INVITED REVIEW ARTICLE

Nagoya J. Med. Sci. **75**. 29 ~ 36, 2013

THE REGULATION OF SLEEP AND WAKEFULNESS BY THE HYPOTHALAMIC NEUROPEPTIDE OREXIN/HYPOCRETIN

AYUMU INUTSUKA and AKIHIRO YAMANAKA

Department of Neuroscience II, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan

ABSTRACT

Orexins, also known as hypocretins, are neuropeptides that are exclusively expressed by neurons in the lateral hypothalamic area. Although originally recognized as regulators of feeding behavior, orexins are now mainly regarded as key modulators of the sleep/wakefulness cycle. In addition, anatomical studies of neural networks and analyses of transgenic mice have revealed integrated roles for orexin neurons in the coordination of emotion, energy homeostasis, and the reward system. A functional link between the limbic system and orexin neurons may be important for increasing vigilance in response to emotional stimuli. These findings suggest that orexin neurons relay information about an organism's environment to maintain the proper amount of sleep and wakefulness in animals.

Key Words: orexin/hypocretin, sleep, hypothalamus, optogenetics, neuropeptide

INTRODUCTION

Studies in genetically modified mice clearly indicate a critical role for orexin neurons in the regulation of sleep and wakefulness. Prepro-orexin knockout mice, orexin-2 receptor knockout mice, and orexin neuron-ablated transgenic mice all show fragmentation in sleep cycles[1-3]. Additionally, degeneration of orexin neurons was found in the narcoleptic brain post-mortem[4-6]. Orexins (orexin-A and -B) are neuropeptides expressed exclusively by neurons in the lateral hypothalamic area (LHA), an area that controls feeding and arousal. Although the number of orexin neurons is small (approximately 4,000 cells in the mouse brain), these neurons project axons throughout the central nervous system[7,8] and consequently affect various homeostatic functions. Dense projections from orexin neurons are observed in the serotonergic dorsal raphe nucleus (DR), noradrenergic locus coeruleus (LC), and histaminergic tuberomammillary nucleus (TMN), and all of these nuclei are involved in promoting arousal[9]. In this review, we discuss the basic features of orexins and orexin receptors. We describe how the orexin system regulates sleep and wakefulness, briefly addressing other physiological functions that are regulated by orexin neurons.

Received: January 9, 2013; accepted: January 10, 2013

Corresponding author: Akihiro Yamanaka, PhD
Research Institute of Environmental Medicine, Nagoya University, Nagoya 464-8601, Japan
Tel: +81-52-789-3864, Fax: +81-52-789-3889, E-mail: yamank@riem.nagoya-u.ac.jp

OREXINS AND OREXIN RECEPTORS

Two independent groups identified orexins in 1998. Using reverse pharmacology, Sakurai and colleagues identified a novel family of neuropeptides that bind to two closely related orphan G-protein coupled receptors (GPCRs). Intracerebroventricular injection of the synthetic neuropeptides induced feeding behavior[10], and the ligands were therefore named "orexin" after the Greek word *orexis*, which means appetite. At the same time, de Lecea and colleagues isolated complementary DNA (cDNA) expressed exclusively in the hypothalamus. Two peptides corresponding to the cDNAs showed substantial amino acid sequence homology to the gut peptide secretin and were therefore named "hypocretins."[11]. It is now known that orexin and hypocretin are synonymous for the same set of peptides.

Orexin-A and -B are produced by proteolysis of a common precursor polypeptide, prepro-orexin (Fig. 1). In rats, orexin-A is a 33 amino acid peptide with an N-terminal pyroglutamyl residue and C-terminal amidation. The four cysteine residues in orexin-A form two intra-chain disulfide bonds, and this structure is conserved among mammalian species. Orexin-B is a 28 amino acid peptide with an amidated C-terminus that is 46% identical in sequence to orexin-A. The 3.2 kb fragment of the 5'-upstream region of the human prepro-orexin gene is utilized to express genes of interest in orexin neurons[12,13]. Prepro-orexin mRNA has been shown to be

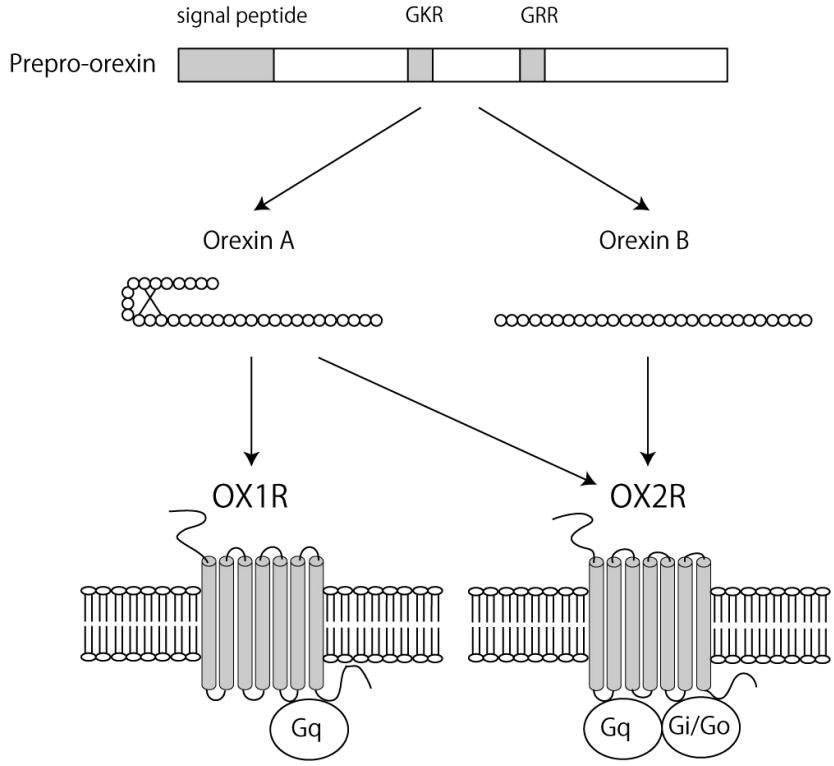

Fig. 1 Schematic representation of orexins and orexin receptors. Prepro-orexin is proteolyzed into two mature neuropeptides, orexin-A and orexin-B. Orexin-A acts on both OX_1R and OX_2R, while orexin-B mainly acts on OX_2R. OX_1R is coupled exclusively to the Gq subclass of G proteins, whereas OX_2R is coupled to either Gi or Gq.

upregulated under fasting conditions[10], and the forkhead box transcription factor Foxa2, a downstream target of insulin signaling, is reported to be involved in this transcriptional regulation[14]. These findings suggest that the activities of orexin neurons are regulated by metabolic balance.

Orexins bind two GPCRs, orexin receptor-1 and -2 (OX_1R and OX_2R). Orexin-A acts on both OX_1R and OX_2R, while orexin-B acts selectively on OX_2R[10]. Although orexin neurons are localized within the LHA, they have widespread projections throughout the brain[7,8], and orexin receptors are expressed in many regions. *In situ* hybridization of OX_1R and OX_2R shows differences in their distribution[15]. OX_1R mRNA is observed in the hippocampus, paraventricular thalamic nucleus (PVN), ventromedial hypothalamic nucleus, DR, and LC. OX_2R mRNA is found primarily in the cerebral cortex, hippocampus, DR, and many hypothalamic nuclei, including the PVN and TMN. Of these regions, the DR, LC, and TMN are well known to be involved in the onset of wakefulness. Orexin receptors are also found in many hypothalamic regions that are strongly implicated in the modulation of feeding, including the LHA, PVN, and the arcuate nucleus.

INPUT TO OREXIN NEURONS

Orexin neurons not only send projections to various brain regions, but they also receive multiple innervations. Retrograde tracers, such as the nontoxic C-terminal fragment of tetanus toxin (TTC) and the cholera toxin B subunit (CT-B), were utilized to show synaptic connections involving orexin neurons. Sakurai *et al*. (2005) generated a transgenic mouse line expressing a green fluorescent protein (GFP)-labeled TTC. The construct was expressed exclusively in orexin neurons using the human prepro-orexin promoter. Using this mouse line, they identified several brain regions including the basal forebrain cholinergic neurons, GABAergic neurons in the ventrolateral preoptic nucleus, and serotonergic neurons in the median raphe and paramedian raphe nucleus[16]. The regions associated with emotion, including, the amygdala, the shell region of the nucleus accumbens, and the bed nucleus of the stria terminalis (BST), were also found to innervate orexin neurons. Yoshida *et al*. (2006) injected CT-B into the LHA and counted labeled cells in rats. Projections from the lateral septum, preoptic area, BST, and posterior hypothalamus were identified. Interestingly, it was shown that hypothalamic regions preferentially innervate orexin neurons in the medial and perifornical parts of the field, and that most projections from the brainstem target the lateral part of the field[17]. This indicates that subpopulations of orexin neurons have different physiological functions.

Electrophysiological experiments have been used to identify factors that regulate orexin neurons. Recordings from transgenic mice expressing GFP in orexin neurons demonstrate that agonists of ionotropic glutamate receptors activate orexin neurons, while glutamate antagonists inhibit their activity[18,19]. These results indicate that orexin neurons are tonically activated by glutamate. In addition, monoamine neurotransmitters such as dopamine, noradrenaline, and serotonin (5-HT) hyperpolarize and inhibit orexin neurons via alpha 2-adrenergic and 5-HT1A receptors[18,20,21]. Other factors that reportedly influence the activity of orexin neurons include corticotrophin-releasing factor[23], ATP[24], neuropeptide Y[25], and physiological fluctuations in acid and carbon dioxide levels[26]. It should be noted that factors involved in feeding (such as glucose, ghrelin, and leptin) inhibit the activity of orexin neurons[27]. The large variety of factors that regulate the activity of orexin neurons demonstrates the role of these neurons in diverse processes, such as circadian rhythms, energy balance, and vigilance level.

FUNCTIONS IN SLEEP AND WAKEFULNESS

Among the multiple projections from orexin neurons, dense innervations to the DR, LC, and TMN are important for the regulation of sleep and wakefulness. Noradrenergic neurons of the LC[28], serotonergic neurons of the DR[29,30], and histaminergic neurons of the TMN[31,32] are activated by orexins, and OX_1R and/or OX_2R are expressed in these regions. These findings suggest that the activity of monoaminergic neurons in the brainstem and the hypothalamus are at least partly regulated by orexins. Orexins also have a strong, direct, excitatory effect on cholinergic neurons of the basal forebrain[33], which is hypothesized to play an important role in arousal. Cholinergic neurons of the pedunculopontine tegmental nucleus (PPN) and the laterodorsal tegmental nucleus (LDT) play a pivotal role in the regulation of REM sleep and wakefulness. These areas are also strongly innervated by orexin neurons[7,8]. Microinjection of orexin-A into the LDT increases awake time and decreases REM sleep in cats[34]. Additionally, injection of orexin-A into the PPN in cats, causes the PPN to require increased stimulus to induce muscle atonia[35]. It should be noted that the LDT and PPN contain neuronal subtypes other than cholinergic neurons that show activity associated with sleep/wake cycles[36].

The physiological importance of the orexin system in sleep is clearly demonstrated in genetically modulated mice. Prepro-orexin knockout mice[2], OX_2R knockout mice[3], and orexin neuron-ablated transgenic mice (*orexin/ataxin-3* mice)[1] show severe sleep fragmentation similar to narcolepsy. Additionally, it has been reported that the number of orexin neurons is greatly reduced and that orexin peptide levels are decreased to undetectable levels in the cerebrospinal fluid of narcoleptic patients[4-6]. *Orexin/ataxin-3* mice are a well-studied model for narcolepsy; however, orexin neurons are absent from birth in *orexin/ataxin-3* mice. Therefore, other neuronal mechanisms likely compensate for the function of orexin neurons during development. Indeed, the frequency of cataplexy is low in these mice. To address this problem, timing-controlled neuronal ablation models, such as the tetracycline transactivator regulatable system, may be useful.

Recently, new techniques in optogenetics made it possible to manipulate the activity of orexin neurons in freely moving animals. Selective photostimulation of orexin neurons expressing channelrhodopsin-2 increases the probability of transition from non-REM or REM sleep to wakefulness[37] and activates downstream wake-promoting nuclei such as the LC and TMN[38]. Consistently, photoinhibition of orexin neurons expressing halorhodopsin induces non-REM sleep[39]. Furthermore, optogenetic stimulation of the LC produces immediate sleep-to-wake transitions, whereas the inhibition causes a decrease in wakefulness[40]. These findings indicate that the LC is a major effector of orexin neurons in the regulation of sleep and wakefulness. However, it should be noted that LC noradrenergic neurons only express OX_1R, and that OX_1R knockout mice show only weak fragmentation in sleep and no cataplexy[41].

Designer receptors exclusively activated by designer drugs (DREADDs) are another approach for regulating the activity of specific neuronal circuits *in vivo*. This method employs modified muscarinic receptors (hM3Dq for excitation, and hM4Di for inhibition) that have lost their affinity for endogenous acetylcholine but can be activated by the synthetic ligand, clozapine-N-oxide[42,43]. Because stimulation of GPCRs has a longer effect on cellular signaling than optical stimulation, DREADDs are suitable for observing the chronic effects of modulating the activity of specific neurons. Using the DREADD technique, it was reported that excitation of orexin neurons significantly increases the amount of wakefulness time, and decreases both non-REM and REM sleep times. Additionally, it was shown that inhibition of orexin neurons decreases wakefulness time and increases non-REM sleep time[44].

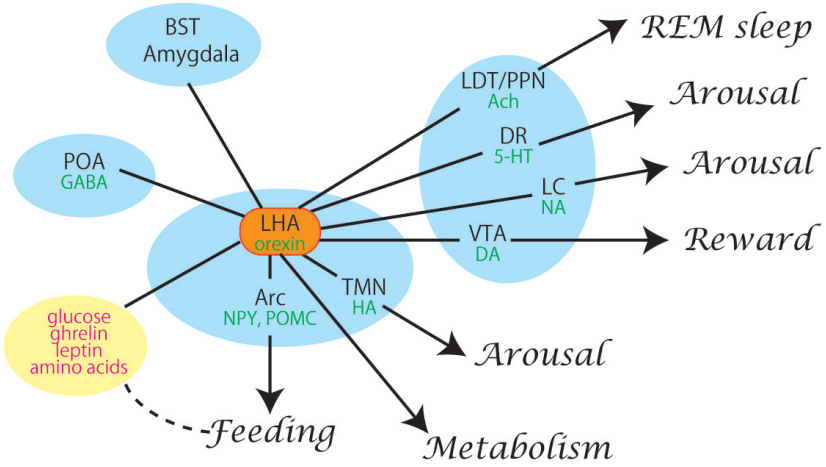

Fig. 2 A schematic diagram illustrating the integrative physiological roles of orexin neurons. Orexin neurons regulate the hypothalamic nuclei involved in feeding behavior. At the same time, they promote wakefulness through monoaminergic nuclei and other sleep-related nuclei in the brain stem. Energy levels influence orexin neuronal activity to coordinate arousal and energy homeostasis. Input from the limbic system may be important for regulating the activity of orexin neurons to evoke emotional arousal or fear-related responses. Abbreviations: 5-HT, serotonin; ACh, Acetylcholine; Arc, arcuate nucleus; BST, bed nucleus of the stria terminalis; DA, dopamine; DR, dorsal raphe nucleus; GABA, gamma-aminobutyric acid; HA, histamine; LC, locus coeruleus; LDT, laterodorsal tegmental nucleus; NA, noradrenaline; NPY, neuropeptide Y; POA, preoptic area; POMC, proopiomelanocortin; PPN, pedunculopontine tegmental nucleus; TMN, tuberomammillary nucleus; VTA, ventral tegmental area

CONCLUDING REMARKS

In this review, we mainly discuss how orexin neurons regulate sleep and wakefulness, yet orexin neurons are also implicated in the reward system, the autonomic nervous system, stress response and feeding behavior (which are discussed in detail in other reviews[45-47]). Orexin neurons in the LHA provide an anatomical link between the limbic system, energy homeostasis, and brain stem monoaminergic, or cholinergic neurons. Similar to the hypothalamus where orexin neurons exist, orexin neurons monitor physiological conditions and coordinate various behaviors in response to environmental changes (Fig. 2). These findings show that the orexin system regulates vigilance states according to internal and external cues.

ACKNOWLEDGEMENT

This study was supported by JST PRESTO program and Grant-in-Aid for Scientific Research (B) (23300142), Grant-in-Aid for Scientific Research on Innovative Area “Mesoscopic Neurocircuitry” (23115103) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan (A. Y.), and Grant-in-Aid for Young Scientists (B) (24790192) (A. I.).

REFERENCES

1) Hara J, Beuckmann CT, Nambu T, Willie JT, Chemelli RM, Sinton CM, Sugiyama F, Yagami K, Goto K, Yanagisawa M, Sakurai T. Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron* 2001; 30: 345–354.
2) Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuki Y, Fitch TE, Nakazato M, Hammer RE, Saper CB, Yanagisawa M. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* 1999; 98: 437–451.
3) Willie JT, Chemelli RM, Sinton CM, Tokita S, Williams SC, Kisanuki YY, Marcus JN, Lee C, Elmquist JK, Kohlmeier KA, Leonard CS, Richardson JA, Hammer RE, Yanagisawa M. Distinct narcolepsy syndromes in Orexin receptor-2 and Orexin null mice: molecular genetic dissection of Non-REM and REM sleep regulatory processes. *Neuron* 2003; 38: 715–730.
4) Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, Siegel JM. Reduced number of hypocretin neurons in human narcolepsy. *Neuron* 2000; 27: 469–474.
5) Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, Li R, Hungs M, Pedrazzoli M, Padigaru M, Kucherlapati M, Fan J, Maki R, Lammers GJ, Bouras C, Kucherlapati R, Nishino S, Mignot E. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat Med* 2000; 6: 991–997.
6) Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. *Lancet* 2000; 355: 39–40.
7) Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, Kilduff TS. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci* 1998; 18: 9996–10015.
8) Nambu T, Sakurai T, Mizukami K, Hosoya Y, Yanagisawa M, Goto K. Distribution of orexin neurons in the adult rat brain. *Brain Res* 1999; 827: 243–260.
9) Saper CB, Scammell TE, Lu J. Hypothalamic regulation of sleep and circadian rhythms. *Nature* 2005; 437: 1257–1263.
10) Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richarson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 1998; 92: 1 page following 696.
11) de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett FS, 2nd, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A* 1998; 95: 322–327.
12) Sakurai T, Moriguchi T, Furuya K, Kajiwara N, Nakamura T, Yanagisawa M, Goto K. Structure and function of human prepro-orexin gene. *J Biol Chem* 1999; 274: 17771–17776.
13) Moriguchi T, Sakurai T, Takahashi S, Goto K, Yamamoto M. 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* 2002; 277: 16985–16992.
14) Silva JP, von Meyenn F, Howell J, Thorens B, Wolfrum C, Stoffel M. Regulation of adaptive behaviour during fasting by hypothalamic Foxa2. *Nature* 2009; 462: 646–650.
15) Marcus JN, Aschkenasi CJ, Lee CE, Chemelli RM, Saper CB, Yanagisawa M, Elmquist JK. Differential expression of orexin receptors 1 and 2 in the rat brain. *J Comp Neurol* 2001; 435: 6–25.
16) Sakurai T, Nagata R, Yamanaka A, Kawamura H, Tsujino N, Muraki Y, Kageyama H, Kunita S, Takahashi S, Goto K, Koyama Y, Shioda S, Yanagisawa M. Input of orexin/hypocretin neurons revealed by a genetically encoded tracer in mice. *Neuron* 2005; 46: 297–308.
17) Yoshida K, McCormack S, Espana RA, Crocker A, Scammell TE. Afferents to the orexin neurons of the rat brain. *J Comp Neurol* 2006; 494: 845–861.
18) Yamanaka A, Muraki Y, Tsujino N, Goto K, Sakurai T. Regulation of orexin neurons by the monoaminergic and cholinergic systems. *Biochem Biophys Res Commun* 2003; 303: 120–129.
19) Li Y, Gao XB, Sakurai T, van den Pol AN. Hypocretin/Orexin excites hypocretin neurons via a local glutamate neuron-A potential mechanism for orchestrating the hypothalamic arousal system. *Neuron* 2002; 36: 1169–1181.
20) Li Y, van den Pol AN. Direct and indirect inhibition by catecholamines of hypocretin/orexin neurons. *J Neurosci* 2005; 25: 173–183.
21) Muraki Y, Yamanaka A, Tsujino N, Kilduff TS, Goto K, Sakurai T. Serotonergic regulation of the orexin/hypocretin neurons through the 5-HT1A receptor. *J Neurosci* 2004; 24: 7159–7166.

22) Yamanaka A, Muraki Y, Ichiki K, Tsujino N, Kilduff TS, Goto K, Sakurai T. Orexin neurons are directly and indirectly regulated by catecholamines in a complex manner. *J Neurophysiol* 2006; 96: 284–298.
23) Winsky-Sommerer R, Yamanaka A, Diano S, Borok E, Roberts AJ, Sakurai T, Kilduff TS, Horvath TL, de Lecea L. Interaction between the corticotropin-releasing factor system and hypocretins (orexins): a novel circuit mediating stress response. *J Neurosci* 2004; 24: 11439–11448.
24) Wollmann G, Acuna-Goycolea C, van den Pol AN. Direct excitation of hypocretin/orexin cells by extracellular ATP at P2X receptors. *J Neurophysiol* 2005; 94: 2195–2206.
25) Fu LY, Acuna-Goycolea C, van den Pol AN. Neuropeptide Y inhibits hypocretin/orexin neurons by multiple presynaptic and postsynaptic mechanisms: tonic depression of the hypothalamic arousal system. *J Neurosci* 2004; 24: 8741–8751.
26) Williams RH, Jensen LT, Verkhratsky A, Fugger L, Burdakov D. Control of hypothalamic orexin neurons by acid and CO2. *Proc Natl Acad Sci U S A* 2007; 104: 10685–10690.
27) Yamanaka A, Beuckmann CT, Willie JT, Hara J, Tsujino N, Mieda M, Tominaga M, Yagami K, Sugiyama F, Goto K, Yanagisawa M, Sakurai T. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. *Neuron* 2003; 38: 701–713.
28) Hagan JJ, Leslie RA, Patel S, Evans ML, Wattam TA, Holmes S, Benham CD, Taylor SG, Routledge C, Hemmati P, Munton RP, Ashmeade TE, Shah AS, Hatcher JP, Hatcher PD, Jones DN, Smith MI, Piper DC, Hunter AJ, Porter RA, Upton N. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. *Proc Natl Acad Sci U S A* 1999; 96: 10911–10916.
29) Liu RJ, van den Pol AN, Aghajanian GK. Hypocretins (orexins) regulate serotonin neurons in the dorsal raphe nucleus by excitatory direct and inhibitory indirect actions. *J Neurosci* 2002; 22: 9453–9464.
30) Brown RE, Sergeeva OA, Eriksson KS, Haas HL. Convergent excitation of dorsal raphe serotonin neurons by multiple arousal systems (orexin/hypocretin, histamine and noradrenaline). *J Neurosci* 2002; 22: 8850–8859.
31) Yamanaka A, Tsujino N, Funahashi H, Honda K, Guan JL, Wang QP, Tominaga M, Goto K, Shioda S, Sakurai T. Orexins activate histaminergic neurons via the orexin 2 receptor. *Biochem Biophys Res Commun* 2002; 290: 1237–1245.
32) Huang ZL, Qu WM, Li WD, Mochizuki T, Eguchi N, Watanabe T, Urade Y, Hayaishi O. Arousal effect of orexin A depends on activation of the histaminergic system. *Proc Natl Acad Sci U S A* 2001; 98: 9965–9970.
33) Eggermann E, Serafin M, Bayer L, Machard D, Saint-Mleux B, Jones BE, Muhlethaler M. Orexins/hypocretins excite basal forebrain cholinergic neurones. *Neuroscience* 2001; 108: 177–181.
34) Xi MC, Morales FR, Chase MH. Effects on sleep and wakefulness of the injection of hypocretin-1 (orexin-A) into the laterodorsal tegmental nucleus of the cat. *Brain Res* 2001; 901: 259–264.
35) Takakusaki K, Takahashi K, Saitoh K, Harada H, Okumura T, Kayama Y, Koyama Y. Orexinergic projections to the cat midbrain mediate alternation of emotional behavioural states from locomotion to cataplexy. *J Physiol* 2005; 568: 1003–1020.
36) Sakai K. Discharge properties of presumed cholinergic and noncholinergic laterodorsal tegmental neurons related to cortical activation in non-anesthetized mice. *Neuroscience* 2012; 224C: 172–190.
37) Adamantidis AR, Zhang F, Aravanis AM, Deisseroth K, de Lecea L. Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature* 2007; 450: 420–424.
38) Carter ME, Adamantidis A, Ohtsu H, Deisseroth K, de Lecea L. Sleep homeostasis modulates hypocretin-mediated sleep-to-wake transitions. *J Neurosci* 2009; 29: 10939–10949.
39) Tsunematsu T, Kilduff TS, Boyden ES, Takahashi S, Tominaga M, Yamanaka A. Acute optogenetic silencing of orexin/hypocretin neurons induces slow-wave sleep in mice. *J Neurosci* 2011; 31: 10529–10539.
40) Carter ME, Yizhar O, Chikahisa S, Nguyen H, Adamantidis A, Nishino S, Deisseroth K, de Lecea L. Tuning arousal with optogenetic modulation of locus coeruleus neurons. *Nat Neurosci* 2010; 13: 1526–1533.
41) Mieda M, Hasegawa E, Kisanuki YY, Sinton CM, Yanagisawa M, Sakurai T. Differential roles of orexin receptor-1 and -2 in the regulation of non-REM and REM sleep. *J Neurosci* 2011; 31: 6518–6526.
42) Alexander GM, Rogan SC, Abbas AI, Armbruster BN, Pei Y, Allen JA, Nonneman RJ, Hartmann J, Moy SS, Nicolelis MA, McNamara JO, Roth BL. Remote control of neuronal activity in transgenic mice expressing evolved G protein-coupled receptors. *Neuron* 2009; 63: 27–39.
43) Armbruster BN, Li X, Pausch MH, Herlitze S, Roth BL. Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. *Proc Natl Acad Sci U S A* 2007; 104: 5163–5168.
44) Sasaki K, Suzuki M, Mieda M, Tsujino N, Roth B, Sakurai T. Pharmacogenetic modulation of orexin neurons alters sleep/wakefulness states in mice. *PLoS One* 2011; 6: e20360.
45) Harris GC, Aston-Jones G. Arousal and reward: a dichotomy in orexin function. *Trends Neurosci* 2006; 29:

571–577.
46) Kuwaki T. Orexin links emotional stress to autonomic functions. *Auton Neurosci* 2011; 161: 20–27.
47) Saper CB. Staying awake for dinner: hypothalamic integration of sleep, feeding, and circadian rhythms. *Prog Brain Res* 2006; 153: 243–252.