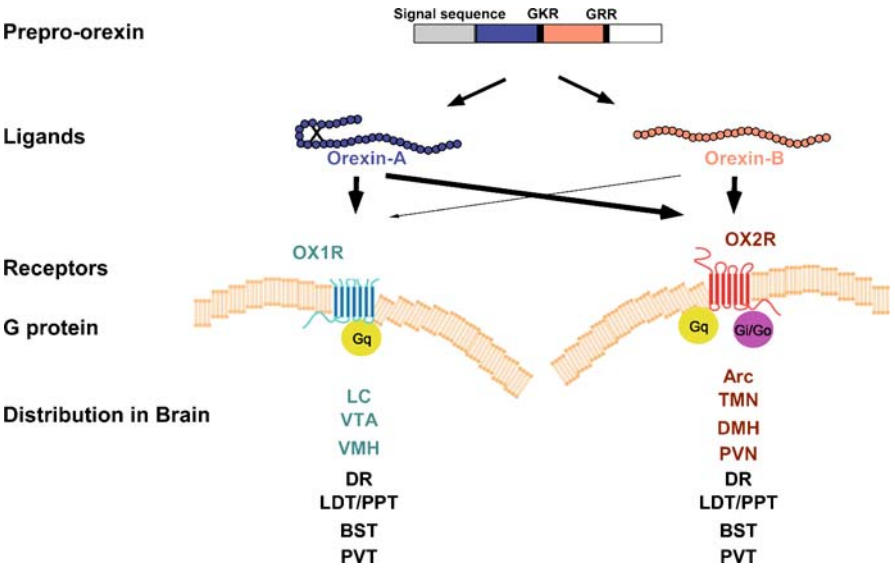

Fig. 1 Overview of orexin system. Orexin A and orexin B are derived from a common precursor peptide, *prepro-orexin*. The actions of orexins are mediated via two G protein-coupled receptors named orexin-1 (OX_1R) and orexin-2 (OX_2R) receptors. OX_1R is selective for orexin A, whereas OX_2R is a nonselective receptor for both orexin A and orexin 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.

tion (Sakurai et al. 1998). The four Cys residues of orexin A form two sets of intrachain disulfide bonds. The primary structure predicted from the cDNA sequences of the genes is conserved among several mammalian species (human, rat, mouse, cow, sheep, dog, and pig). On the other hand, rat orexin B is a 28-amino-acid, C-terminally amidated linear peptide of 2937 Da, which is 46% (13/28) identical in sequence to orexin A. The C-terminal half of orexin B is very similar to that of orexin A (73%; 11/15), while the N-terminal half is more variable. Mouse orexin B is identical to rat orexin B. Compared to the rodent sequence, human orexin B has two amino acid substitutions within the 28-residue stretch. Other than mammalian species, the structures of fish, xenopus, and chicken orexin A and orexin B have been elucidated, and they have also conserved structures as compared to mammalian sequences. (Alvarez and Sutcliffe 2002; Sakurai 2005; Shibahara et al. 1999).

The *prepro-orexin* cDNA sequences reveal that both orexins are produced from a common precursor polypeptide, prepro-orexin, by usual proteolytic processing (Fig. 1). An mRNA encoding the same precursor peptide was independently isolated by de Lecea et al. as a hypothalamus-specific transcript (de Lecea et al. 1998). De Lecea et al. predicted that this transcript potentially encodes two neuropeptides, named hypocretin-1 and hypocretin-2. The names "hypocretin" and "orexin" are currently used as synonyms in many papers.

2.2 Orexin Receptors

OX_1R was initially identified as an expressed sequence tag (EST) from human brain (US Patent WO96/34877 (Sakurai et al. 1998)). The *Orexin-2 receptor* (*OX_2R*) was identified by searching an EST database by tBLASTn with the *OX_1R* sequence as a query (Sakurai et al. 1998). *OX_1R* has one order of magnitude greater affinity for orexin A than for orexin B. In contrast, orexin A and orexin B bind *OX_2R* with similar affinity (Sakurai et al. 1998) (Fig. 1). *OX_1R* is thought to transmit signals through activation of the $G_{q/11}$ class of G proteins, which results in activation of phospholipase C (PLC) with subsequent triggering of the phosphatidylinositol cascade. *OX_2R* is shown to be coupled to both $G_{q/11}$ and inhibitory G_i proteins (Zhu et al. 2003) (Fig. 1).

2.3 Distribution of Orexin Neurons

Orexin-producing neurons (orexin neurons) are located exclusively in the lateral posterior hypothalamic regions, including the perifornical area (PFA), lateral hypothalamus, and posterior hypothalamus. The lateral hypothalamic area (LHA) has classically been implicated in a wide variety of behavioral and homeostatic regulatory systems, and thus the localization of *orexin*-expressing neurons has generated hypotheses as to their physiological rele-

vance (Bernardis and Bellinger 1996; Elmquist et al. 2005). Specific localization of orexin neurons is regulated by the *orexin* gene promoter (Moriguchi et al. 2002). The human *prepro-orexin* gene fragment, which contains the 3149-bp 5′-flanking region and 122-bp 5′-non-coding region of exon 1, has the ability to express *lacZ* in orexin neurons without ectopic expression in transgenic mice, suggesting that this genomic fragment contains most of the cis-elements necessary for appropriate expression of the gene (Sakurai et al. 1999).

From these *orexin*-expressing regions, these cells widely project to the entire brain, except for the cerebellum (Date et al. 1999; Nambu et al. 1999; Peyron et al. 1998). Dense staining of orexin-immunoreactive nerve endings in the brain was present in the central medial nucleus (CM) and paraventricular nucleus of the thalamus (PVT); arcuate nucleus (Arc), ventomedial nucleus (VMH), dorsomedial nucleus (DMH), and tuberomammillary nucleus (TMN) of the hypothalamus; locus coeruleus (LC); and ventral tegmental area (VTA).

2.4
Distributions of Orexin Receptors

Orexin receptors are expressed in regions which contain dense orexin projections, as described above. OX_1R and OX_2R mRNAs show partially overlapping and partially distinct distribution patterns, suggesting that they play different physiological roles.

OX_1R is expressed in many brain regions, such as the prefrontal and infralimbic cortex (IL), hippocampus (CA2), amygdala and bed nucleus stria terminalis (BST), PVT, anterior hypothalamus, dorsal raphe (DR), VTA, LC, and laterodorsal tegmental nucleus (LDT)/ pedunculopontine nucleus (PPT) (Lu et al. 2002; Marcus et al. 2001). OX_2R is expressed in the amygdala and BST, PVT, DR, VTA, and LDT/PPT (Lu et al. 2002; Marcus et al. 2001). On the other hand, OX_2R is prominent in the Arc, TMN, DMH, paraventricular nucleus (PVN), LHA in the hypothalamus, CA3 in the hippocampus, and medial septal nucleus (Lu et al. 2002; Marcus et al. 2001). These histological findings suggest that orexins and their receptors are involved in feeding, sleep, memory, and reward systems. These regions are major effector sites of orexins, as later described in detail.

3
Neural Circuits of Orexin Neurons

3.1
Neuronal Afferents

Recently, upstream neuronal populations that make innervations to orexin neurons in rodents were revealed in several studies (Sakurai et al. 2005;

Yoshida et al. 2006). These studies showed that orexin neurons are innervated by the lateral parabrachial nucleus (LPB), ventrolateral preoptic nucleus (VLPO), medial and lateral preoptic areas, basal forebrain (BF), posterior/dorsomedial hypothalamus, VTA, and median raphe nuclei (MnR). Many neurons were identified in regions associated with emotion, including the IL, amygdala, shell region of the nucleus accumbens, lateral septum (LS), and BST.

From these regions, neurons send input to orexin neurons and regulate orexin neuronal activity by secretion of particular neuromodulators. Many electrophysiological and histological studies have identified several neurotransmitters and neuromodulators that activate or inhibit orexin neurons (summarized in Table 1).

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, suggesting functional dichotomy of orexin neurons. Another work also suggested that the lateral part of orexin neurons is strongly linked to preferences for cues associated with drug and food reward (Harris et al. 2005).

Table 1 Factors that influence the activity of orexin neurons

Excitation	***Receptor involved***
Glutamate	AMPA-R, NMDA-R mGluRs
Ghrelin	GHS-R
CCK	CCK-A
Neurotensin	N.D.
Vasopressin	V1a
Oxytocin	OTR
CRH	CRHR1
TRH	TRHR
Ach (muscarinic) (20%)	M3
GLP1	GLP1-R
GALP	N.D.
Dopamine	(D1)
ATP	P2X
Inhibition	***Receptor involved***
Glucose	unknown
GABA	GABA-A, GABA-B
Serotonin	5HT1A
Noradrenaline	$\alpha 2$
Dopamine	D2
Leptin	ObR
Ach (muscarinic) (6%)	N.D.
NPY	Y1 (postsynaptic), Y2/Y5 (presynaptic)
Adenosine	A1

3.2
Local Interneurons

GABAergic input from local interneurons to orexin neurons is also important for organization of neuronal activity, because genetic disruption of this input resulted in marked sleep/wake abnormality (Matsuki and Sakurai 2008, unpublished). Both postsynaptic and presynaptic GABAergic input to orexin neurons strongly inhibits activity of orexin neurons. At the same time, glutamatergic input might be important for regulation of orexin neuronal activity. Overnight fasting promotes the formation of more excitatory synapses and synaptic currents to orexin neurons (Horvath and Gao 2005). Additionally, the fasting-induced increase of miniature excitatory presynaptic currents (mEPSCs) is blocked by leptin signals (Horvath and Gao 2005). Orexin increases local glutamate signaling by facilitation of glutamate release from presynaptic terminals (Li et al. 2002). On the other hand, orexins activate local GABA input to orexin neurons (Matsuki and Sakurai 2008, unpublished). These local GABAergic and glutamatergic interneuron networks might play critical roles in the regulation of orexin neurons.

3.3
Efferents of Orexin Neurons

As discussed earlier, the densest staining of orexin-immunoreactive nerve endings from orexin neurons in the LHA/PFA were found in the PVT, Arc, VMH, VTA, LC, and TMN. There are many reports on the effects of orexins on these neurons (summarized in Table 2). These regions are important for maintenance of arousal, feeding, and reward systems.

4
Roles of Orexins in Regulation of Sleep/Wake States

4.1
Interaction with Sleep and Arousal Centers

The POA, 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 inhibitory projections to wake-active neurons producing wake-promoting neurotransmitters, including histamine, norepinephrine, 5-HT, and acetylcholine (Lu et al. 2002; Sherin et al. 1998). 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

Table 2 Summary of orexin output effects

Region	Cell type	Effect	Function	Refs.
Prefrontal cortex	Layer V pyramidal neurons (Glu)	Glutamate release	Attention	(Lambe and Aghajanian 2003)
Prefrontal cortex	Layer 6b (Glu)	Activation	Arousal system	(Bayer et al. 2004)
Hippocampus/ Septum (LS)	Acetylcholine GABA Schaffer collateral CA3-CA1	Activation LTP & theta rhythm plasticity	Arousal system REM sleep	(Selbach et al. 2004; Wu et al. 2004, 2002)
Amygdala	Central medial nucleus	Activation	Fear/emotion	(Bisetti et al. 2006)
BST		Activation	Fear/emotion, Autonomic nervous system	(Mullett et al. 2000)
Thalamus	Rhomboid nucleus, centromedial nucleus	Activation	Arousal system	(Bayer et al. 2002; Huang et al. 2006)
Basal forebrain	Acetylcholine	Activation	Arousal system	(Eggermann et al. 2001)
Arc	POMC	Inhibition	Feeding/energy regulation	(Ma et al. 2007)
Arc	NPY	Activation	Feeding/energy regulation	(Li et al. 2002; van den Top et al. 2004)
Arc	GABA	Activation	Feeding/energy regulation	(Burdakov et al. 2003)
VMH	(Glucose-responsive neurons)	Activation	Autonomic responses	(Monda et al. 2005; Muroya et al. 2004)
PVN	CRF	Activation	Stress/ autonomic response	(Kuru et al. 2000; Sakamoto et al. 2004)
TMN	Histamine	Activation (direct and indirect)	NREM sleep, Arousal system	(Bayer et al. 2001; Yamanaka et al. 2002)
LHA	Orexin	Activation (indirect)	Feeding, Arousal, Reward systems	(Li et al. 2002; Li and van den Pol 2006)
LHA	MCH	Activation (direct and indirect)	Feeding/energy regulation	(Li and van den Pol 2006; van den Pol et al. 2004)
LHA	Glu	Activation	Feeding, Arousal, Reward	(Li et al. 2002; van den Pol et al. 1998)
LHA	GABA	Activation	Feeding, Arousal, Reward	(van den Pol et al. 1998; Matsuki 2008, unpublished)

Table 2 (continued)

Region	Cell type	Effect	Function	Refs.
SNr	GABA	Inhibition	Locomotion	(Thorpe and Kotz 2005)
VTA/NAc	Dopamine GABA	Activation	Locomotion, Reward system	(Borgland et al. 2006; Boutrel et al. 2005; Harris et al. 2005; Korotkova et al. 2003; Nakamura et al. 2000; Narita et al. 2006)
LC	Noradrenaline	Activation	Arousal system	(Hagan et al. 1999; Horvath et al. 1999; van den Pol et al. 2002)
DR	Serotonin	Activation	Arousal system	(Brown et al. 2001, 2002)
LDT/PPT	Acetylcholine	Activation (direct and indirect)	Locomotors, REM sleep	(Burlet et al. 2002; Takakusaki et al. 2005; Xi et al. 2001)
Spinal cord	Lamina 1 & X	Activation	Pain and thermal sensation	(van den Pol et al. 2002; Yamamoto et al. 2002)
Spinal cord	Sympathtic preganglionic neurons	Activation	Heart rate, blood pressure	(Antunes et al. 2001; van den Top et al. 2003)

Abbreviations:
BF; basal forebrain, BST; bed nucleus stria terminalis, CM; central medial nucleus, PVT; paraventricular nucleus of the thalamus, Arc; arcuate nucleus, VMH; ventomedial nucleus, DMH; dorsomedial nucleus, LHA; lateral hypothalamic area, PFA; perifornical area, PVN; paraventricular nucleus, TMN; tuberomammillary nucleus of the hypothalamus, LC; locus coeruleus, VTA; vetral tegmental area, NAc; nucleus accumbens, LS; lateral septum, DR; dorsal raphe, LDT; laterodorsal tegmental nucleus, PPT; pedunculopontine nucleus, DRG; dorsal root ganglion, GABA; γ-aminobutyric acid, POMC; proopiomelanocortin, NPY; neuropeptide Y, MCH; melanin concentrating hormone, CRF; corticotropin-releasing factor, Glu; glutamate

acetylcholine (Gallopin et al. 2000). GABAergic neurons in the POA densely innervate orexin neurons (Sakurai et al. 2005; Yoshida et al. 2006). Orexin neurons are strongly inhibited by both a $GABA_A$ receptor agonist, muscimol, and a $GABA_B$ receptor agonist, baclofen (Xie et al. 2006; Yamanaka et al. 2003a). These observations suggest that VLPO neurons send GABAergic inhibitory projections to orexin neurons (Fig. 2). This pathway might be important in turning off orexin neurons during sleep. Also, orexin neu-

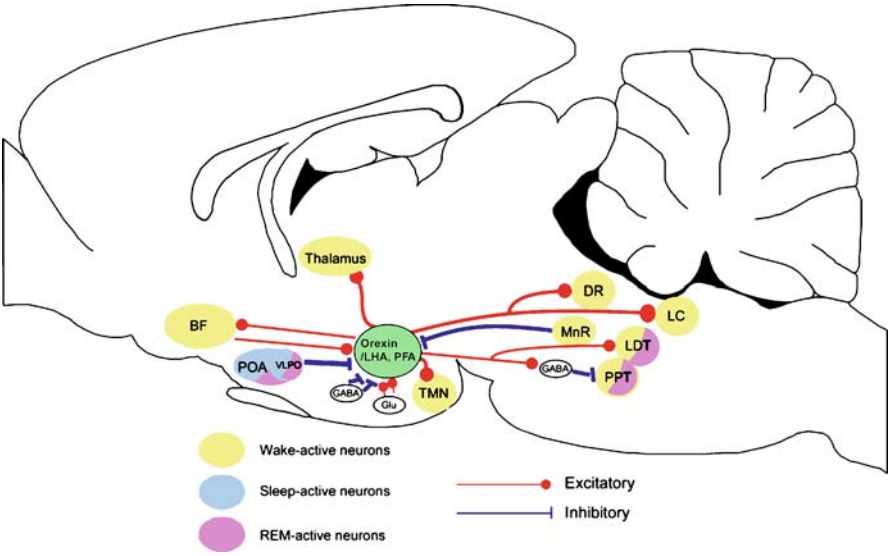

Fig. 2 Input and output of orexin neurons in sleep/wake regulation. Orexin neurons regulate cortical arousal and promote wakefulness through the aminergic nuclei and other arousal-related nuclei. On the other hand, in the sleep center, VLPO-GABAergic neurons innervate orexin neurons. BF-cholinergic neurons also project to orexin neurons.

rons are innervated by BF-cholinergic neurons (Sakurai et al. 2005) (Fig. 2). A cholinergic agonist, carbachol, 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 inhibitory feedback mechanisms might also be important for the stability of orexin neuronal activity (Fig. 2).

4.2 Interaction with Waking Centers

How do the orexins regulate sleep/wake states, and why does lack of orexin signaling result in narcolepsy-cataplexy?

The activity of monoaminergic neurons in the hypothalamus and brain stem, including neurons in the TMN, LC, and DR, is known to be synchronized and strongly associated with sleep/wake states. These groups play a significant role in arousal maintenance (Saper et al. 2005). They fire tonically during the awake state, less during NREM sleep, and not at all during REM sleep (Vanni-Mercier et al. 1984). Since orexin neurons innervate and

their receptors are expressed in these regions, it is suggested that orexin neurons might be activated during the wakeful period, and that they exert an excitatory influence on these wake-active neurons to sustain their activity (Fig. 2). Consistent with this hypothesis, orexin neurons discharge during active waking, and virtually cease firing during sleep, including the NREM and REM periods, in vivo (Lee et al. 2005). In vitro, 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 (Fig. 2). Orexins also have a strong direct excitatory effect on cholinergic neurons of the BF (Eggermann et al. 2001), which also play an important role in regulating arousal (Alam et al. 1999). In addition, orexin neurons 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). In addition, orexin A induces long-lasting excitation of cholinergic neurons in the LDT (Takahashi et al. 2002). On the contrary, 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 ability of these cells to control wakefulness and REM sleep (Fig. 2).

Some reports have shown that the effect of orexins on awake time is largely mediated by activation of the TMN histaminergic system through OX_2R. In rats, intracerebroventricular (icv) 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). Furthermore, 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, while OX_1R is strongly expressed in the LC. Therefore, the TMN-histaminergic pathway seems to be an important effector site of orexin for sleep/wake regulation.

Several findings indicate that signaling through OX_1R is also important for the proper regulation of vigilance states. However, OX_2R knockout mice exhibit characteristics of narcolepsy (Willie et al. 2003), and OX_1R knockout mice do not have any overt behavioral abnormalities and exhibit only very mild fragmentation of the sleep/wake cycle (Willie et al. 2001). Interestingly, the phenotype of OX_2R knockout mice is less severe than that found in *prepro-orexin* knockout mice and double receptor knockout (OX_1R- and OX_2R-null) mice, which appear to have the same phenotype as *prepro-orexin* knockout mice. Importantly, both OX_2R knockout and *prepro-orexin* knockout mice are similarly affected by behaviorally abnormal attacks of NREM sleep ("sleep attacks") and show a similar degree of disrupted wakefulness (Willie et al. 2003). In contrast, 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, while lack of OX_2R cannot be 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-dependent and OX_2R-dependent pathways.

5
Roles of Orexins in Feeding Behavior

The finding of decreased caloric intake combined with an increased body mass index in narcolepsy patients suggests that they have a feeding abnormality with reduced energy expenditure, or a low metabolic rate (Lammers et al. 1996; Schuld et al. 2000). Consistently, orexin neuron-ablated mice also show hypophagia and late-onset obesity, although the degree of the obese phenotype critically depends on their genetic background (Hara et al. 2001, 2005). The altered energy homeostasis in narcolepsy patients and mouse narcolepsy models suggests a role of orexin in the regulation of energy homeostasis (Honda et al. 1986; Schuld et al. 2000).

5.1
Interaction with Hypothalamic Neurons

The LHA has long been recognized as a "feeding center", because electrical stimulation of this area causes hyperphagia and obesity, whereas lesions yield the opposite results (Anand and Brobeck 1951). Other hypothalamic regions, including the Arc, VMH, DMH, and PVN, are also involved in energy homeostasis (Elmquist et al. 1999). Leptin-responsive or leptin-sensitive neurons exist in the Arc, VMH, DMH, and LHA regions, in which the functional leptin receptor (*ObRb*) and *STAT3* are expressed (Elmquist et al. 2005; Hakansson et al. 1999; Hakansson and Meiste 1998).These regions reciprocally project to LHA neurons.

Intracerebroventricular injection of orexin increases feeding, and *orexin* mRNA is increased upon fasting (Sakurai et al. 1998). POMC neurons and NPY neurons in the Arc have been shown to innervate to orexin neurons (Elias et al. 1999). Injection of agouti related protein (Agrp), which is an endogenous antagonist for MC3/4Rs, resulted in the activation of orexin neu-

rons, but not MCH 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.

5.2 Regulation of Orexin Neurons by Humoral Factors

Orexin neurons respond to humoral and neuroendocrine factors as 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 the glucose concentration induces depolarization and increases the frequency of action potentials in these same neurons (Burdakov et al. 2005; Yamanaka et al. 2003a). Orexin neuron-ablated *ataxin-3* Tg mice cannot respond to fasting by increased locomotor activity and waking time. *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). Importantly, this mechanism is sufficiently sensitive to encode variations in glucose levels, reflecting those occurring physiologically between normal meals (Burdakov et al. 2005). A recent study demonstrated that inhibition of orexin neurons by glucose is mediated by tandem-pore K^+ (K_{2P}) channels (Burdakov et al. 2006).

When applied in superfused solution, the orexigenic peptide, ghrelin, activated 60% of dispersed orexin neurons, 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 sensors for the nutritional status of the body (Sakurai et al. 1998). Consistently, *prepro-orexin* expression of normal and *ob/ob* mice is negatively correlated with changes in blood glucose, leptin, and food intake (Yamanaka et al. 2003a).

5.3 Mechanism of Orexin-mediated Feeding

Supporting the physiological relevance of orexin in the control of feeding, icv administration of anti-orexin antibody or an OX_1R-selective antagonist reduced food intake (Haynes et al. 2000; Yamada et al. 2000). *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. Orexin neurons densely project to the Arc

(Date et al. 1999; Peyron et al. 1998; Yamanaka et al. 2000), and *Fos* expression was induced in NPY neurons of the arcuate nucleus by icv 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 (Li and van den Pol 2006; van den Top et al. 2004), but inhibited proopiomelanocortin (POMC) neurons (Ma et al. 2007; Muroya et al. 2004). Furthermore, the orexin A-induced increase in food intake was partly inhibited by prior administration of BIBO3340, 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 it 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 wake states might also be important in supporting feeding behavior, 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 (Challet et al. 1997; Itoh et al. 1990). Consistently, transgenic mice with ablated orexin neurons fail to respond to fasting with increased wakefulness and activity (Yamanaka et al. 2003a). This suggests that orexin neurons have a critical role in maintenance of arousal during the period in which the energy balance is negative. These mechanisms may modulate the activity of orexin neurons according to energy stores in order to maintain wakefulness.

5.4
Orexin as Effector of Food Entrainable Oscillator (FEO)

The activity of orexin neurons also contributes to the promotion and maintenance of food anticipatory activity (FAA) (Akiyama et al. 2004; Mieda et al. 2004). Daily restricted feeding produces an anticipatory locomotor activity rhythm and entrains the peripheral molecular oscillator, which is independent of the central clock located in the suprachiasmatic nucleus (SCN). 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. 2004). Formation of the FAA is severely impaired in orexin neuron-ablated, *orexin/ataxin-3*, transgenic mice (Akiyama et al. 2004; Mieda et al. 2004). Expression of *mNpas2* mRNA, a transcription factor thought to be involved in regulation of the 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. The DMH was shown to have marked oscillation of *mPer* expression only under restricted feeding (Mieda et al. 2006). Consistently, Gooley et al. also demonstrated that lesions in the DMH in rats blocked food entrainment of wakefulness, locomotor activity, and core body temperature (Gooley et al. 2006). However, Landry et al. reported that complete ablation of the dorsomedial hypothalamic nucleus does not affect food anticipatory activity rhythms in rats (Landry et al. 2006). The nature of the discrepancy between these reports is unclear. 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.

The circuit for neurons in the hypothalamus and other regions in energy homeostasis is summarized in Fig. 3.

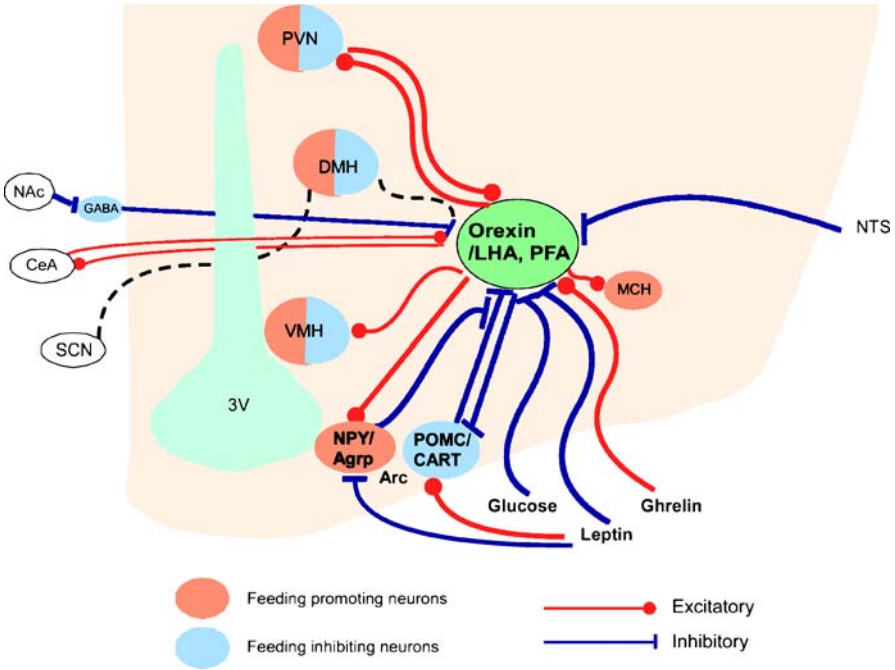

Fig. 3 Input and output of orexin neurons in energy homeostasis. Orexin neurons regulate the hypothalamic nuclei involved in feeding behavior. Peripheral metabolic signals, leptin, ghrelin, and glucose, and circadian rhythms influence orexin neuronal activity to coordinate arousal and energy homeostasis. Input from the limbic system may also influence feeding behavior.

6
Roles of Orexins in Reward Systems

6.1
Input from Reward Systems

Orexin neurons receive projections from the VTA, NAc, and LS, regions involved in reward systems (Yoshida et al. 2006). Dopamine inhibits orexin neurons (Yamanaka et al. 2003b). In the LHA/PFA, dopamine has an inhibitory influence on food intake and the reward pathways (Yang et al. 1997). On the contrary, VTA neurons receive a projection from a part of the LHA/PFA including orexin neurons (Fadel and Deutch 2002; Marcus et al. 2001). These reciprocal interactions might constitute regulatory mechanisms of reward systems (Fig. 4).

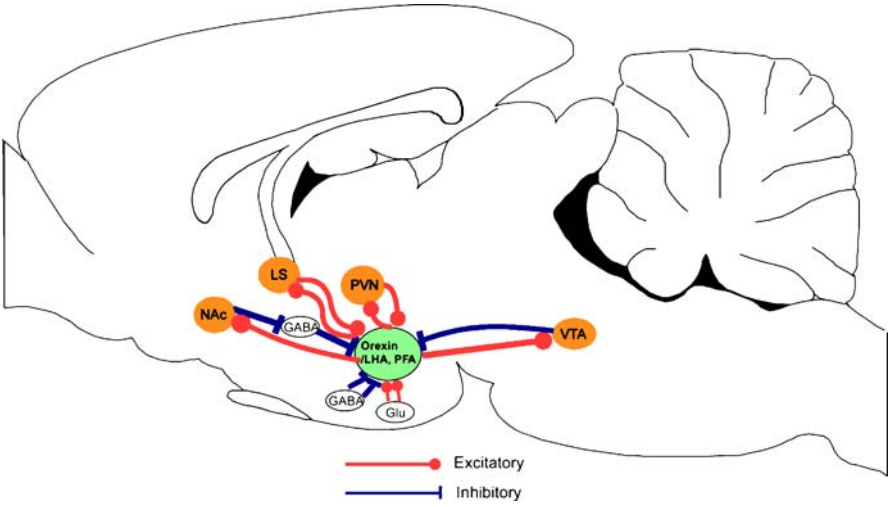

Fig. 4 Input and output of orexin neurons in reward systems. Stimulation of dopaminergic, limbic, and cholinergic centers by orexins can modulate reward systems, motor activity, and emotional arousal.

6.2
Output to Reward Systems

Orexin directly activates VTA dopaminergic neurons (Korotkova et al. 2003; Nakamura et al. 2000), and orexin neurons abundantly innervate the VTA (Fadel and Deutch 2002; Peyron et al. 1998). The dopamine receptor antagonist, haloperidol, blocks hyperlocomotion and stereotypy induced by icv orexin (Nakamura et al. 2000). Intracerebroventricular or local VTA infu-

sion of orexin is shown to reinstate drug-seeking 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 *N*-methyl-D-aspartate receptor (NMDAR)-mediated neurotransmission via PLC/PKC-dependent recruitment of NMDARs in VTA dopamine neuron synapses in slice preparations (Borgland et al. 2006). 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 the VTA in neural plasticity, and they imply 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 (Fig. 4).

These mechanisms are similar to CRF-induced sensitization of the activation of dopaminergic neurons by glutamate. 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). 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) (Fig. 4).

7
Orexins in Emotion, Stress Responses and Autonomic Nervous System

7.1
Input from Mesolimbic System

Input from the limbic system to orexin neurons might be involved in the regulation of feeding and/or motivating behavior, because some of the affective content of the perception of food is thought to be processed in the amygdala and limbic system (Morton et al. 2006). In narcoleptic dogs, cataplexy is triggered by recognition of highly palatable food and excited play, but neither noxious stimuli nor unfamiliar environments (John et al. 2004; Siegel and Boehmer 2006). In rodents, cataplexy is most frequently linked to exploration, burrowing, and investigation of the environment (Chemelli et al. 1999; Hara et al. 2001). In humans, cataplexy is most frequently elicited by laughter, but not sadness or pain (Guilleminault and Gelb 1995; Guilleminault et al. 1998; Siegel and Boehmer 2006). A recent report showed that orexin neurons have maximal activity during exploratory behavior, compared to grooming and eating behavior in unanesthetized and freely moving rats (Mileykovskiy

et al. 2005). They also reported that firing of orexin neurons decreased during food aversion, a state characterized by high levels of attention and motor activity.

On the other hand, the PFA, a region in which orexin neurons exist, has been known as the center for defense responses, or "fight or flight" response (Geerling et al. 2003; Jansen et al. 1995). The defense response is characterized by a coordinated rise in arterial blood pressure, heart rate, and respiratory frequency. Pioneering work showed that electrical stimulation of the posterior hypothalamus in cats elicited behavioral rage, along with specific autonomic responses. These defense responses are induced by activation of the BST and amygdala, or other regions involved in emotional responses. Furthermore, the BST and amygdala nearly project to orexin neurons (Sakurai et al. 2005). These pathways might be important for regulation of the activity of orexin neurons upon emotional stimuli to evoke emotional arousal or fear-related responses. The importance of these inputs is readily apparent in the defense response. In an awake and freely moving condition, *prepro-orexin* knockout mice showed diminished cardiovascular and behavioral responses to emotional stress in the resident-intruder paradigm (Kayaba et al. 2003). These findings suggest that emotional states modulate the activity of orexin neurons (Fig. 5).

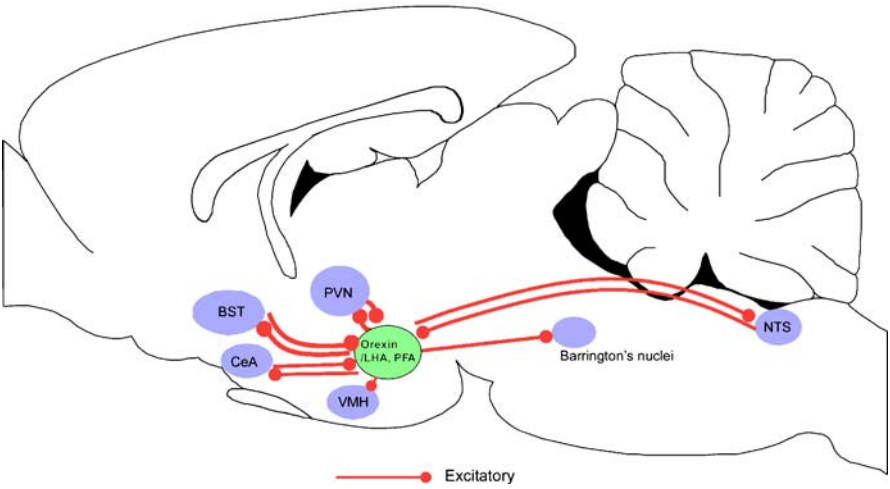

Fig. 5 Input and output of orexin neurons in stress response and autonomic regulation. Orexin neurons regulate the PVN and BST nucleus, which are involved in stress responses and the autonomic nervous system. Orexin A administered into the LH significantly increase cFos-immunoreactivity in the lateral septal area, CeA, shell of NAc, and BST. The CeA, BST, and NAc also strongly project to orexin neurons. Input from the limbic system may be important to regulate the activity of orexin neurons upon emotional stimuli to evoke emotional responses.

7.2 Stress Response

Orexin neurons are activated by cold exposure or immobilized stress (Sakamoto et al. 2004). Orexin also enhances the pituitary release of adrenocorticotropic hormone (ACTH). This response is mediated by CRF and AVP in the PVN (Engelmann et al. 2004). The CRF peptidergic system in the PVN provides an anatomical input to orexin neurons expressing *CRF receptors CRFR1* and *CRFR2* (Winsky-Sommerer et al. 2004). CRF directly activates a population of orexin neurons by acting on CRFR1. The number of orexin neurons expressing *c-Fos* was markedly reduced in *CRFR1* knockout mice in both foot-shock challenge and restraint stress (Winsky-Sommerer et al. 2004). These results suggest that CRFR1 signaling is important for the activation of orexin neurons by physical stress.

Orexin neurons innervate the PVN, in which OX_2R is abundantly expressed (Lu et al. 2002; Marcus et al. 2001). Orexin enhances *c-Fos* expression, *CRF*, and *AVP* mRNA expression in the PVN (Al-Barazanji et al. 2001; Brunton and Russell 2003; Kuru et al. 2000). Orexin A strongly activates *CRF*-expressing neurons in the PVN and the central nucleus of the amygdala (CeA) (Sakamoto et al. 2004). These interactions between the orexin system and CRF neurons might regulate arousal in stressful environments.

7.3 Orexin in Autonomic Nervous System

Intracerebroventricular orexin injection increases blood pressure and heart rate, and these effects are abolished by prior administrations of an α or β blocker (Shirasaka et al. 1999). Moreover, orexin-deficient mice show 10–15 mmHg lower blood pressure than wild-type littermates (Kayaba et al. 2003; Zhang et al. 2006). These results suggest that orexins physiologically stimulate sympathetic outflow. Therefore, *orexin* deficiency might decrease sympathetic tone, which might result in decreased energy expenditure. As suggested from the effects on sympathetic tone, although orexins stimulate feeding behavior, they do not slow the metabolic rate, as might be expected in a system geared for weight gain. Instead, orexins both increase food intake and increase the metabolic rate (Lubkin and Stricker-Krongrad 1998). Because animals must be aware and active when they seek and eat food, this function might be important for feeding behavior. This suggests that the function of orexins might support reward-seeking behavior with an increase in vigilance, awareness, and sympathetic outflow.

Barrington's nucleus in the midbrain also has dense innervations of orexin neurons, and these neurons express OX_2R (Marcus et al. 2001; Nambu et al. 1999; Peyron et al. 1998). These neurons are the center for the autonomic nervous system involved in urinary function (Mitsuyoshi 2005). It remains

unknown how these physiological functions are influenced by the orexin system.

These networks for emotion and autonomic responses are summarized in Fig. 5.

8
Clinical Implications

8.1
Orexin Deficiency and Narcolepsy-Cataplexy

Narcolepsy-cataplexy is a disabling sleep disorder affecting 0.02% of adults worldwide (Dauvilliers et al. 2007). It is characterized by severe, irresistible daytime sleepiness and sudden loss of muscle tone (cataplexy), and can be associated with sleep-onset paralysis and hypnagogic hallucinations, frequent movement and awakening during sleep, and weight gain. Sleep monitoring during night and day shows rapid sleep onset and abnormal, shortened REM sleep latency. The onset of narcolepsy-cataplexy usually occurs during teenage and young adulthood and persists throughout life. Since narcolepsy-cataplexy has no cure, its management relies on symptomatic treatment. This includes psycho-stimulants, including modafinil, methylphenidate, amphetamine, and caffeine for excessive daytime sleepiness and sleep attacks, and tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRI) for cataplexy and other REM-associated symptoms. Hypnotics are also used for disturbed night-time sleep. Pathophysiological studies have shown that the disease is caused by early loss of orexin-producing neurons in the hypothalamus. The cause of neuronal loss could be autoimmune, since most patients have the HLA DQB1*0602 allele, which predisposes to the disorder. Because narcolepsy-cataplexy is a disorder of organization of the sleep/wake cycle, resulting from an absence of orexin, replacement therapy using orexin receptor agonists may provide an effective treatment for narcolepsy.

8.2
Orexin Agonists

Chronic overproduction of orexin peptides from an ectopically expressed transgene prevented the development of narcolepsy syndrome in orexin neuron-ablated *orexin/ataxin-3* mice (Mieda et al. 2004) (schematic model in Fig. 6A). Acute administration of orexin A also maintained wakefulness, suppressed sleep, and inhibited cataplectic attacks in narcoleptic mice (Mieda et al. 2004). However, chronic overexpression of orexin in an unregulated fashion results in fragmentation of non-REM sleep. Therefore, if orexin agon-

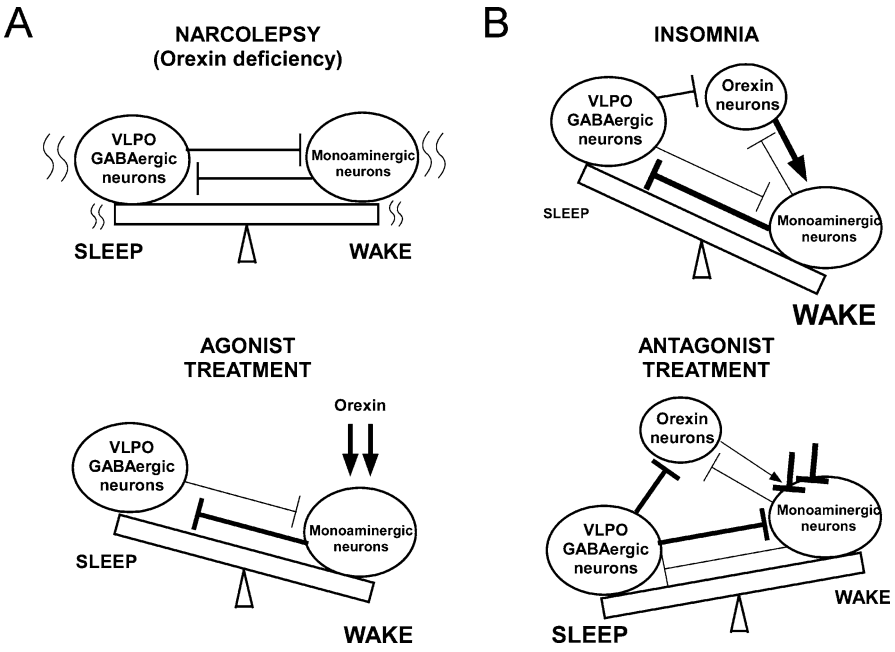

Fig. 6 Mechanism by which orexin system stabilizes sleep and wakefulness. Schematic summary of interactions between orexin neurons and monoaminergic waking center and VLPO sleep center under normal, agonist, and antagonist treatment states. Arrows show excitation and bars show inhibition. **A** Agonist treatment. In narcolepsy, monoaminergic neurons and VLPO neurons set up a mutually inhibitory circuit, which can cause unwanted abrupt transitions between each state. Administration of orexin agonists activates and has an increased excitatory influence on monaminergic cells to maintain their activity. These monoaminergic cells send excitatory projections to the forebrain cortex, and inhibitory projections to the VLPO sleep center. These mechanisms maintain awake states and a balanced sleep/awake state. **B** Antagonist treatment. Orexin neurons send excitatory influences to monaminergic neurons, and monoaminergic neurons send inhibitory feedback projections to orexin neurons. This system might maintain the activity of monoaminergic neurons. Then, administration of an orexin antagonist results in inhibition of monoaminergic neurons. This effect maintains sleep states.

ists were available, a short half-life (<12 hr) might be desirable. Attempts at using orexin-based treatment after peripheral administration have been disappointing, since the peptides do not cross the blood—brain barrier (Mignot and Nishino 2005). Unfortunately, currently there are no reported non-peptide orexin receptor agonists. Orexin-based therapy, such as direct use of orexin receptor agonists (Mieda et al. 2004) and orexin neuron transplantation, is currently under investigation in animal models.

Orexin-immunoreactive fibers innervate the spinal cord, especially dorsal root ganglion (DRG) 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). Thus, orexin receptor agonists could possibly also be useful for pain control (Kajiyama et al. 2005; Mobarakeh et al. 2005).

8.3 Orexin Antagonists

There is much evidence suggesting that the orexin system is involved in the regulation of feeding, wakefulness, and reward. Several pharmaceutical companies have shown interest in the potential therapeutic application of non-peptide, low molecular weight orexin receptor antagonists (review; (Bingham et al. 2006). Orexin receptor antagonists might be effective for inducing sleep and treating insomnia patients (schematic model in Fig. 6B). Recently, it was reported that a new dual orexin receptor antagonist (ACT-078573) selectively blocks both OX_1R and OX_2R at nanomolar concentrations (Brisbare-Roch et al. 2007). The drug is orally active and rapidly enters the brain. Although the drug thoroughly blocks orexin signaling and produces sleepiness, it does not appear to produce cataplexy. This drug 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. Accordingly, this drug 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 progressed to phase II clinical trials for insomnia (Actelion Ltd. Press Release 2006).

Another possible use of orexin receptor antagonists includes withdrawal from drug addiction. A selective OX_1R antagonist, SB334867A, significantly suppressed morphine-induced place preference and hyperlocomotion (Harris et al. 2005; Narita et al. 2006), and it blocked the reinstatement of previously extinguished cocaine-seeking behavior and locomotor sensitization to cocaine (Borgland et al. 2006; Boutrel et al. 2005). These studies indicate that the orexin system is directly implicated in reward systems.

Several research groups have demonstrated that an effect of the orexin-induced increase of food consumption is blocked by an OX_1R antagonist (SB-334867) (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-DG- and orexin A-induced gastric acid secretion in rats. 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.

9 Conclusion

Symptoms of narcolepsy unequivocally show that orexins and orexin receptors play highly important roles in regulating sleep/wake states and the maintenance of arousal by regulating monoaminergic/cholinergic nuclei in the brain. At the same time, this system is also related to the limbic system, reward systems, and hypothalamic mechanisms that regulate energy homeostasis.

The link between the limbic system and orexin neurons might be important for emotional arousal and sympathetic responses during emotional events. On the other hand, the responsiveness of orexin neurons to peripheral metabolic cues, leptin, and glucose suggests that these cells might act as a sensor for the metabolic status of animals. These findings indicate that orexin neurons provide a crucial link between energy balance, emotion, reward systems, and arousal.

References

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

Al-Barazanji KA, Wilson S, Baker J, Jessop DS, Harbuz MS (2001) Central orexin-A activates hypothalamic-pituitary-adrenal axis and stimulates hypothalamic corticotropin releasing factor and arginine vasopressin neurones in conscious rats. J Neuroendocrinol 13:421–424

Alam MN, Szymusiak R, Gong H, King J, 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, Sutcliffe JG (2002) Hypocretin is an early member of the incretin gene family. Neurosci Lett 324:169–172

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

Antunes VR, Brailoiu GC, Kwok EH, Scruggs P, Dun NJ (2001) Orexins/hypocretins excite rat sympathetic preganglionic neurons in vivo and in vitro. Am J Physiol Regul Integr Comp Physiol 281:R1801–1807

Baldo BA, Gual-Bonilla L, Sijapati K, Daniel RA, Landry CF, 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

Bayer L, Eggermann E, Saint-Mleux B, Machard D, Jones BE, Muhlethaler M, Serafin M (2002) Selective action of orexin (hypocretin) on nonspecific thalamocortical projection neurons. J Neurosci 22:7835–7839

Bayer L, Eggermann E, Serafin M, Saint-Mleux B, Machard D, Jones B, Muhlethaler M (2001) Orexins (hypocretins) directly excite tuberomammillary neurons. Eur J Neurosci 14:1571–1575

Bayer L, Serafin M, Eggermann E, Saint-Mleux B, Machard D, Jones BE, Muhlethaler M (2004) Exclusive postsynaptic action of hypocretin-orexin on sublayer 6b cortical neurons. J Neurosci 24:6760–6764

Bernardis LL, Bellinger LL (1996) The lateral hypothalamic area revisited: ingestive behavior. Neurosci Biobehav Rev 20:189–287

Bingham MJ, Cai J, 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

Bisetti A, Cvetkovic V, Serafin M, Bayer L, Machard D, Jones BE, Muhlethaler M (2006) Excitatory action of hypocretin/orexin on neurons of the central medial amygdala. Neuroscience 142:999–1004

Borgland SL, Taha SA, Sarti F, Fields HL, 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, de Lecea L (2005) Role for hypocretin in mediating stress-induced reinstatement of cocaine-seeking behavior. Proc Natl Acad Sci USA 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

Brown RE, Sergeeva O, Eriksson KS, Haas HL (2001) Orexin A excites serotonergic neurons in the dorsal raphe nucleus of the rat. Neuropharmacology 40:457–459

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

Brunton PJ, Russell JA (2003) Hypothalamic-pituitary-adrenal responses to centrally administered orexin-A are suppressed in pregnant rats. J Neuroendocrinol 15:633–637

Burdakov D, Gerasimenko O, 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, Verkhratsky A (2006) Tandem-pore K+ channels mediate inhibition of orexin neurons by glucose. Neuron 50:711–722

Burdakov D, Liss B, Ashcroft FM (2003) Orexin excites GABAergic neurons of the arcuate nucleus by activating the sodium–calcium exchanger. J Neurosci 23:4951–4957

Burlet S, Tyler CJ, Leonard CS (2002) Direct and indirect excitation of laterodorsal tegmental neurons by hypocretin/orexin peptides: Implication for wakefulness and Narcolepsy. J Neurosci 22:2862–2872

Challet E, Pevet P, 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 TE, 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

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

Dauvilliers Y, Arnulf I, Mignot E (2007) Narcolepsy with cataplexy. Lancet 369:499–511
de Lecea L, Jones BE, Boutrel B, Borgland SL, Nishino S, Bubser M, 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 et al. (1998) The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci USA 95:322–327
Eggermann E, Serafin M, Bayer L, Machard D, Saint-Mleux B, Jones BE, Muhlethaler M (2001) Orexins/hypocretins excite basal forebrain cholinergic neurones. Neuroscience 108:177–181
Elias CF, Aschkenasi C, Lee C, Kelly J, Ahima RS, Bjorbaek C, Flier JS, Saper CB, Elmquist JK (1999) Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. Neuron 23:775–786
Elmquist JK, Coppari R, Balthasar N, Ichinose M, Lowell BB (2005) Identifying hypothalamic pathways controlling food intake, body weight, and glucose homeostasis. J Comp Neurol 493:63–71
Elmquist JK, Elias CF, Saper CB (1999) From lesions to leptin: hypothalamic control of food intake and body weight. Neuron 22:221–232
Engelmann M, Landgraf R, Wotjak CT (2004) The hypothalamic-neurohypophysial system regulates the hypothalamic-pituitary-adrenal axis under stress: an old concept revisited. Front Neuroendocrinol 25:132–149
Fadel J, Deutch AY (2002) Anatomical substrates of orexin-dopamine interactions: lateral hypothalamic projections to the ventral tegmental area. Neuroscience 111:379–387
Gallopin T, Fort P, Eggermann E, Cauli B, Luppi PH, Rossier J, Audinat E, Muhlethaler M, Serafin M (2000) Identification of sleep-promoting neurons in vitro. Nature 404:992–995
Geerling JC, Mettenleiter TC, Loewy AD (2003) Orexin neurons project to diverse sympathetic outflow systems. Neuroscience 122:541–550
Gooley JJ, Schomer A, Saper CB (2006) Nat Neurosci 9:398–407
Griffond B, Risold PY, Jacquemard C, Colard C, Fellmann D (1999) Insulin-induced hypoglycemia increases preprohypocretin (orexin) mRNA in the rat lateral hypothalamic area. Neurosci Lett 262:77–80
Guilleminault C, Gelb M (1995) Clinical aspects and features of cataplexy. Adv Neurol 67:65–77
Guilleminault C, Heinzer R, Mignot E, Black J (1998) Investigations into the neurologic basis of narcolepsy. Neurology 50:S8–15
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 USA 96:10911–10916
Hakansson M, de Lecea L, Sutcliffe JG, Yanagisawa M, Meister B (1999) Leptin receptor- and STAT3-immunoreactivities in hypocretin/orexin neurones of the lateral hypothalamus. J Neuroendocrinol 11:653–663
Hakansson ML, Meiste RB (1998) Transcription factor STAT3 in leptin target neurons of the rat hypothalamus. Neuroendocrinology 68:420–427
Hara J, Beuckmann CT, Nambu T, Willie JT, Chemelli RM, Sinton CM, Sugiyama F, Yagami K, Goto K, Yanagisawa M, Sakurai T (2001) Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. Neuron 30:345–354
Hara J, Yanagisawa M, Sakurai T (2005) Difference in obesity phenotype between orexin-knockout mice and orexin neuron-deficient mice with same genetic background and environmental conditions. Neurosci Lett 380:239–242

Harris GC, Wimmer M, 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, 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, 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, Leslie RA (2001) Gene expression and protein distribution of the orexin-1 receptor in the rat brain and spinal cord. Neuroscience 103:777–797

Honda Y, Doi Y, Ninomiya R, Ninomiya C (1986) Increased frequency of non-insulin-dependent diabetes mellitus among narcoleptic patients. Sleep 9:254–259

Horvath TL, Gao XB (2005) Input organization and plasticity of hypocretin neurons: possible clues to obesity's association with insomnia. Cell Metab 1:279–286

Horvath TL, Peyron C, Diano S, Ivanov A, Aston-Jones G, Kilduff TS, van Den Pol AN (1999) Hypocretin (orexin) activation and synaptic innervation of the locus coeruleus noradrenergic system. J Comp Neurol 415:145–159

Huang H, Ghosh P, van den Pol AN (2006) Prefrontal cortex-projecting glutamatergic thalamic paraventricular nucleus-excited by hypocretin: a feedforward circuit that may enhance cognitive arousal. J Neurophysiol 95:1656–1668

Huang ZL, Qu WM, Li WD, Mochizuki T, Eguchi N, Watanabe T, Urade Y, Hayaishi O (2001) Arousal effect of orexin A depends on activation of the histaminergic system. Proc Natl Acad Sci USA 98:9965–9970

Itoh T, Murai S, Nagahama H, Miyate H, Abe E, Fujiwara H, 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

Jansen AS, Nguyen XV, Karpitskiy V, Mettenleiter TC, Loewy AD (1995) Central command neurons of the sympathetic nervous system: basis of the fight-or-flight response. Science 270:644–646

John J, Wu MF, Boehmer LN, Siegel JM (2004) Cataplexy-active neurons in the hypothalamus: implications for the role of histamine in sleep and waking behavior. Neuron 42:619–634

Kajiyama S, Kawamoto M, Shiraishi S, Gaus S, Matsunaga A, Suyama H, 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

Kayaba Y, Nakamura A, Kasuya Y, Ohuchi T, Yanagisawa M, Komuro I, Fukuda Y, Kuwaki T (2003) Attenuated defense response and low basal blood pressure in orexin knockout mice. Am J Physiol Regul Integr Comp Physiol 285:R581–593

Korotkova TM, Sergeeva OA, Eriksson KS, Haas HL, Brown RE (2003) Excitation of ventral tegmental area dopaminergic and nondopaminergic neurons by orexins/hypocretins. J Neurosci 23:7–11

Kuru M, Ueta Y, Serino R, Nakazato M, Yamamoto Y, Shibuya I, Yamashita H (2000) Centrally administered orexin/hypocretin activates HPA axis in rats. Neuroreport 11:1977–1980

Lambe EK, Aghajanian GK (2003) Hypocretin (orexin) induces calcium transients in single spines postsynaptic to identified thalamocortical boutons in prefrontal slice. Neuron 40:139–150

Lammers GJ, Pijl H, Iestra J, Langius JA, Buunk G, Meinders AE (1996) Spontaneous food choice in narcolepsy. Sleep 19:75–76

Landry GJ, Simon MM, Webb IC, 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:R1527–1534

Lee MG, Hassani OK, 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, 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, van den Pol AN (2006) Differential target-dependent actions of coexpressed inhibitory dynorphin and excitatory hypocretin/orexin neuropeptides. J Neurosci 26:13037–13047

Liu RJ, van den Pol AN, 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

Lu J, Bjorkum AA, Xu M, Gaus SE, Shiromani PJ, Saper CB (2002) Selective activation of the extended ventrolateral preoptic nucleus during rapid eye movement sleep. J Neurosci 22:4568–4576

Lubkin M, Stricker-Krongrad A (1998) Independent feeding and metabolic actions of orexins in mice. Biochem Biophys Res Commun 253:241–245

Ma X, Zubcevic L, Bruning JC, Ashcroft FM, 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, Elmquist JK (2001) Differential expression of orexin receptors 1 and 2 in the rat brain. J Comp Neurol 435:6–25

Mieda M, Williams SC, Richardson JA, Tanaka K, Yanagisawa M (2006) The dorsomedial hypothalamic nucleus as a putative food-entrainable circadian pacemaker. Proc Natl Acad Sci USA 103:12150–12155

Mieda M, Williams SC, Sinton CM, Richardson JA, Sakurai T, Yanagisawa M (2004) Orexin neurons function in an efferent pathway of a food-entrainable circadian oscillator in eliciting food-anticipatory activity and wakefulness. J Neurosci 24:10493–10501

Mieda M, Willie JT, Hara J, Sinton CM, Sakurai T, Yanagisawa M (2004) Orexin peptides prevent cataplexy and improve wakefulness in an orexin neuron-ablated model of narcolepsy in mice. Proc Natl Acad Sci USA 101:4649–4654

Mignot E, Nishino S (2005) Emerging therapies in narcolepsy-cataplexy. Sleep 28:754–763

Mileykovskiy BY, Kiyashchenko LI, Siegel JM (2005) Behavioral correlates of activity in identified hypocretin/orexin neurons. Neuron 46:787–798

Mitsuyoshi S (2005) Role of Barrington's nucleus in micturition. J Comp Neurol 493:21–26

Mobarakeh JI, Takahashi K, Sakurada S, Nishino S, Watanabe H, Kato M, Yanai K (2005) Enhanced antinociception by intracerebroventricularly and intrathecally-administered orexin A and B (hypocretin-1 and -2) in mice. Peptides 26:767–777

Monda M, Viggiano AN, Viggiano A, Viggiano E, Lanza A, De Luca V (2005) Hyperthermic reactions induced by orexin A: role of the ventromedial hypothalamus. Eur J Neurosci 22:1169–1175

Moriguchi T, Sakurai T, Nambu T, Yanagisawa M, 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

Moriguchi T, Sakurai T, Takahashi S, Goto K, Yamamoto M (2002) The human prepro-orexin gene regulatory region that activates gene expression in the lateral region

and represses it in the medial regions of the hypothalamus. J Biol Chem 277:16985–16992

Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW (2006) Central nervous system control of food intake and body weight. Nature 443:289–295

Mullett MA, Billington CJ, Levine AS, Kotz CM (2000) Hypocretin I in the lateral hypothalamus activates key feeding-regulatory brain sites. Neuroreport 11:103–108

Muraki Y, Yamanaka A, Tsujino N, Kilduff TS, Goto K, 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 T, Uramura K, Nambu T, Yada T, Goto K, Yanagisawa M, 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, 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, Suzuki T (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

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

Sakamoto F, Yamada S, Ueta Y (2004) Centrally administered orexin-A activates corticotropin-releasing factor-containing neurons in the hypothalamic paraventricular nucleus and central amygdaloid nucleus of rats: possible involvement of central orexins on stress-activated central CRF neurons. Regul Pept 118:183–191

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

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

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, Moriguchi T, Furuya K, Kajiwara N, Nakamura T, Yanagisawa M, Goto K (1999) Structure and function of human prepro-orexin gene. J Biol Chem 274:17771–17776

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

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

Selbach O, Doreulee N, Bohla C, Eriksson KS, Sergeeva OA, Poelchen W, Brown RE, Haas HL (2004) Orexins/hypocretins cause sharp wave- and theta-related synaptic

plasticity in the hippocampus via glutamatergic, gabaergic, noradrenergic, and cholinergic signaling. Neuroscience 127:519–528
Sherin JE, Elmquist JK, Torrealba F, 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, Goto K (1999) Structure, tissue distribution, and pharmacological characterization of Xenopus orexins. Peptides 20:1169–1176
Shirasaka T, Nakazato M, Matsukura S, Takasaki M, Kannan H (1999) Sympathetic and cardiovascular actions of orexins in conscious rats. Am J Physiol 277:R1780–R1785
Siegel JM, Boehmer LN (2006) Narcolepsy and the hypocretin system – where motion meets emotion. Nat Clin Pract Neurol 2:548–556
Takahashi K, Koyama Y, Kayama Y, Yamamoto M (2002) Effects of orexin on the laterodorsal tegmental neurones. Psychiat Clin Neurosci 56:335–336
Takakusaki K, Takahashi K, Saitoh K, Harada H, Okumura T, Kayama Y, Koyama Y (2005) Orexinergic projections to the midbrain mediate alternation of emotional behavioral states from locomotion to cataplexy. J Physiol 568(Pt 3):1003–1020
Thorpe AJ, Kotz CM (2005) Orexin A in the nucleus accumbens stimulates feeding and locomotor activity. Brain Res 1050:156–162
van den Pol AN (1999) Hypothalamic hypocretin (orexin): robust innervation of the spinal cord. J Neurosci 19:3171–3182
van den Pol AN, Acuna-Goycolea C, Clark KR, Ghosh PK (2004) Physiological properties of hypothalamic MCH neurons identified with selective expression of reporter gene after recombinant virus infection. Neuron 42:635–652
van den Pol AN, Gao XB, Obrietan K, Kilduff TS, Belousov AB (1998) Presynaptic and postsynaptic actions and modulation of neuroendocrine neurons by a new hypothalamic peptide, hypocretin/orexin. J Neurosci 18:7962–7971
van den Pol AN, Ghosh PK, Liu RJ, Li Y, Aghajanian GK, Gao XB (2002) Hypocretin (orexin) enhances neuron activity and cell synchrony in developing mouse GFP-expressing locus coeruleus. J Physiol 541:169–185
van den Top M, Lee K, Whyment AD, Blanks AM, Spanswick D (2004) Orexigen-sensitive NPY/AgRP pacemaker neurons in the hypothalamic arcuate nucleus. Nat Neurosci 7:493–494
van den Top M, Nolan MF, Lee K, Richardson PJ, Buijs RM, Davies CH, Spanswick D (2003) Orexins induce increased excitability and synchronisation of rat sympathetic preganglionic neurones. J Physiol 549:809–821
Vanni-Mercier G, Sakai K, Jouvet M (1984) Neurons specifiques de l'eveil dans l'hypothalamus posterieur du chat. CR Acad Sci III 298:195–200
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, 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, 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

Wu M, Zaborszky L, Hajszan T, van den Pol AN, Alreja M (2004) Hypocretin/orexin innervation and excitation of identified septohippocampal cholinergic neurons. J Neurosci 24:3527–3536

Wu M, Zhang Z, Leranth C, Xu C, van den Pol AN, Alreja M (2002) Hypocretin increases impulse flow in the septohippocampal GABAergic pathway: implications for arousal via a mechanism of hippocampal disinhibition. J Neurosci 22:7754–7765

Xi M, Morales FR, 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, Kilduff TS (2006) GABA(B) receptor-mediated modulation of hypocretin/orexin neurones in mouse hypothalamus. J Physiol 574:399–414

Yamada H, Okumura T, Motomura W, Kobayashi Y, 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, 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, Sakurai T (2000) Orexin-induced food intake involves neuropeptide Y pathway. Brain Res 24:404–409

Yamanaka A, Muraki Y, Ichiki K, Tsujino N, Kilduff TS, Goto K, 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, 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, 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, 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, Espana RA, Crocker A, Scammell TE (2006) Afferents to the orexin neurons of the rat brain. J Comp Neurol 494:845–861

Zhang W, Sakurai T, Fukuda Y, 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:R1654–1663

Zheng H, Corkern MM, Crousillac SM, Patterson LM, Phifer CB, Berthoud HR (2002) Neurochemical phenotype of hypothalamic neurons showing Fos expression 23 h after intracranial AgRP. Am J Physiol Regul Integr Comp Physiol 282:R1773–1781

Zhu Y, Miwa Y, Yamanaka A, Yada T, Shibahara M, Abe Y, Sakurai T, 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