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Integrative Physiology of Orexins and Orexin Receptors

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Abstract

Recent studies have established that the orexin system is a critical regulator of sleep/wake states. Deficiency of orexin signaling results in the sleep disorder narcolepsy-cataplexy in humans, dogs, and rodents. These findings have brought about the possibility of novel therapies for sleep disorders including narcolepsy-cataplexy. Furthermore, accumulating evidence has indicated that the orexin system regulates sleep and wakefulness through interactions with neuronal systems that regulate emotion, reward, and energy homeostasis. This review presents and discusses the current understanding of the integrative physiology of the orexin system.

Keywords: orexin, orphan GPCR, hypothalamus, narcolepsy-cataplexy, sleep/wake, energy homeostasis, reward, emotion

Introduction

Since its discovery in 1998, the field of orexin biology has grown rapidly. In the last decade, more than 1700 articles on orexin research have been published. Information on the role of the orexin system in the pathophysiology of narcolepsy-cataplexy has had a huge impact on studies of sleep and wakefulness **and on other areas of inquiry**. Scientists have used a multidisciplinary approach to understand various aspects of the physiological functions of orexin peptides, and their efforts have uncovered crucial roles of the orexin system in the integrative physiology of sleep/wake, energy homeostasis, and the reward systems. Orexin biology has also been applied to the diagnosis and treatment of sleep-related disorders. Living in the post-genome era, the great success story of orexin biology has driven many researchers to dig up novel bioactive peptides and their receptors for further discovery of novel physiology and clinical treatments.

Discovery of orexin peptides

Orexin peptides (orexin-A and orexin-B) were initially identified by our group as endogenous ligands for two orphan G-protein coupled receptors (GPCR) [1]: GPCRs, for which endogenous ligands are unknown, are referred to as “orphan” GPCRs. Since intracerebroventricular (ICV) injection of these peptides in rats acutely stimulated food consumption (discussed below), they were named orexin-A and -B after the Greek word ‘orexis’, meaning appetite. Orexin-A and -B are produced by cleavage of a single precursor polypeptide, prepro-orexin. Mammalian orexin-A is a 33 amino acid peptide that has two sets of intrachain disulfide bonds and undergoes pyroglutamylation and amidation at its N- and C-terminals, respectively, while orexin-B is a 28 amino acid linear peptide with C-terminal amidation. Around the same time,

de Lecea et al. searched mRNA expressed specifically within the hypothalamus and identified a cDNA encoding a polypeptide identical to prepro-orexin, and named the putative mature peptides hypocretin-1 (orexin-A) and -2 (orexin-B) [2]. Although the initial estimated structures of hypocretin-1 and hypocretin-2 were not the same as those of orexin A and orexin B, the terms ‘orexin’ and ‘hypocretin’ are used as synonyms in many papers.

The actions of orexins are mediated by two receptors, named orexin 1 (OX₁R) and orexin 2 (OX₂R) receptors (also named HCRTR1 and HCRTR2) [1]. OX₁R has one-order higher affinity for orexin-A than for orexin-B, while OX₂R binds orexin-A and orexin-B with similar affinities. OX₁R primarily couples to the G_{q/11} subclass of heterotrimeric G proteins, while OX₂R coupled to G_{q/11} or G_{i/o} in a neuronal cell line [3].

Neurons expressing orexins (orexin neurons) are distributed within an area consisting of three contiguous hypothalamic regions: lateral hypothalamus (LH), perifornical area (PFA) and dorsomedial hypothalamic nucleus (DMH) [1, 2, 4-6]. The number of these neurons has been estimated to total around 3000 in the rat and 50,000 in the human brain. In contrast to the restricted localization of their cell bodies, orexin neurons send projections widely throughout the central nervous system (CNS), including the cerebral cortex, limbic system (such as the amygdala, bed nucleus of stria terminalis (BST) and hippocampus), hypothalamus (such as the arcuate nucleus (ARC) and tuberomammillary nucleus (TMN)), and brain stem area (such as the central gray, locus coeruleus (LC), and raphe nuclei) [4-7]. Consistent with the broad projections of orexin neurons, OX₁R and OX₂R show partly overlapping but distinct distributions of their mRNA throughout the CNS [8-10]. For instance, nuclei such as the LC, laterodorsal tegmental nucleus (LDT), and pedunculopontine tegmental

nucleus (PPT) mainly express OX₁R mRNA, while those including the TMN, nucleus accumbens (NAc), and septal nuclei mainly express OX₂R mRNA. These distributions suggest partly overlapping and partly distinct roles of these two receptors.

Disruption of orexin system causes narcolepsy-cataplexy

Soon after the discovery of orexin peptides, two independent studies utilizing dog forward genetics and mouse reverse genetics elucidated a causal linkage between disruption of the orexin system and narcolepsy-cataplexy. Subsequent studies showed that loss of orexin neurons is observed in human narcolepsy-cataplexy patients. These studies have revealed that orexin is essential for stability of sleep/wake states in various species, namely dog, mouse and human.

Human narcolepsy-cataplexy

Human narcolepsy is a debilitating neurological disease that affects approximately 1 in 2000 individuals in the United States [11]. Onset of the condition is usually during adolescence (around 12-14 years old). A cardinal symptom of the disorder is excessive daytime sleepiness (an insurmountable urge to sleep), which manifests itself primarily when the subject falls asleep at inappropriate times (“sleep attacks”). When normal individuals fall asleep, a certain period of non-REM (NREM) sleep (approximately 90 minutes) usually precedes rapid eye movement (REM) sleep. However, the latency of REM sleep is markedly reduced in narcolepsy patients. REM sleep is sometimes observed immediately after wakefulness (sleep-onset REM). The existence of “sleep-onset REM periods” is one of the diagnostic criteria of narcolepsy.

In patients, nocturnal sleep is also fragmented and is often accompanied by hypnagogic hallucinations, vivid dreaming, and sleep paralysis, which usually occur

near sleep onset. Narcolepsy patients often suffer from a condition called “cataplexy”, which is characterized by a sudden weakening of muscle tone (muscle atonia), ranging from jaw dropping and speech slurring to complete bilateral collapse of the postural muscles. These attacks are often triggered by emotional stimuli, such as laughter, excitement, and pleasure. Consciousness is preserved during cataplexy. Around 10% of narcolepsy patients do not suffer from cataplexy, although they experience excessive daytime sleepiness. Therefore, narcolepsy with cataplexy is sometimes referred to as “narcolepsy-cataplexy” to stress the occurrence of cataplexy.

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

Discovery of a causal link between loss of orexin signaling and narcolepsy-cataplexy

Orexin knockout mice exhibit frequent sudden collapses during the dark phase, the portion of the circadian rhythm during which there is the most time awake and spent in activity. These attacks resemble human cataplexy attacks [12]. Electroencephalogram/electromyogram (EEG/EMG) recordings correlated these

attacks with direct transitions from wakefulness to REM sleep, suggesting that they are homologous to cataplexy (Fig. 1). Quantitative sleep state parameters in orexin^{-/-} mice revealed significantly decreased waking time, increased NREM and REM sleep time, decreased REM sleep latency, and, most importantly, a markedly decreased duration of waking episodes during the dark phase (i.e. inability to maintain a long awake period) (Fig. 1). Around the same time, Mignot and colleagues identified mutations in the OX₂R gene responsible for inherited canine narcolepsy-cataplexy by positional cloning [13]. This canine model of narcolepsy-cataplexy displays emotionally triggered cataplexy, fragmented sleep patterns, and excessive daytime sleepiness. The phenotype is transmitted as a single autosomal recessive trait (canarc-1) with full penetrance. These studies in mice and dogs established that genetic disruption of orexin signaling causes narcolepsy-cataplexy.

Subsequently, deficiency of orexin in human narcolepsy-cataplexy, especially when accompanied by cataplexy, was confirmed. In contrast to normal control individuals, the vast majority of narcoleptic individuals have low or undetectable levels of orexin neuropeptides in the cerebrospinal fluid (CSF) [14]. Marked reductions of orexin mRNA and immunoreactivity in postmortem brains of narcoleptic patients was also shown [15, 16]. No mutation has been found in either the prepro-orexin or orexin receptor genes of human narcolepsy-cataplexy patients except for an unusually severe, early onset case associated with a mutation in the prepro-orexin gene that impairs peptide trafficking and processing [15]. A recent finding showing concomitant loss of dynorphin, neuronal activity-regulated pentraxin, and orexin, which colocalize in orexin neurons, further indicates a loss of orexin neurons, rather than selective inhibition of orexin expression, in narcolepsy-cataplexy [17]. Based on these observations, as well as the strong association of human narcolepsy-

cataplexy with certain Human leukocyte antigen (HLA) alleles [18], it has been speculated that narcolepsy-cataplexy may result from selective autoimmune degeneration of orexin neurons.

$OX_2R^{-/-}$ mice have characteristics of narcolepsy-cataplexy, although their behavioral and EEG phenotype is less severe than that found in orexin $^{-/-}$ mice (Fig. 1) [19]. They show behavioral arrests that are less frequent and severe than those in orexin $^{-/-}$ mice. Electroencephalographically, these mice exhibit fragmentation of sleep/wake states but lack frequent direct transitions to REM sleep. $OX_1R^{-/-}$ mice do not have overt behavioral abnormalities and exhibit only increased fragmentation of sleep-wakefulness states (Fig. 1) [20]. Double deletion, $OX_1R^{-/-};OX_2R^{-/-}$, mice appear to be a phenocopy of orexin knockout mice (Fig. 1) [20], implying that these two receptors are sufficient to mediate regulation of sleep/wake by orexins. Results of these mouse reverse genetic studies suggest that normal regulation of wake/NREM sleep transitions depends critically on OX_2R activation, whereas the profound dysregulation of REM sleep control unique to the narcolepsy-cataplexy syndrome emerges from loss of signaling through both OX_1R - and OX_2R -dependent pathways.

Hara *et al.* produced transgenic mice in which orexin neurons are ablated by expression of a neurotoxic form of ataxin-3, which induces postnatal apoptotic death of all orexin neurons by adulthood (orexin neuron-ablated mice) [21]. These adult mice show essentially the same phenotype of sleep/wake regulation as orexin $^{-/-}$ mice. Therefore, although orexin neurons produce other neurotransmitters such as glutamate and dynorphin [22-24], these results demonstrate that orexin is important for regulation of sleep/wake states by these neurons.

Clinical potential of orexin peptides

The finding of a low CSF orexin-A level in patients with narcolepsy-cataplexy led to the development of a novel, definitive diagnostic test for this disease [14]. Currently, a low orexin-A level in CSF is one of the diagnostic criteria for narcolepsy-cataplexy according to the 2nd edition of the International Classification of Sleep Disorders [25].

In addition, the discovery of a causal link between loss of orexin signaling and human narcolepsy-cataplexy has brought about the possibility of novel therapies for this disease. Currently, excessive sleepiness is treated using psychostimulants, while cataplexy is treated with tricyclic antidepressants. γ -hydroxybutyrate (sodium oxybate, GHB) is also used to consolidate nocturnal sleep and reduce cataplexy [26]. This therapeutic regimen is problematic due to limited effectiveness (psychostimulants, tricyclic antidepressants, and GHB), undesirable side effects such as insomnia (psychostimulants) or symptom rebound (tricyclic antidepressants and psychostimulants), and the potential for abuse (psychostimulants and GHB). Since the etiology and disease course in orexin neuron-ablated mice (orexin/ataxin-3 mice) are similar to those of human narcolepsy-cataplexy, these mice may represent the most accurate pathophysiological model of narcolepsy-cataplexy available [21]. We demonstrated rescue of the phenotype of orexin neuron-ablated mice by genetic and pharmacological means [27]. Chronic overproduction of orexin peptides from an ectopically expressed transgene prevented the development of narcolepsy syndrome in orexin neuron-ablated mice. Furthermore, acute ICV administration of orexin-A maintained wakefulness, suppressed sleep, and inhibited cataplectic attacks in these mice. Intriguingly, ICV administration of orexin-A had stronger arousal effects in orexin neuron-ablated mice than in wild-type controls. These results indicate that

orexin neuron-ablated mice retain the ability to respond to orexin neuropeptides and that spatially targeted secretion of orexin is not necessary to prevent narcoleptic symptoms. A similar result was also obtained by Fujiki *et al.*, demonstrating that orexin-A intravenously administered at an extremely high dose induced a very short-lasting anticataplectic effect in an orexin-deficient narcoleptic dog [28]. Unfortunately, constitutive production of orexin peptides from a prepro-orexin transgene in mice *per se* caused fragmentation of NREM sleep episodes in the light period, when mice spend the most time asleep (T.S., unpublished observation). These results indicate that orexin neurons should be turned on and switched off to maintain consolidated wakefulness and NREM sleep, respectively. Thus, orexin receptor agonists with half-lives of several hours (< 12 hours) would be of potential value for treating human narcolepsy-cataplexy. Such agonists might also be useful in the treatment of other conditions of excessive daytime sleepiness in humans. Conversely, orexin receptor antagonists might be useful as safe hypnotics. Indeed, Almorexant, an orally available antagonist of OX₁R and OX₂R, has been reported to increase subjective and objective electrophysiological signs of sleep in humans [29], and it is now in Phase III studies.

Regulation of wakefulness by orexin

In light of the evidence that deficiency of orexin signaling is associated with narcolepsy-cataplexy in animals and humans, there are additional pharmacological, anatomical, and electrophysiological data that support the role of the orexin system in the maintenance of wakefulness.

Neural mechanism of sleep/wake regulation

Wakefulness is maintained by a complex process involving multiple neurotransmitters. Included in this system are histaminergic, noradrenergic and serotonergic neurons that diffusely innervate the forebrain, regulating cortical function. Other important wake-inducing signals are cholinergic fibers from the basal forebrain (BF) and the brain stem (PPT/LDT) that interconnect with key forebrain targets [30, 31]. Monoaminergic neurons, including LC noradrenergic, raphe serotonergic, and TMN histaminergic neurons, project diffusely to the cerebral cortex, thalamus, and brainstem, and are thought to promote arousal. They are highly active during wakefulness, while their activities are reduced during NREM sleep and their discharge almost ceases during REM sleep (REM-off). These monoaminergic neurons are believed to suppress activities of PPT/LDT cholinergic neurons. A subset of PPT/LDT neurons is active during both wakefulness and REM sleep (W-REM on) and project to the thalamus, including the intralaminar nuclei, thalamic relay nuclei, and the reticular nucleus of the thalamus. The reticular nucleus is thought to play a key role in regulating thalamic activity, and the cholinergic influence is thought to be crucial in activating thalamocortical transmission to generate EEG desynchronization characteristics of wakefulness and REM sleep. Others are active exclusively during REM sleep (REM-on) and are thought to induce REM sleep and REM atonia. GABA/galaninergic neurons in the ventrolateral preoptic nucleus (VLPO) of the hypothalamus are active during sleep, especially non-REM sleep [31], and are considered to comprise a sleep center. VLPO neurons and monoaminergic neurons reciprocally inhibit each other. This reciprocal interplay of stimulation and inhibition maintains states of sleep and wakefulness.

Orexin neurons in regulatory circuit of sleep/wake

Orexin neurons send projections to nuclei involved in sleep/wake regulation, including the LC noradrenergic, raphe serotonergic, TMN histaminergic, PPT/LDT and basal forebrain cholinergic neurons (Figs. 2, 3) [12, 32, 33]. Neurons in these nuclei express orexin receptors [10]. ICV administration of orexin-A in rodents reduces REM and non-REM sleep, and increases wakefulness [34]. More recently, Adamantidis *et al.* demonstrated that direct and selective photostimulation of orexin neurons in freely moving mice, in which orexin neurons were genetically targeted to express a photo-activatable cation channel (channelrhodopsin-2), increased the probability of transitions to wakefulness from either NREM or REM sleep [35].

Application of orexin directly into the LC [36], TMN [37], BF cholinergic area [38], LDT [39], and lateral preoptic area [40] has effects similar to those of ICV injection on sleep/wake states. Especially, several reports demonstrated that histaminergic neurons in the TMN play an important role in the arousal-promoting effect of orexin. The effect of ICV orexin-A administration is markedly attenuated by the histamine H₁ receptor antagonist pyrilamine [33], and is absent in H₁ histamine receptor knockout mice [37]. Taken in conjunction with the facts that OX₂R is abundantly expressed in the TMN and that OX₂R knockout mice exhibit fragmentation of sleep/wake states [10, 19], these results suggest that the TMN histaminergic system plays a prominent role in mediating the waking effect of orexin-A.

In vitro slice electrophysiology studies have shown that orexin increases the firing rates of monoaminergic neurons in the LC [32, 41], raphe [42, 43], and TMN [33, 44, 45], and in cholinergic neurons in the BF and LDT [46, 47], but has no effect on GABAergic neurons in the VLPO [46]. More recent work in cats has shown that

orexin-A inhibits cholinergic neurons in the PPT in vivo through activation of GABAergic interneurons and GABAergic neurons in the substantia nigra pars reticulata [48]. These results indicate that orexin neurons affect the activity of PPT/LDT cholinergic neurons both directly and indirectly to regulate arousal and REM sleep.

At the same time, orexin neurons receive projections from nuclei involved in sleep/wake regulation. GABAergic neurons in the preoptic area, including the VLPO, densely innervate orexin neurons (Fig. 3) [49, 50]. Orexin neurons are strongly inhibited by both the GABA_A receptor agonist muscimol and the GABA_B receptor agonist baclofen [51-53]. Orexin neurons are also innervated by BF cholinergic neurons [49]. Carbacol, an agonist at muscarinic receptors, activates a subset of orexin neurons [49, 54]. Thus, orexin neurons are inhibited by sleep-promoting neurons and activated by wake-promoting BF neurons: this regulation of orexin neurons is consistent with their proposed function to stabilize wakefulness. In contrast, wake-active serotonergic neurons in the medial raphe nuclei send inhibitory projections to orexin neurons [49, 51, 55]. Noradrenergic neurons also have inhibitory effects on orexin neurons [51, 54, 56]. Serotonergic and noradrenergic inputs hyperpolarize orexin neurons through activation of G-protein-regulated inwardly rectifying K⁺ (GIRK or Kir3) channels mediated by 5-HT_{1A} receptors and α_2 -adrenoceptors, respectively [51, 54, 56]. These negative feedback mechanisms may also be important for fine adjustment of orexin neuronal activity to stabilize wakefulness. Histamine has little effect on orexin neurons [54]. Interestingly, a short 2-hour period of total sleep deprivation was reported to change the action of noradrenaline on orexin neurons from excitation to inhibition in rats [57]. This mechanism may contribute to the

growing sleepiness that accompanies sleep deprivation, although this phenomenon was not observed in mice [56].

Feedback circuits mediated by local interneurons may also play important roles in the regulation of orexin neurons. In slice preparations, orexin-A and orexin-B were reported to depolarize orexin neurons [51]. This effect appears to be mediated by orexin-induced excitation of local glutamatergic neurons that regulate orexin neuronal activity, in part by presynaptic facilitation of glutamate release. On the other hand, GABAergic input from local interneurons to orexin neurons is also important for organization of orexin neuronal activity [58]. Orexins activate local GABAergic input to orexin neurons. Genetic disruption of this input was reported to produce marked sleep/wake abnormality [58].

Horvath and Gao reported unusual synaptic organization of orexin neurons in which excitatory synaptic currents and asymmetric synapses exert control on the perikarya of these long projective neurons with minimal inhibitory input, which is in sharp contrast to the fact that neuronal cell bodies in the central nervous system are either dominated by inhibitory inputs (long-projective neurons) or have an approximate ratio of excitatory to inhibitory inputs of 1:1 [59]. Arousal is a vital behavior in all species. This unique input organization of orexin neurons may be a necessary element for the maintenance of a low threshold for arousal and alertness. On the other hand, this circuitry may also be an underlying cause of insomnia. Consistent with these results, an electrophysiological slice study indicated that orexin neurons are tonically activated by glutamatergic neurons [51]. By contrast, the basal tone of GABAergic input seems to be low, as GABA antagonists do not influence basal activity of orexin neurons. Furthermore, Rao *et al.* reported that sleep

deprivation induced long-term potentiation of synaptic strength at glutamatergic synapses on orexin neurons [60].

Based on the known pathophysiology of narcolepsy-cataplexy, orexin neurons are expected to be active during wakefulness and to be silent during sleep. Fos expression (an indicator of neuronal activity) in orexin neurons in rats is higher during the dark phase (active period) than during the light phase (rest period) [61]. Moreover, orexin level in CSF peaks during the dark period and decreases during the light period [62]. *In vivo* single unit recordings further confirmed activity patterns of orexin neurons across sleep/wake cycles with high temporal resolution. Mileykovskiy *et al.* first developed criteria for identification of orexin neurons based on electrophysiological characteristics in anesthetized rats, then recorded the activity of orexin neurons in unanesthetized, unrestrained rats [63]. Lee *et al.*, as well as Takahashi *et al.*, also recorded from orexin neurons in the LHA and posterior hypothalamus of head-fixed rats and mice, respectively, identified through a combination of neurobiotin labeling and immunohistochemical staining [64, 65]. Essentially, orexin neurons fired most actively during active waking, showed decreased discharge during quiet waking, were virtually silent during NREM sleep, and were almost silent but exhibited occasional firing during REM sleep. During the transition from sleep to wakefulness, orexin neurons fired prior to the onset of EEG activation, the EEG sign of wakefulness [64, 65]. In addition, they responded with a short latency to an arousing sound stimulus given during sleep, causing EEG activation [63, 65]. These characteristics of orexin neurons clearly contrast with those of histaminergic neurons, which display waking-specific discharge. Although the numbers of cells examined were too small to provide a complete picture of the temporal pattern of orexin neuronal activity across sleep/wake cycles, these seminal

and laborious studies provide the strongest evidence that these neurons are activated during wakefulness and inhibited during sleep.

Orexin neurons as stabilizers of sleep/wake states

As discussed above, instability of wake episodes is the characteristic abnormality in narcoleptics. Meanwhile, narcoleptics can be roused from sleep and their daily amount of wakefulness is relatively similar to that in normal controls. How do orexin neurons stabilize wakefulness (Fig. 4)?

Sleep-active VLPO neurons and wake-active monoaminergic neurons reciprocally inhibit each other, constituting a “flip-flop” circuit [31]. In this type of circuit, when activity on either side begins to overcome the other, the system will flip to one of two possible extremes. Although it is well suited to avoid intermediate states, a small perturbation of the activity on one side can easily cause abrupt switching between two states, resulting in frequent state transitions. Such a condition resembles narcoleptic phenotypes, flip-flopping between wakefulness and sleep. Orexin neurons seem to function as a stabilizer of this circuit by enhancing the activity of monoaminergic neurons during wakefulness, avoiding state instability caused by small perturbations. At the same time, negative feedback input to orexin neurons from serotonergic and noradrenergic neurons acts to maintain the activities of orexin and monoaminergic neurons within appropriate ranges. During sleep, orexin neurons together with monoaminergic neurons are turned off by VLPO GABA/galaninergic neurons activated by sleep substances such as adenosine. Adenosine may also inhibit orexin neurons directly via the adenosine A₁ receptor [66].

Roles of orexin peptides beyond mere global arousal

As described earlier, the fact that orexin neurons are much more active during active waking than during quiet waking *in vivo* clearly suggests that the roles of orexin extend beyond mere global arousal. Recently, Deadwyler *et al.* reported that systemic and nasal delivery of orexin-A reduced the effects of sleep deprivation on cognitive performance in nonhuman primates; interestingly, orexin-A did not produce facilitative effects if the animals were not sleep deprived [67]. In slice preparations from rat prefrontal cortex, orexin was reported to induce calcium transients in single spines postsynaptic to identified thalamocortical boutons [68, 69]. By this cellular mechanism, orexinergic projections to the prefrontal cortex may play a role in prefrontal or “executive” aspects of alertness and attention. In addition, Deadwyler’s finding that the nasal delivery method was significantly more effective than the highest dose of intravenous orexin-A tested provides strong evidence for the effectiveness of intranasal orexin-A in alleviating the cognitive deficit produced by loss of sleep, as well as in the treatment of narcolepsy-cataplexy [67].

Integrative physiology of the orexin system

Orexin peptides and feeding behavior: A link between energy homeostasis and wakefulness regulation

Since orexins are expressed in the lateral hypothalamic area, which has long been identified as a “feeding center”, and ICV injection of orexin-A increases food intake, orexins were initially characterized as orexigenic (appetite-stimulating) factors [1, 70-73]. In support of this idea, ICV administration of anti-orexin antibody or an OX₁R-selective antagonist reduced food intake [74, 75]. In addition, an OX₁R-selective antagonist reduced food intake and ameliorated obesity in leptin-deficient

ob/ob mice [76]. Moreover, orexin knockout mice and orexin neuron-ablated mice ate less than control wild-type mice [20, 21]. Human narcolepsy patients also have a decreased caloric intake, although paradoxically, they show an increased body mass index [77, 78]. Similarly, orexin neuron-ablated mice exhibit late-onset obesity [21], although the degree of obesity in these mice critically depends on their genetic background [79]. This apparent inconsistency will be further discussed later.

Orexin neurons have dense and reciprocal connections with other hypothalamic nuclei regulating feeding behavior, such as the ARC and ventromedial hypothalamic nucleus (VMH) [5, 6, 49, 50, 80-82]. Consistent with the dense projections of orexin neurons to the ARC, several studies have suggested that the increased food intake following orexin-A administration is at least partly mediated by activation of neuropeptide Y (NPY) neurons in the ARC [83, 84]. Other events involved in orexin-induced feeding behavior include the inhibition of proopiomelanocortin neurons in the ARC, which are thought to have an important role in leptin-mediated inhibition of food intake [84]. Recent reports also showed that infusion of orexin-A into the shell of the nucleus accumbens increased feeding behavior [85]. In addition, infusion of the GABA_A receptor agonist muscimol into the NAc shell strongly induced food intake and simultaneously increased Fos expression specifically in orexin neurons [86]. These findings indicate that reciprocal interactions between the orexin and reward systems have a role in the regulation of feeding.

An important difference in effects on feeding between orexins and other orexigenic factors, such as NPY and melanin-concentrating hormone (MCH), is that orexin increases both food intake and energy expenditure [21, 78, 87], while other feeding peptides generally decrease energy expenditure [88]: the latter response is more adaptive to conserving energy under food scarcity. Increased energy expenditure

by orexin administration seems to be caused by increased wakefulness and locomotor activity, as well as an increase in activity of the autonomic nervous system. ICV orexin injection increases blood pressure and heart rate. These effects are abolished by the administration of blockers of α - or β -adrenoceptors [89]. Moreover, blood pressure is 10-15 mmHg lower in orexin knockout mice compared to wild-type mice [90, 91]. These results suggest that orexins physiologically stimulate sympathetic outflow. In other words, orexin deficiency decreases sympathetic tone, resulting in reduced energy expenditure. This may explain why human and mouse narcolepsy-cataplexy is associated with an increase of body weight despite their hypophagia [21, 77, 78].

Thus, orexin neurons do not simply act as a system that maintains long-term body weight homeostasis. Rather, they seem to be necessary for food seeking and feeding behavior, especially when animals are faced with food scarcity. Food seeking and food intake require more vigilant states and more energy expenditure. Recent evidence has suggested that orexin neurons are capable of sensing indicators of energy balance and are activated under negative energy balance (Fig. 3). Analysis of Fos expression revealed that orexin neurons are activated by hypoglycemia [92]. In slice preparations, decreasing the extracellular glucose concentration produced depolarization and increased the frequency of action potentials in orexin neurons, whereas increasing extracellular glucose concentration induced marked hyperpolarization and cessation of action potentials in the same neurons [52, 93]. Importantly, this mechanism is sufficiently sensitive to encode variations in glucose levels reflecting those occurring physiologically between normal meals [52, 93]. Inhibition of orexin neurons by glucose is mediated by tandem-pore K^+ (K_{2p}) channels [94]. Glucose appears to act at an extracellular site on orexin neurons [94]. The

intracellular mechanism that transmits information on extracellular glucose to ion channels has not been elucidated, although ATP, Ca^{2+} , and glucose itself have been excluded from candidate messengers [94]. Ghrelin, a stomach-derived orexigenic peptide, activated 60% of dispersed orexin neurons when applied in a superfused solution, with depolarization and an increase in action potential frequency [52]. In contrast, bath-application of leptin, an anorexigenic protein hormone secreted by adipocytes, was found to robustly inhibit most of the orexin neurons examined, causing hyperpolarization and a decrease in firing rate [52]. Notably, insulin exerted no direct effect on orexin neurons.

These abilities of orexin neurons to sense humoral metabolic cues, as well as innervations from regions that control metabolism and feeding, including the ARC, VMH, and nucleus of the solitary tract (NTS) [49, 50, 80-82], might enable orexin neurons to control arousal related to the peripheral energy balance. When faced with a negative energy balance due to reduced food availability, mammals respond behaviorally with phases of increased wakefulness and alertness, which presumably enhances their ability to find food [52, 95, 96]. We previously demonstrated that orexin neuron-ablated mice are incapable of this fasting-induced arousal, indicating that orexin neurons are necessary for evoking adaptive behavioral arousal during fasting [52]. Orexin neurons also regulate the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis, as discussed earlier and below, respectively. Coordinated increases of sympathetic and HPA tone in response to fasting-induced arousal directed by orexin neurons may further help animals to execute adaptive behavior along with autonomic and neuroendocrine responses.

Horvath and Gao recently showed that overnight food deprivation increased the number of excitatory synapses and synaptic currents onto orexin neurons, which

was reversed by re-feeding and blocked by leptin administration [59]. This surprising plasticity of orexin neurons further indicates the importance of their role in linking energy homeostasis to arousal regulation. Meanwhile, these mechanisms may hinder attempts to treat obesity by food restriction.

Another way in which orexin neurons contribute to feeding behavior is mediating efferent signals from the so-called “food-entrainable circadian oscillator (FEO)” to elicit food-anticipatory activity (FAA) and wakefulness, which will be discussed in “Orexin peptides and circadian rhythms” [97, 98].

Orexin peptides and drinking behavior

Orexins may act as a mediator that regulates drinking behavior, since ICV administration of orexins increases water intake in rats, and orexin-immunoreactive varicose axons were observed in the subfornical organ and area postrema - regions implicated in drinking behavior [99]. In addition, anti-diuretic hormone arginine-vasopressin (AVP) directly depolarizes orexin neurons through the V1a receptor [100, 101]. Similar to food deprivation, wild-type mice display increased locomotor activity when dehydrated, presumably increasing the ability of the animals to search for water, as well after ICV AVP administration. In contrast, orexin neuron-ablated mice failed to exhibit dehydration- and AVP-induced increases in locomotor activity, suggesting a key role of orexin neurons in water deprivation-induced hyperlocomotor activity [100].

Orexin peptides and the reward system

The roles of orexin neurons in the reward system have been a focus of recent attention. Dopaminergic projections of neurons in the midbrain ventral tegmental area

(VTA) to the forebrain, particularly to the NAc, have classically been identified as the “reward pathway”. Drugs of abuse stimulate this pathway. Orexin neurons have reciprocal connections with both the VTA and NAc (Fig. 3) [5, 50, 102]. Orexin directly activates VTA dopaminergic neurons through OX₁R [103, 104]. On the other hand, dopamine can inhibit orexin neurons by acting on α_2 -adrenoceptors and/or the dopamine D2 receptor in slice preparations [54, 56, 105].

Harris *et al.* recently demonstrated that activation of orexin neurons in the LH (but not in the PFA) was strongly linked to preferences for cues associated with drug and food rewards in rats [106]. Systemic administration of the selective OX₁R antagonist SB334867 during training significantly reduced place preference for morphine (a μ -opioid receptor agonist). In addition, chemical activation of lateral hypothalamic orexin neurons, as well as local administration of orexin-A peptide directly into the VTA, reinstated extinguished drug-seeking behavior [106]. Similarly, Narita *et al.* reported that morphine-induced place preference and hyperlocomotion observed in wild-type mice were abolished in orexin knockout mice [107]. Development of place preference was also blocked by injection of an OX₁R antagonist into the VTA. Synaptic plasticity induced by orexin in the VTA seems to underlie behavioral sensitization to cocaine. Borgland *et al.* demonstrated that orexin-A input to VTA dopamine neurons potentiates NMDAR (N-methyl-D-aspartate receptor)-mediated neurotransmission through a protein kinase C-dependent insertion of NMDARs in synapses on these neurons in slice preparations [108]. Furthermore, *in vivo* administration of an OX₁R antagonist blocked locomotor sensitization to cocaine and prevented cocaine-induced potentiation of excitatory currents in VTA dopamine neurons. These observations indicate a strong functional interaction between the orexinergic pathways and the dopaminergic system in the mechanisms of reward and

drug addiction. This interaction may underlie the hedonic control of feeding, as well as the facilitating role of orexin in male sexual behavior of rats [109].

Meanwhile, Boutrel *et al.* suggested that orexin-A also reinstates cocaine seeking by mechanisms different from increased dopamine release [110]. Similarly to the results described above, ICV infusion of orexin-A, although ineffective on cocaine intake in rats, led to dose-related reinstatement of previously extinguished cocaine-seeking and food-seeking behavior, and antagonism of OX₁R blocked foot shock-induced reinstatement of extinguished cocaine-seeking. In sharp contrast to the well-known threshold-lowering effect of cocaine, a drug of abuse that stimulates the VTA-forebrain dopaminergic pathway, orexin-A significantly elevated the intracranial self-stimulation (ICSS) threshold in rats, reflecting a decrease in the activity of brain reward systems. Orexin-induced reinstatement of cocaine seeking was prevented by blockade of the noradrenergic and corticotropin releasing factor (CRF) systems, suggesting that orexin-A reinstated drug seeking through induction of a stress-like state (see below). Therefore, the orexin-A and stress systems may closely interact to regulate cocaine-seeking behavior [110].

These findings highlight the key role of orexin in the mechanisms of reward and drug addiction. Indeed, orexin knockout mice develop attenuated morphine dependence as compared to wild-type controls, as measured by physical withdrawal responses [111]. Interestingly, narcolepsy patients with daytime sleepiness who were treated with amphetamine-like stimulants and/or GHB for a long time rarely developed drug addiction [112].

Orexin peptides and emotional states

Under fearful conditions, animals exhibit increased arousal and vigilance levels, accompanied by increased sympathetic outflow and HPA axis activity. Orexin neurons are likely to be involved in the coordinated regulation of these responses in stressful environments (Fig. 3). The PFA, in which orexin neurons reside, is known to be the center of defense responses, or the “fight or flight” response, which is characterized by a coordinated rise in arterial blood pressure, heart rate, and respiratory frequency [113, 114]. These defense responses are induced by activation of the BST and amygdala, or other regions involved in emotional responses. Recent tracing studies identified innervation to orexin neurons from several regions of the limbic system (a set of brain structures critical for processing of emotional information), including the amygdala, infralimbic cortex, NAc shell, lateral septum, and BST [49, 50]. The importance of projections from the limbic system to orexin neurons is readily apparent in defense responses. As compared with wild-type mice, orexin knockout mice and orexin neuron-ablated mice exhibited diminished cardiovascular and locomotor responses to the emotional stress evoked by resident-intruder tests and air-jet stress, respectively [90, 91].

Orexin also affects the HPA axis. Orexin neurons innervate the paraventricular hypothalamic nucleus (PVN) [5, 6], which abundantly expresses OX₂R [9, 10] and regulates hypothalamic outflow to the pituitary and the autonomic system. ICV administration of orexin-A increases the blood concentrations of corticotropin (ACTH) and corticosterone [34, 115] and strongly activates CRF-expressing neurons in the PVN and the central nucleus of the amygdala [116]. Consistent with their expected roles in the stress response, orexin neurons were activated by cold exposure or immobilization stress [116]. On the other hand, orexin neurons receive projections

from CRF neurons, and some of these cells (8 out of 32 examined) are directly activated by CRF through the CRF-R1 receptor [117]. Indeed, activation of orexin neurons by foot shock stress is severely impaired in CRF-R1-deficient mice [117]. Thus, a reciprocal link between the CRF system and orexin neurons may play an important role in coordinated regulation of arousal and the HPA axis.

As for positive emotional stimuli, neural input from the limbic system to orexin neurons may be implicated in the pathophysiology of cataplexy. In narcoleptic dogs, cataplexy is triggered by recognition of highly palatable food and excited play, but not by noxious stimuli or unfamiliar environments [118]. In mice, cataplexy is most frequently observed when animals are grooming, exploring, burrowing, and investigating the environment [12, 21]. In humans, cataplexy is most frequently elicited by laughter or pleasure, but not sadness or pain [118-120]. These facts suggest that activation of orexin neurons by positive emotion is required to prevent undesirable muscle atonia. Orexin neurons project to the mesencephalic locomotor region (MLR) and the PPT/LDT in the midbrain; cholinergic neurons in the PPT/LDT are implicated in REM-related atonia [48]. Local injection of orexin-A into the MLR enhances its activity, while injection into the PPT inhibits REM-related atonia in cats. Thus, limbic signals to the midbrain may induce locomotor activity and muscle tone when the orexinergic system functions normally, but elicit atonia (cataplexy) when orexinergic function is disturbed [48]. Indeed, orexin neurons are maximally active during exploratory behavior, and are less but still highly active during grooming and eating, whereas they are much less active during food aversion, which is manifested as repeated approaches to and withdrawal from a novel food and would not be expected to reflect positive emotion, despite the presence of comparable motor activity among these behaviors [63].

Projections to orexin neurons from the limbic system may also be important for maintaining activity of orexin neurons during the active period by conveying various emotional stimuli to orexin neurons. In addition, the limbic input to orexin neurons may be involved in the regulation of feeding behavior as well, since some of the affective content of the perception of food processed in the amygdala and limbic system may be passed on to orexin neurons [121].

Orexin peptides and nociception

Orexin-immunoreactive fibers innervate the spinal cord, especially dorsal root ganglion (DRG) neurons and lamina I and X surrounding the central canal [7]. OX₁R is localized on C-fibers in the spinal cord [122]. These data suggest that the spinal orexin system is involved in transmission of nociceptive information. Several studies have shown that orexin-A produces analgesic effects in rats [123, 124]. A recent study reported a critical role of orexin neurons in stress-induced analgesia (SIA) [125]. Orexin neuron-ablated mice did not exhibit SIA, although analgesia was induced by ICV administration of orexin-A. Nociceptin/orphanin FQ (N/OFQ) peptide blocked SIA in wild-type mice, while coadministration of orexin-A overcame N/OFQ inhibition of SIA. N/OFQ-containing fibers form synaptic contacts on orexin neurons and inhibit them. These results suggest that the CRF and the N/OFQ systems modulate orexin neurons in a coordinated manner to regulate SIA, a key component of the defensive behavioral “fight or flight” response [125].

Orexin peptides and circadian rhythm

Direct input to orexin neurons from the suprachiasmatic nucleus (SCN), in which the central circadian clock is located, seems to be sparse. Orexin neurons,

however, receive abundant innervations from the BST, the supraventricular zone, and the DMH [49, 50], all of which receive input from the SCN [126]. This indicates that orexin neurons may receive signals indirectly from the SCN via these regions to regulate the circadian rhythm of the sleep/wake cycle.

For animals to survive effectively, the timing of feeding-related behavior should be appropriately coordinated both with the environmental conditions, such as food availability and risk of predation, and with the internal physiological state, such as gastrointestinal function and energy balance. The central circadian clock in the SCN is regulated according to environmental light/dark cues conveyed from the eye (light-entrainable oscillator, LEO) [127]. Independently of LEO, daily restricted feeding (in which food availability is restricted to a single period scheduled at a fixed time of the day) produces an anticipatory increase in locomotor activity (FAA), as well as in many physiological events, in the hours preceding food availability [128]. These changes in biological rhythms have been postulated to be brought about by the FEO, independent of the central circadian clock in the SCN. Orexin neuron-ablated mice showed a severe deficit in the normal food anticipatory increases in wakefulness and locomotor activity under restricted feeding [97, 98]. Moreover, activity of orexin neurons markedly increased during the food-anticipatory period under restricted feeding in wild-type mice. Since entrainment to feeding per se seemed to be normal in both genotypes, orexin neurons are suggested to function in an efferent pathway of a FEO in eliciting food-anticipatory activity and wakefulness [97].

Integration of external and internal environmental information by orexin neurons

In summary, not only do orexin neurons contribute to sleep/wake regulation by stabilizing the activity of wake-promoting neural circuits, they are involved in sensing the body's external and internal environments, and regulate states of sleep and wakefulness accordingly, which is beneficial for survival (Fig. 3). They further coordinate autonomic tone and hormonal balance with arousal to maintain homeostasis or homeodynamics. In addition, orexin neurons may have a direct impact on feeding and the reward system in a manner independent of sleep/wake regulation. Thus, the reciprocal interactions between orexin neurons and multiple neuronal systems raise the possibility that orexin neurons function as an interface between multiple regulatory systems including feeding, reward, emotional, circadian, autonomic, and endocrine systems.

As yet unrecognized physiological roles of orexin peptides

Using transgenic mice in which a genetically encoded calcium indicator (Yellow Cameleon, Yc2.1) is expressed specifically in orexin neurons, we have screened for factors that affect the activity of these neurons [101]. This screen not only confirmed the effects of several factors, including GABA, glucose, serotonin, noradrenaline, and leptin, that had been shown to have an influence on orexin neurons by slice electrophysiological studies, but also identified a sulphated octapeptide form of cholecystinin (CCK-8S), neurotensin, oxytocin, and AVP as novel activators of orexin neurons, leading to further elucidation of the physiological interaction between AVP and orexin neurons in water deprivation-derived hyperactivity as described earlier [100]. Studies using electrophysiological, pharmacological, behavioral, and

physiological techniques on other activators of orexin neurons in wild-type mice, as well as mutant mice in which receptors for these activators are deleted specifically in orexin neurons, would further uncover unexpected roles of orexin neurons in the regulation of behavior and physiology.

Conclusion and perspective

Symptoms of narcolepsy-cataplexy unequivocally reveal that orexins and orexin receptors play crucial roles in regulation of sleep/wake states. In addition, the existence of reciprocal connections between the orexin system and multiple neuronal systems indicates that orexin neurons provide crucial links between multiple brain functions, such as energy homeostasis, the reward processing, emotion, and arousal. Future studies with full use of mouse molecular genetics, such as selective deletion of the gene for a particular receptor or signaling molecule in orexin neurons, might lead to further understanding of the integrative physiology orchestrated by the orexin system.

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

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Abbreviations

ACTH = corticotropin
ARC = arcuate nucleus
AVP = arginine-vasopressin
BF = basal forebrain
BST = bed nucleus of the stria terminalis
CCK-8S = sulphated octapeptide form of cholecystokinin
CNS = central nervous system
CRF = corticotropin releasing factor
CSF = cerebrospinal fluid
DMH = dorsomedial hypothalamic nucleus
DRG = dorsal root ganglion
EEG/EMG = electroencephalogram/electromyogram
FAA = food-anticipatory activity
FEO = food-entrainable circadian oscillator
GHB = γ -hydroxybutyrate
GIRK = G-protein-regulated inwardly rectifying K⁺ channel
GPCR = G-protein coupled receptors
HLA = Human leukocyte antigen
HPA = hypothalamic-pituitary-adrenal
ICSS = intracranial self-stimulation
ICV = intracerebroventricular
LC = locus coeruleus
LDT = laterodorsal tegmental nucleus
LEO = light-entrainable oscillator
LH = lateral hypothalamus
MCH = melanin-concentrating hormone
MLR = mesencephalic locomotor region
N/OFQ = nociceptin/orphanin FQ
NAc = nucleus accumbens
NMDAR = N-methyl-D-aspartate receptor
NPY = neuropeptide Y

NREM = non-REM
NTS = nucleus of the solitary tract
OX1R = orexin 1 receptor
OX2R = orexin 2 receptor
PFA = perifornical area
PPT = pedunculo pontine tegmental nucleus
PVN = paraventricular hypothalamic nucleus
REM = rapid eye movement
SB334867 = (1-(2-methylbenzoxazol-6-yl)-3-[1,5]naphthydrin-4-yl urea HCl
SCN = suprachiasmatic nucleus
SIA = stress-induced analgesia
SSRI = selective serotonin reuptake inhibitors
TCA = tricyclic antidepressants
TMN = tuberomammillary nucleus
VLPO = ventrolateral preoptic nucleus
VMH = ventromedial hypothalamic nucleus
VTA = ventral tegmental area

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Figure legends

Fig. (1). Sleep state abnormalities in orexin receptor-knockout mice.

Typical representative 12 hour dark period (20:00–08:00) hypnograms for wild-type (WT), OX₁R knockout (OX₁R KO), OX₂R knockout (OX₂R KO) and double-receptor knockout mice (OX₁R/OX₂R DKO), all on a C57B/6J background, are shown. The different levels above the baseline indicate states of sleep and wakefulness (REM, rapid eye movement (REM) sleep; NREM, non-REM (NREM) sleep; Wake, awake) of the mouse at the time. Episodes of direct transition from wakefulness to REM sleep are shown by red arrowheads. Note the greater awake/NREM sleep episode fragmentation and reduced duration of wakefulness in the hypnograms of OX₂R knockout and double-receptor knockout mice compared with wild-type and OX₁R knockout mice. Episodes of direct transition from wakefulness to REM sleep were not observed in OX₁R knockout mice, and were hardly observed in OX₂R knockout mice, whereas they were frequently observed in double-receptor knockout mice. Hypnograms were obtained by simultaneous electroencephalographic (EEG) and electromyographic (EMG) recording for 4 weeks (N = 18–40).

Fig. (2). Schematic drawing showing main projections of orexin neurons.

This figure summarizes predicted orexinergic projections in the human brain. The distributions of orexin fibres and receptors (OX₁R, OX₂R) are predicted from the results of studies on rodent brains, as most histological studies on the orexin system have been carried out on rodents. Circles show regions with strong receptor expression and dense orexinergic projections. Orexin neurons originating in the lateral hypothalamic area (LHA) and posterior hypothalamus (PH) regulate sleep and wakefulness and the maintenance of arousal by sending excitatory projections to the

entire CNS, excluding the cerebellum, with particularly dense projections to monoaminergic and cholinergic nuclei in the brain stem and hypothalamic regions [4-6, 10, 32, 42, 43, 103, 129], including the locus coeruleus (LC, containing noradrenaline), tuberomammillary nucleus (TMN, containing histamine), raphe nuclei (raphe, containing serotonin) and laterodorsal/pedunculopontine tegmental nuclei (LDT/PPT), containing acetylcholine). Orexin neurons also have links with the reward system through the ventral tegmental area (VTA, containing dopamine) and with the hypothalamic nuclei that stimulate feeding behavior. Anatomical image adapted, with permission, from [130].

Fig. (3). Interactions of orexin neurons with other brain regions implicated in sleep and wakefulness.

Orexin neurons in the lateral hypothalamic area (LHA) and posterior hypothalamus (PH) are anatomically well placed to provide a link between the limbic system, systems involved in energy homeostasis, and monoaminergic and cholinergic neurons in the brain stem. Solid arrows show excitatory projections, and broken lines inhibitory ones. Wake-active regions, sleep-active regions and REM-active regions are shown by red, blue and green boxes, respectively. Orexin neurons promote wakefulness through the monoaminergic nuclei that are wake-active. Stimulation of dopaminergic centers by orexins can modulate reward systems (purple). Peripheral metabolic signals such as leptin, ghrelin and glucose influence orexin neuronal activity to coordinate arousal and energy homeostasis. The nucleus suprachiasmaticus (SCN), the central body clock, sends signals to orexin neurons via the dorsomedial hypothalamus (DMH). Input from the limbic system (amygdala and bed nucleus of the stria terminalis (BST)) might regulate the activity of orexin neurons upon

presentation of emotional stimuli that evoke emotional arousal or fear-related responses. VLPO, ventrolateral preoptic area; DR, dorsal raphe; GABA, γ -aminobutyric acid; LC, locus coeruleus; LDT, laterodorsal tegmental nucleus; PPT, pedunculopontine tegmental nucleus; SNr, substantia nigra pars reticulata; TMN, tuberomammillary nucleus.

Fig. (4). Mechanisms by which the orexin system stabilizes sleep and wakefulness.

The figure represent the functional interactions between orexin neurons, monoaminergic wake-active centers and the ventrolateral preoptic area (VLPO) sleep-active center during various states of sleep and wakefulness. Red arrows show excitatory input, and blue arrows inhibitory input. The thickness of arrows represents the relative strength of excitatory and inhibitory input, respectively. Circle sizes represent relative activities of each region. **(a) Awake state.** Orexin neurons send excitatory influences to monoaminergic neurons, which send inhibitory feedback projections to orexin neurons. This system might maintain the activity of monoaminergic neurons. A slight decrease in input to the monoaminergic neurons results in decreased inhibitory influence on orexin neurons. Orexin neurons, therefore, are disinhibited and have an increased excitatory influence on monoaminergic cells to maintain their activity. These monoaminergic cells send excitatory projections to the thalamus and cerebral cortex, and send inhibitory projections to the VLPO sleep center. These mechanisms maintain wakefulness states. **(b) Sleep state.** VLPO sleep-active neurons are activated and send inhibitory projections to monoaminergic neurons and orexin neurons to maintain sleep. **(c) Narcolepsy.** If orexin neurons are removed, monoaminergic neurons and VLPO neurons set up a mutually inhibitory

circuit, which can cause unwanted and abrupt transitions between the wake/sleep states. Activity in one of the competing sides shuts down inhibitory input from the other side, and therefore disinhibits its own action. So, when either side begins to overcome the other, there is an abrupt switch to the alternative state.

Fig.(1)

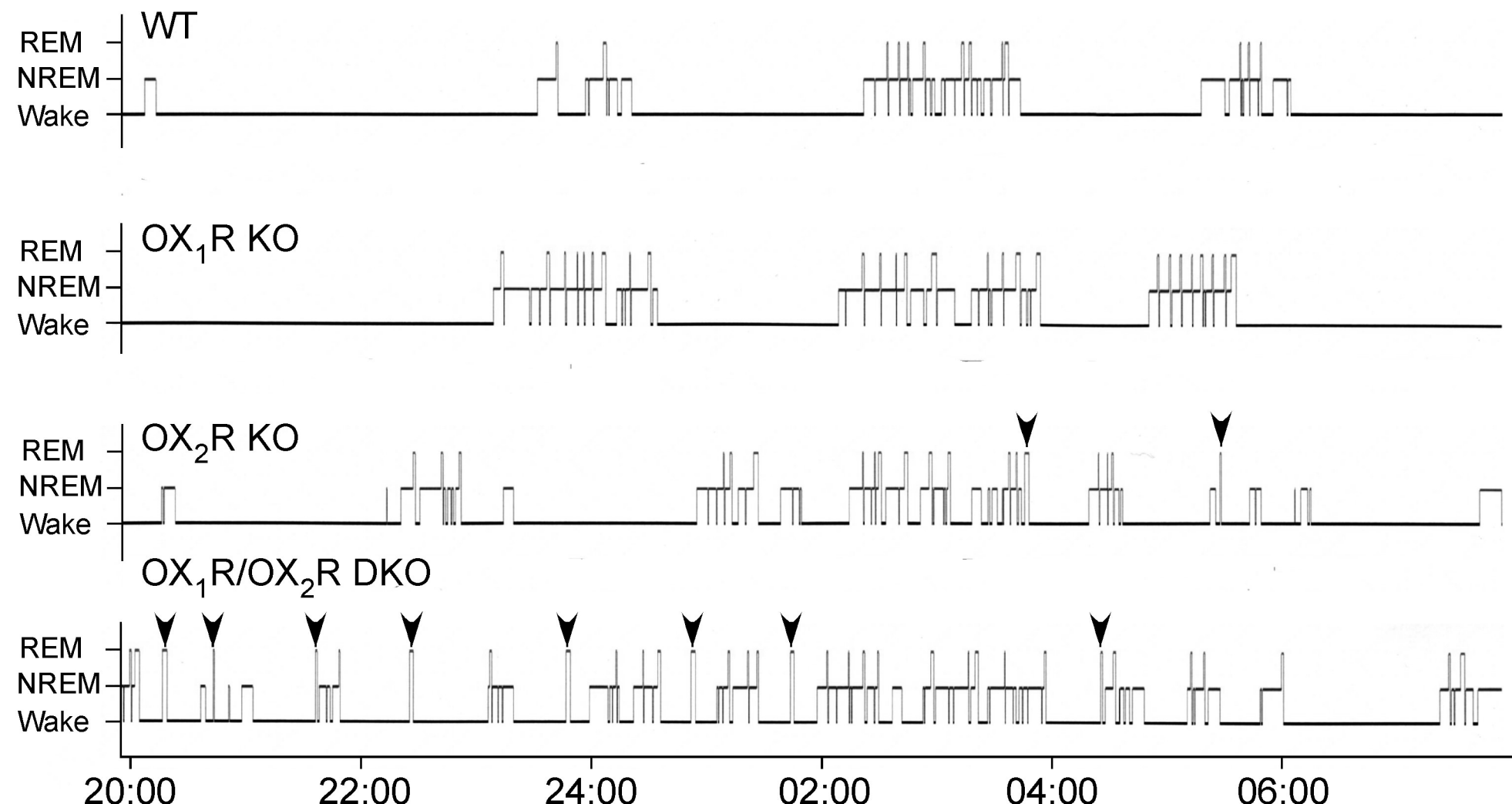


Fig.(2)

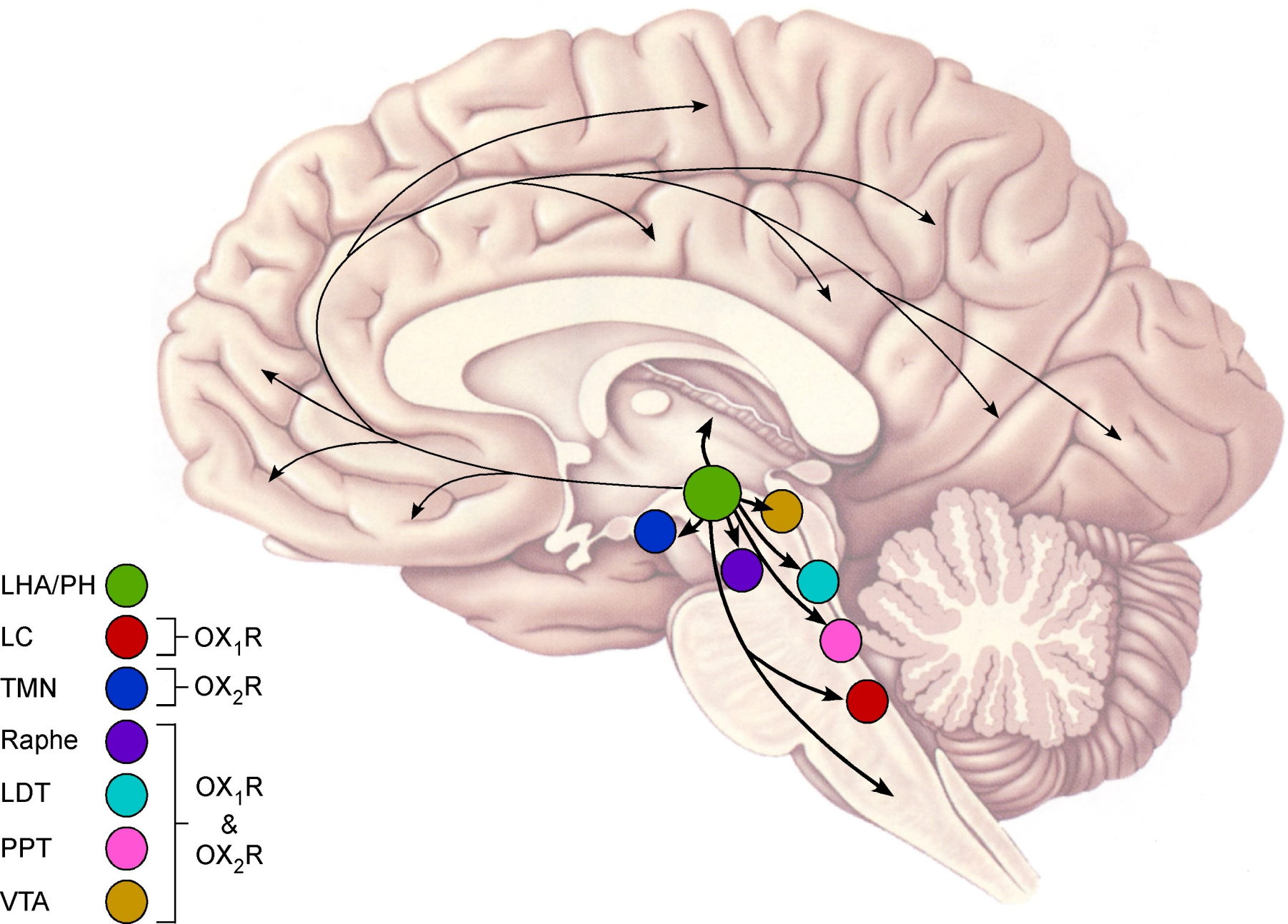


Fig.(3)

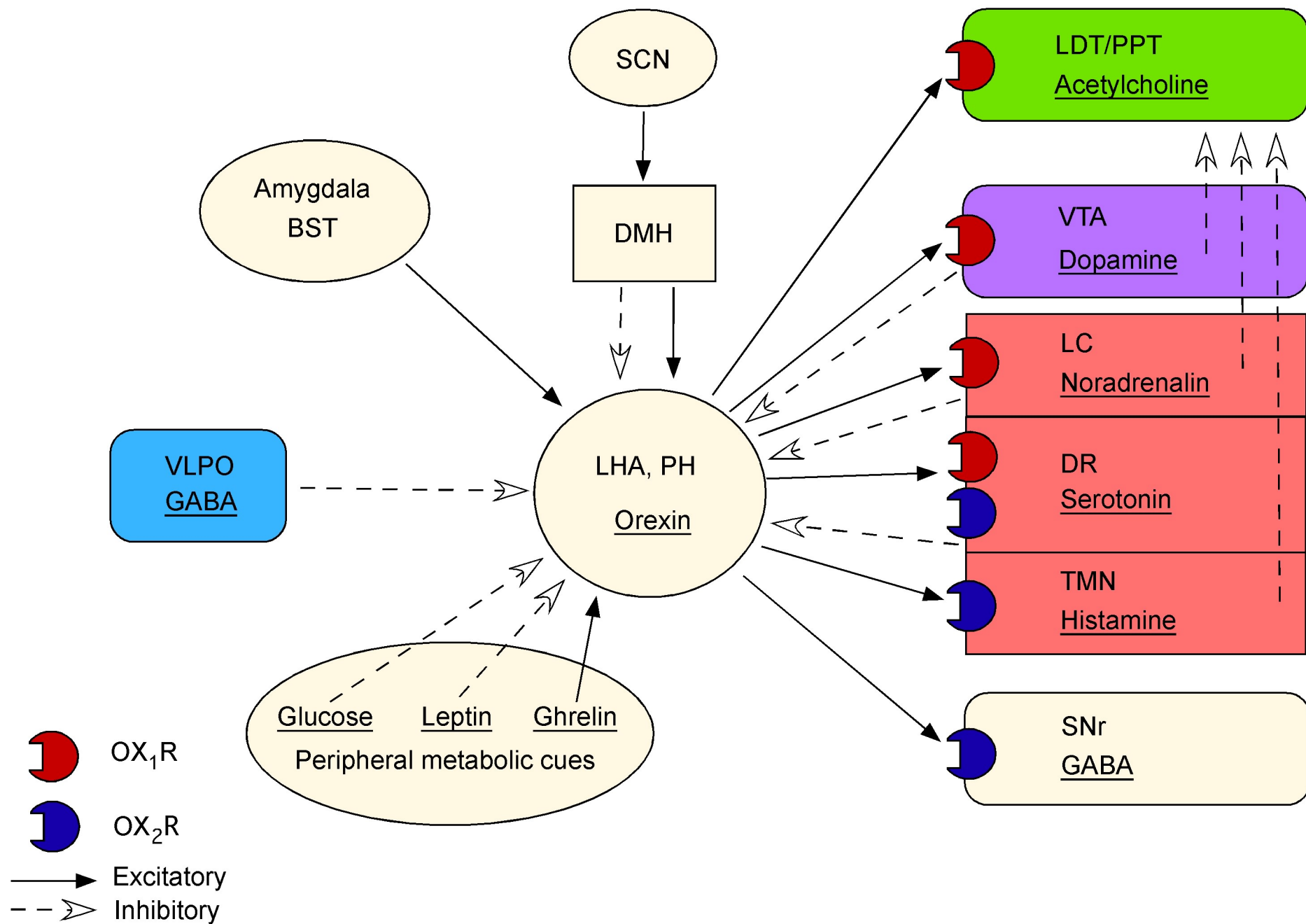


Fig.(4)

