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·Review·

Central functions of the orexinergic system

Xiao-Yang Zhang, Lei Yu, Qian-Xing Zhuang, Jing-Ning Zhu, Jian-Jun Wang
Department of Biological Science and Technology and State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing 210093, China
Corresponding authors: Jian-Jun Wang and Jing-Ning Zhu. E-mail: jjwang@nju.edu.cn; jnzhu@nju.edu.cn

The neuropeptide orexin is synthesized by neurons exclusively located in the hypothalamus. However, these neurons send axons over virtually the entire brain and spinal cord and therefore constitute a unique central orexinergic system. It is well known that central orexin plays a crucial role in the regulation of various basic non-somatic and somatic physiological functions, including feeding, energy homeostasis, the sleep/wake cycle, reward, addiction, and neuroendocrine, as well as motor control. Moreover, the absence of orexin results in narcolepsy-cataplexy, a simultaneous somatic and non-somatic dysfunction. In this review, we summarize these central functions of the orexinergic system and associated diseases, and suggest that this system may hold a key position in somatic–non-somatic integration.

Keywords: orexin; sleep/wake cycle; motor control; narcolepsy-cataplexy; integration of somatic–non-somatic responses

Introduction

In 1998, a novel family of neuropeptides, named orexins or hypocretins, was isolated from the central nervous system by two groups[1,2]. Using subtraction hybridization, de Lecea *et al.*[1] first identified the mRNA sequence encoding prepro-hypocretin, the putative precursor of hypocretins (named for their exclusive expression within the hypothalamus and sharing substantial amino-acid identity with the gut hormone secretin). Almost simultaneously, while searching for endogenous peptide ligands for multiple orphan G protein-coupled receptors, Sakurai *et al.*[2] identified the same neuropeptides. In view of their stimulation of food intake by central administration, Sakurai *et al.*[2] called these peptides “orexins”, derived from the Greek word “orexis” meaning appetite. It soon became clear that the peptides isolated by the two groups were, in principle, identical. In this review, we use the orexin nomenclature.

Orexin is synthesized by neurons located exclusively in the hypothalamus (prefornical area, lateral hypothalamic area (LHA) and posterior hypothalamus)[1-3]. From this restricted region, the orexinergic neurons project extensively to almost the whole brain, constituting the central orexinergic system[3-5]. Moreover, orexin and orexin receptors are also found outside the central nervous system, such as in the pancreas and gastrointestinal tract, where they affect insulin release, intestinal motility and secretion[6]. Intriguingly, accumulating evidence reveals that the central orexinergic system holds a key position in many important basic physiological functions, including non-somatic regulation, such as feeding, energy homeostasis, the sleep/wake cycle and neuroendocrine, as well as somatic motor control. Clearly, an intact behavior comprises both somatic (motor) and non-somatic (e.g., visceral, emotional, and cognitive) components and requires somatic–non-somatic integration involving various brain regions and discrete neuronal pathways[7,8]. By linking the non-somatic centers to somatic motor structures, the central orexinergic system may actively participate in somatic–non-somatic integration, which is crucial for the generation and execution of appropriate and coordinated behavioral responses to changes in the internal and external environments.

Orexins and Orexin Receptors

There are two splice variants of orexin, A and B (Fig. 1), both of which are derived from a common precursor peptide, prepro-orexin. Orexin A is composed of 33 amino-acids (mol. wt. 3562 Da) and orexin B of 28 (2937 Da), with 46% amino-acid identity. Strong homology is found in their C-terminal halves (73%). The amino-acid sequences of orexins A and B in both non-mammalian vertebrates and mammalian species are conserved[9,10], indicating that they are phylogenetically old neurotransmitters/neuromodulators.

Two orexin receptors subtypes, orexin 1 receptor (OX1R) and orexin 2 receptor (OX2R), have been identified (Fig. 1). Both belong to the family of G protein-coupled receptors[2], and they share an overall 64% sequence identity. OX1R has an order-of-magnitude greater affinity for orexin A than for orexin B, whereas OX2R is relatively nonselective between the two peptides[2,11]. It is also known that both receptor genes are highly conserved among mammalian species[9,10]. While orexins are produced in restricted locations of the hypothalamus, orexin receptors are expressed in diverse regions in the brain and spinal cord. Moreover, the distributions of the receptors are generally in agreement with the innervation by orexinergic neurons[12-14]. The distribution patterns of OX1Rs and OX2Rs overlap but are partially distinct in the central nervous system. For instance, regions such as the locus coeruleus, the laterodorsal tegmental nucleus, and the pedunculopontine tegmental nucleus mainly express OX1R, whereas the tuberomammillary nucleus (TMN) of the hypothalamus, the nucleus accumbens, and the septal nuclei mainly express OX2R[15]. The different distribution patterns suggest that the two orexin receptor subtypes play different physiological roles.

It is worthwhile to note that orexinergic neurons typically have varicose axon terminals rather than classical chemical synaptic specializations[16], and orexin receptors are metabotropic and the orexinergic projections are extensively distributed[9,10]. Thus, the central orexinergic system may act as a general modulator for whole-brain activity.

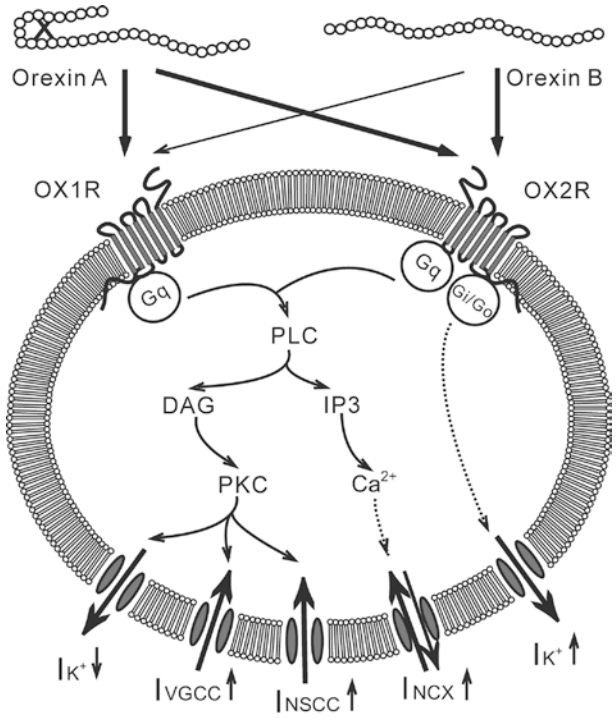

Fig. 1. Orexins, orexin receptors and the underlying signal transduction pathways. The actions of orexin A and orexin B are mediated *via* two G-protein-coupled receptors named OX1R and OX2R. OX1R has an order-of-magnitude greater affinity for orexin A than for B, whereas OX2R binds both with similar affinity. OX1R is coupled exclusively to the Gq subclass of heterotrimeric G-proteins, whereas OX2R can couple to not only Gq but also Gi/Go. Stimulation of Gq by orexins binding to the receptors activates both the PLC–DAG–PKC and PLC–IP3–Ca^{2+} (released from intracellular stores) pathways. The activation of PKC results in the enhancement of nonselective cation channels (NSCC) and voltage-gated Ca^{2+} channels (VGCC) and inhibition of K^+ currents (I_{K+}). And the store-operated Ca^{2+} influx may lead to the enhancement of the Na^+-Ca^{2+} exchanger (NCX), which has yet to be confirmed. In addition, OX2R-mediated activation of G_i/G_o may result in the enhancement of K^+ currents. However, the direct electrophysiological effect of orexin mediated by $G_{i/o}$ protein remains undetected. DAG, diacylglycerol; IP3, inositol-1,4,5-triphosphate; PKC, protein kinase C; PLC, phospholipase C.

Signal Transduction Pathways Coupled to Orexin Receptors

Orexins exert a quite uniform excitatory effect on neurons in the central nervous system, although they can also inhibit neurons through undefined presynaptic mechanisms. The G protein-coupled receptors OX1Rs and OX2Rs are, as yet, the only known receptors that respond to orexins. It is well known that the family of G_q proteins, a subclass of heterotrimeric G proteins, consists of several subtypes, in-

cluding G_q, G_{11}, G_{14} and $G_{15/16}$. The G_q family mainly signals *via* the G_α subunit to activate phospholipase C (PLC), which then induces Ca^{2+} release from the endoplasmic reticulum *via* IP3 and the activation of protein kinase C (PKC) *via* diacylglycerol. Since OX1Rs and OX2Rs elicit either extracellular Ca^{2+} influx by PLC-PKC voltage-gated Ca^{2+} channels/transient receptor potential channel pathway or endoplasmic reticulum Ca^{2+} release by the PLC–IP3 pathway, both orexin receptors are considered to be G_q protein-coupled receptors (Fig. 1)[11,17]. On the other hand, besides the G_q pathway, OX2Rs may also be independently coupled to other signal transduction pathways. In OX2R-transfected neuron-like cells, orexin inhibits forskolin-stimulated cAMP accumulation in a dose-dependent manner, and this is abolished by pretreatment with pertussis toxin, while in OX1R-transfected cells the inhibitory effect of orexin does not occur[18], suggesting that OX2Rs are also coupled to the pertussis toxin-sensitive $G_{i/o}$ pathway (Fig. 1). Even though the contribution of $G_{i/o}$ proteins to orexin-mediated cellular responses in the central nervous system remains unknown, the distinct signal transduction mechanisms may account for the different central physiological functions mediated by the two orexin receptors.

The ionic mechanisms underlying the orexin-induced depolarization/excitation of neurons are quite complicated and distinct in different brain regions/nuclei. The currently-known mechanisms are mainly inhibition of K^+ channels, activation of non-selective cation channels, activation of the electrogenic Na^+/Ca^{2+} exchanger, and activation of voltage-gated Ca^{2+} channels (Fig. 1)[6]. In addition, interactions between orexin receptors and NMDA channels have been reported[19,20].

Central Physiological Functions of Orexin and Related Diseases

Sleep/Wakefulness and Narcolepsy

The central function of orexin that attracts most attention is regulation of the sleep/wake cycle, since orexin- or OX2R-deficient mice and dogs exhibit symptoms strikingly similar to human narcolepsy[21-24], that affects ~1/2 000 persons. Narcolepsy is a chronic sleep disorder characterized by a primary disorganization of behavioral states. A cardinal symptom of narcolepsy is excessive daytime sleepiness, with irresistible sleep attacks at inappropriate times, such as while at work. In narcolepsy patients, the latency for rapid eye-movement (REM) sleep is notably reduced and REM sleep can even intrude directly into wakefulness. It has been reported that human narcoleptics have an 85–95% reduction in the number of orexin neurons[25,26]. Similarly, orexins are undetectable in the cerebrospinal fluid of these patients[27].

Experimental studies have provided substantial evidence for the regulation of sleep and wakefulness by the central orexinergic system. Orexin A or orexin B, intracerebroventricularly administered at the onset of the normal sleep period, produces a significant increase in the time the animal spends awake and a significant decrease in the proportion of REM and non-REM sleep[28,29]. Moreover, c-fos expression in orexin neurons[30] and the orexin levels in cerebrospinal fluid[31] correlate positively with the amount of wakefulness and negatively with the amounts of non-REM and REM sleep. Furthermore, in unanesthetized rats, orexin neurons discharge during active waking, reduce discharges during quiet waking, and virtually cease firing during sleep[32]. All of these findings show that orexin neurons are relatively active during wakefulness and inactive during sleep.

Currently, it is agreed that orexin regulates the sleep/wake cycle through its activation of arousal-promoting monoaminergic systems. Orexin receptors are distributed abundantly in monoaminergic neurons in the raphe nuclei, locus coeruleus and TMN[12-14]. Besides, the regions where monoamine neurons are concentrated receive a large number of projections from orexin neurons[4,5]. These results suggest that the monoaminergic systems are important targets of orexin neurons. Actually, electrophysiological studies have demonstrated that orexin excites monoamine systems, including noradrenergic neurons in the locus coeruleus[28], dopaminergic neurons in the ventral tegmental area (VTA)[33] and serotoninergic neurons in the dorsal raphe nuclei[34,35] as well as histaminergic neurons in the TMN[36]. These monoaminergic neurons project diffusely to the cerebral cortex, thalamus and brainstem, and are highly active during wakefulness, less active during non-REM sleep and rarely active during REM sleep. Thus, it is likely that orexin neurons enhance and stabilize the activity of monoaminergic neurons to promote wakefulness[37]. Be-

sides, orexin has a strong and direct excitatory effect on the cholinergic neurons of the basal forebrain which contribute to the cortical activation associated with wakefulness[38].

Among the monoaminergic systems, the histaminergic neurons in the TMN seem to be the most important target for the orexinergic system in sleep/wake regulation. Several reports demonstrate that orexin promotes arousal mainly by activating histaminergic neurons *via* OX2Rs[36,39]. Intracerebroventricular infusion of orexin A significantly increases the duration of the waking state. Pretreatment with pyrilamine, a histamine H_1 receptor antagonist, strikingly decreases the orexin A-induced wake time[36]. Similarly, orexin has no effect on the wake time in H_1-receptor knockout mice when administered centrally[40]. Interestingly, OX1Rs and OX2Rs may play different roles in the regulation of wakefulness. OX2R knockout mice display clear characteristics of narcolepsy, whereas OX1R knockout mice do not have overt behavioral abnormalities, with only increased fragmentation of sleep/wake states[41]. However, OX1Rs also play an important role in sleep/wake regulation. The narcolepsy syndrome that orexin knockout mice exhibit appears to be much more severe than that of OX2R knockout mice[24]. Considering that histaminergic TMN neurons express mainly OX2Rs but locus coeruleus norepinephrine neurons express mainly OX1Rs[15], the histaminergic system originating from the TMN may be a key target for the central orexinergic system to regulate the sleep/wake cycle.

Besides the innervation of wake-promoting monoaminergic neurons, orexin neurons also receive inhibitory GABAergic projections from sleep-active neurons in the ventrolateral preoptic area (VLPO)[42], which plays a critical role in the initiation of non-REM sleep and the maintenance of both non-REM and REM sleep. Selective deletion of $GABA_B$ receptors in orexin neurons results in highly unstable sleep/wake architecture in mice[43], indicating that the inhibitory pathway from the VLPO may be important for turning off arousal orexin neurons during sleep. Thus, the central orexinergic system may link the VLPO sleep-active neurons with the wake-active monoaminergic neurons and hold a key position in sleep/wake regulation, while loss of the link established by the orexinergic system may result in narcolepsy.

Motor Control and Cataplexy

Among narcoleptic patients, ~70% experience a dangerous complication named cataplexy. Cataplexy is an attack characterized by the sudden loss of muscle tone, which is most often triggered by strong emotional stimuli. During cataplexy, patients remain conscious. Notably, orexin deficiency in humans, dogs, and rodents results in not only narcolepsy but also cataplexy[9,21], highlighting the possibility of a direct modulatory role of orexin in motor control. Since cataplexy is closely associated with narcolepsy and often triggered by strong emotions, the effect of orexin on the motor system has always been considered secondary to its actions on the neuronal circuits controlling sleep or emotions[9,10].

However, numerous neuroanatomical and immunohistochemical studies reveal that essential subcortical motor structures, such as the basal ganglia, cerebellum, and vestibular nucleus, receive direct innervation from orexin neurons[4,5]. Moreover, during movements, orexinergic neurons are particularly active[30,44] and orexin release increases[45]. The evidence suggests that the orexinergic system directly participates in central motor control.

Orexin neurons in the LHA and prefornical area are known to project to regions in the midbrain, including the substantia nigra (SN) and mesopontine tegmentum[4,5]. The latter contains the mesencephalic locomotor region and pedunculopontine nucleus, both of which are involved in the initiation and modulation of locomotion and other stereotyped movements. In decerebrate cats, injecting orexin A into the mesencephalic locomotor region reduces the intensity of electrical stimulation required to induce locomotion on a treadmill or even elicits locomotor movements without electrical stimulation[46]. On the other hand, microinjection of orexin A into the pedunculopontine nucleus increases the stimulus intensity required to induce muscle atonia[46]. Furthermore, orexins dose-dependently and selectively activate GABAergic neurons of the SN pars reticulata and not dopaminergic neurons in the SN pars compacta[47]. Injecting orexin A into the pars reticulata reduces the inhibitory effect of the pedunculopontine nucleus on muscle atonia[46]. In addition, accumbens shell-induced locomotor activity is greatly strengthened by activation of OX2Rs after local orexin A infusion[48].

Since cataplexy is a sudden and transient episode of loss of muscle tone, the central motor areas/nuclei which directly control muscle tone are particularly focused on. A recent study from our laboratory[49] first reported a direct

excitatory effect of orexins on neurons in the cerebellar interpositus nucleus, one of the final outputs of the spinocerebellum, which mainly regulates muscle tone and fine-tunes ongoing movements of the body and limbs. Furthermore, we demonstrated that orexin increases not only the excitability but also the sensitivity of projection neurons in the lateral vestibular nucleus, which directly contributes to the adjustment of muscle tone for both postural maintenance and alternation between extensor and flexor phases during locomotion[50]. The homogeneous excitatory effect of orexin on these neurons involved in motor control suggests that the central orexinergic system acts to excite motor structures uniformly and in parallel. Therefore, common excitation by orexin may help the motor structures maintain excitability at a certain level and also provide for an appropriate level of sensitivity to inputs coding changes in the internal and external environments. Intriguingly, local microinjection of orexin A improves vestibular-related motor behaviors in rats, including posture, balance, and negative geotaxis against the gravitational field[50], implicating orexin and the central orexinergic system in the direct control of somatic motor behavior. More importantly, the direct motor effect of endogenous orexin is critical when an animal faces a major motor challenge as opposed to during rest and general movements[50]. Thus, during a significant behavioral challenge, the increased excitatory drive and sensitivity due to the release of orexin may be essential for ensuring the prompt and appropriate magnitude of motor responses. This may account for why, when encountering an unexpected challenge that requires a strong motor response, orexin deficiency results in cataplexy.

Feeding and Energy Homeostasis

In the earlier publications dealing with orexin, it was reported that intracerebroventricular administration promotes feeding[2]. But the orexin-induced food intake is only an acute effect. Chronic continuous intracerebroventricular infusion of orexin A results in an increase in daytime food intake but a decrease in nighttime food intake in rats[51]. Thus, on the whole, there is almost no increase in the total amount of food intake per day. Many brain regions/nuclei, including the arcuate nucleus (ARC), prefornical area, LHA, dorsomedial hypothalamic nucleus, paraventricular nucleus (PVN), VTA and nucleus accumbens, are effective sites for the injection of orexin to promote feeding[52]. The promotion effect is blocked significantly by central pretreatment with anti-orexin A antibodies and the highly selective OX1R antagonist SB-334867[53,54].

Orexin fibers project densely into the ARC[4,5], which contains first-order neurons responsive to circulating adiposity signals and is involved in the regulation of food intake. Intracerebroventricular administration of orexin A induces high c-fos expression in orexigenic neuropeptide Y (NPY)-containing neurons in the ARC as well as increasing food consumption by animals. Pretreatment with BIBO3304, an NPY-Y1 receptor-specific antagonist, partially inhibits orexin-induced feeding behavior, suggesting that the NPY system may be one of the downstream pathways by which orexin A stimulates feeding[55]. However, since BIBO3304 does not completely abolish the effect of orexin A, other pathways may also be involved in orexin A-induced feeding behavior. Electrophysiological studies have shown a possible involvement of the anorexigenic pro-opiomelanocortin (POMC) system. Apart from exciting NPY-containing neurons[56,57], orexin also inhibits POMC-containing neurons in the ARC by attenuating $[Ca^{2+}]_i$ oscillations and decreasing $[Ca^{2+}]_i$ levels[58,59]. Intriguingly, the central orexinergic system may regulate feeding behavior through its innervation of the limbic system[60]. Infusion of orexin A into the shell of the nucleus accumbens, a limbic forebrain area strongly linked to eating motivation, increases feeding[48]. Moreover, intra-accumbens shell administration of muscimol, the $GABA_A$ receptor agonist, induces intense hyperphagia in rats and high c-fos expression in orexin-containing neurons[61]. These results indicate that orexin may influence food intake by appetitive modulation.

Despite the fact that orexin significantly increases food intake in the daytime, continuous intracerebroventricular administration has no effect on body weight[51] and daily intraparaventricular orexin treatment even induces weight loss[62] in rats. On the other hand, in orexin- or orexin receptor-knockout mice, body weight is increased[23,41,63]. All these seemingly paradoxical results indicate that orexin may be actively involved in energy metabolism rather than only in food intake. Actually, feeding is not an isolated behavior but is closely associated with wakefulness, activity and even higher brain functions. In wild-type mice, food deprivation triggers a drop in circulating glucose and leptin levels and an elevation in ghrelin signaling, which leads to the acti-

vation of orexin neurons to induce a significant increase in vigilance, exploration and locomotor activity[64]. In contrast, orexin neuron-ablated animals fail to exhibit fasting-induced wakefulness as well as locomotor activity[64]. Thus, it is speculated that reduced energy expenditure may be the reason for the increased body weight in orexin-deficient rodents. More recently, Sellayah *et al*.[65] reported that mice deficient in orexin gain more weight when fed the same high-fat diet as normal mice. The underlying mechanism involves brown-fat hypoactivity, which also leads to dampening of energy expenditure. Therefore, by modulating energy expenditure, orexin plays an integral role in adaptive energy homeostasis and body weight regulation.

Reward

Psychostimulants, such as amphetamine and methylphenidate, have been widely used to treat narcolepsy; however, interestingly, the phenomenon of drug addiction rarely occurs in these patients[66]. Therefore, it is suggested that the orexinergic system may be necessary for the formation of addiction and plays important roles in reward processing. This hypothesis was later confirmed in animal models. Subcutaneous morphine-induced place preference and hyperlocomotion are abolished in orexin knock-out mice, demonstrating a crucial link between the central orexinergic system and reward-seeking behavior[67,68].

Orexin neurons receive projections from the VTA and nucleus accumbens[42], both of which are widely implicated in the reward system, drug addiction, and motivation. Correspondingly, the VTA also receives massive inputs from orexin neurons in the LHA and prefornical area[4,5,69]. These reciprocal connections may constitute a basis for the involvement of the orexinergic system in the regulation of reward circuitry. Electrophysiological data have documented that orexins directly activate dopamine neurons in the VTA[70]. In addition, both intracerebroventricular infusion of orexin A and its administration directly into the VTA lead to dose-related reinstatement of drug-seeking[71,72]. Pretreatment of alcohol-preferring rats with the OX1R antagonist SB-334867 completely abolishes the olfactory cue-induced reinstatement of alcohol-seeking behavior[73]. Moreover, *in vitro* application of orexin A potentiates N-methyl-*D*-aspartate receptor (NMDAR)-mediated neurotransmission in the neural plasticity relevant to addiction, *via* PLC/PKC-dependent insertion of NMDARs into VTA dopamine neuron synapses[19]. Furthermore, *in vivo* administration of SB-334867 blocks locomotor sensitization to cocaine and occludes cocaine-induced potentiation of excitatory currents in VTA dopamine neurons[19]. These results provide substantial evidence for a critical role of orexin signaling in the induction of synaptic plasticity associated with addiction, and in the subsequent reward-seeking behaviors.

Intriguingly, the orexinergic system may be also involved in the memory link between stimulus and reward[72]. Harris *et al*. used a two-chamber, nonbiased, conditioned place-preference (CPP) model to measure the rewarding properties of morphine, cocaine and food and thereby evaluate the effect of the orexinergic system on reward processing. In this model, one chamber becomes associated with drug or food reward through repeated pairings, whereas the other chamber is associated with no reward. When the animal becomes addicted, it spends more time in the chamber with consummatory rewards and thereby forms CPP. And once the consummatory rewards in the chamber are withdrawn for a time, the CPP gradually disappears. Chemical activation of LHA orexin neurons reinstates the extinguished drug-seeking behavior. And this reinstatement effect is completely blocked by prior administration of an OX1R antagonist[72]. These results show that the projections from the LHA orexinergic system to the VTA play an important role not only in the formation of an acute desire for reward, but also in the learning and memory of the reward condition.

Neuroendocrine

The release of many hormones is modulated by orexin. Intracerebroventricular administration of orexins markedly increases plasma adrenocorticotropic hormone and corticosterone levels[74], suggesting that central orexin activates the hypothalamo–pituitary–adrenal axis. Moreover, after intracerebroventricular injection of orexins, c-fos mRNA increases in a dose-related manner in the parvocellular division of the PVN, a large part of which contains corticotropin-releasing hormone neurons[28,74]. In addition, recently, Lopez *et al*.[75] found that the mRNA of growth hormone-releasing hormone in PVN neurons decreases significantly after intracerebroventricular injection of orexin A. Furthermore, intracerebroventricular administration of orexin A leads to a decrease in spontaneous growth hormone secretion in rats, indicating an inhibitory role of orexin

A in growth hormone secretion[76]. In addition, the central orexinergic system may also modulate the hypothalamo–pituitary–gonad axis. Campbell *et al.*[77] found that 75–85% of gonadotropin-releasing hormone (GnRH) neurons receive projections from orexin neurons, and 85% of GnRH neurons express OX1Rs or OX2Rs. In addition, orexin A activates the release of GnRH in hypothalamus explants[78]. Moreover, in the brains of narcolepsy patients, the level of luteinizing hormone decreases and the release of luteinizing hormone activated by GnRH declines[79]. All of these results suggest that the central orexinergic system is actively involved in neuroendocrine regulation.

Conclusion

Accumulating evidence reveals that the central orexinergic system, originating from restricted hypothalamic regions, regulates various basic physiological processes, including not only non-somatic regulation, such as feeding, energy homeostasis, the sleep/wake cycle, addiction, and neuroendocrine, but also somatic motor control (Fig. 2). On the other hand, central motor structures project back to the hypothalamus, such as direct cerebellohypothalamic projections[7,8] and indirect projections from the vestibular nuclei to the hypothalamus[80], to influence nonsomatic physiological functions. By bridging the non-somatic centers to somatic motor structures and modulating both non-somatic and somatic activity in parallel, the central orexinergic system, together with the circuits from motor structures to non-somatic centers, may actively participate in the orchestration of somatic–non-somatic integration, which is crucial for the generation and execution of appropriate and coordinated behavioral responses (including both somatic and non-somatic components) to changes in the internal and external environments. Actually, narcolepsy-cataplexy caused by orexin deficiency is a simultaneous somatic and non-somatic dysfunction in which somatic (motor) activity and some non-somatic (sleep and emotional) responses are not

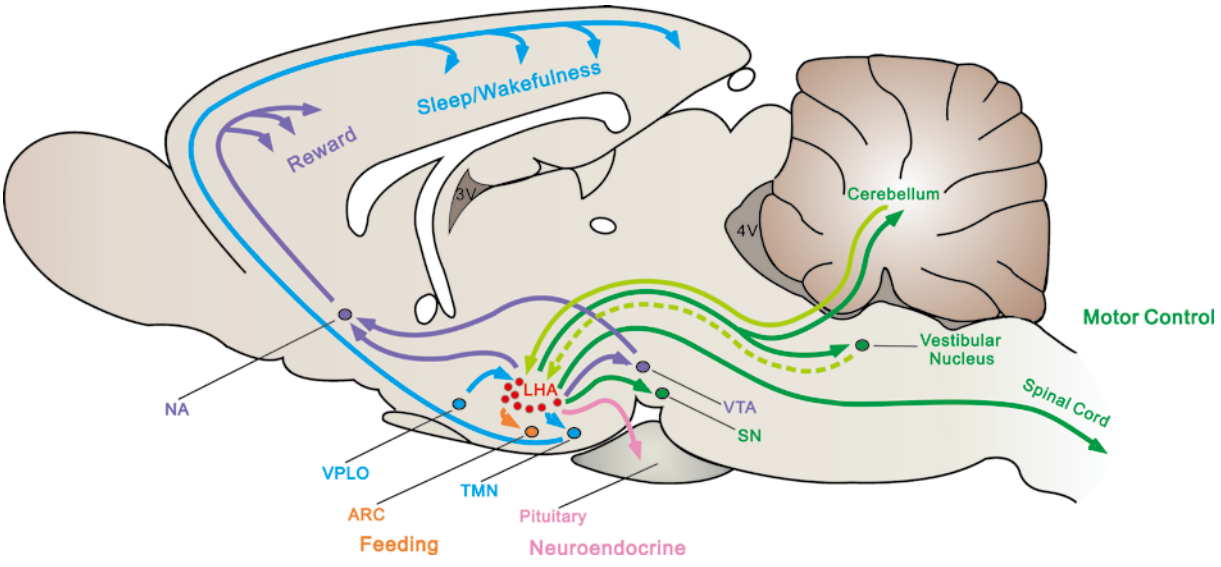

Fig. 2. Central functions of the orexinergic system (diagrammed in the rat brain). Central orexin, secreted exclusively by neurons in the hypothalamus (red), plays a crucial role in the regulation of non-somatic and somatic physiological functions by actions on brain regions involved in feeding (orange), the sleep/wake cycle (blue), reward (purple), and neuroendocrine (pink), as well as motor control (dark green). On the other hand, central motor structures, including both the cerebellum and vestibular nuclei, project directly (light green solid line) and indirectly (light green dashed line) back to the hypothalamus to influence non-somatic functions. Thus, by bridging the non-somatic center to somatic motor structures and modulating both non-somatic and somatic activities in parallel, the central orexinergic system, together with the circuits from motor structures to the hypothalamus, may actively participate in somatic-non-somatic integration. ARC, arcuate nucleus; LHA, lateral hypothalamic area; NA, nucleus accumbens; SN, substantia nigra; TMN, tuberomammillary nucleus; VLPO, ventrolateral preoptic area; VTA, ventral tegmental area; 3V, third ventricle; 4V, fourth ventricle.

correctly integrated and coordinated. Thus, further studies on the somatic and non-somatic functions of the orexinergic system and the underlying mechanisms will assist not only in understanding and reevaluating the functional roles of orexin, but also in comprehending the entire mechanism of somatic–non-somatic integration, which will help to explain the pathogenesis of simultaneous somatic and non-somatic dysfunctions.

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REFERENCES

[1] de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, *et al*. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci U S A 1998, 95: 322–327.

[2] Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, *et al*. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 1998, 92: 573–585.

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

[4] Cutler DJ, Morris R, Sheridhar V, Wattam TA, Holmes S, Patel S, *et al*. Differential distribution of orexin-A and orexin-B immunoreactivity in the rat brain and spinal cord. Peptides 1999, 20: 1455–1470.

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

[6] Kukkonen JP, Holmqvist T, Ammoun S, Akerman KE. Functions of the orexinergic/hypocretinergic system. Am J Physiol Cell Physiol 2002, 283: 1567–1591.

[7] Zhu JN, Yung WH, Kwok-Chong Chow B, Chan YS, Wang JJ. The cerebellar-hypothalamic circuits: potential pathways underlying cerebellar involvement in somatic-visceral integration. Brain Res Rev 2006, 52: 93–106.

[8] Zhu JN, Wang JJ. The cerebellum in feeding control: possible function and mechanism. Cell Mol Neurobiol 2008, 28: 469–478.

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

[10] Tsujino N, Sakurai T. Orexin/hypocretin: a neuropeptide at the interface of sleep, energy homeostasis, and reward system. Pharmacol Rev 2009, 61: 162–176.

[11] Smart D, Jerman JC, Brough SJ, Rushton SL, Murdock PR, Jewitt F, *et al*. Characterization of recombinant human orexin receptor pharmacology in a Chinese hamster ovary cell-line using FLIPR. Br J Pharmacol 1999, 128: 1–3.

[12] Trivedi P, Yu H, MacNeil DJ, van der Ploeg LH, Guan XM. Distribution of orexin receptor mRNA in the rat brain. FEBS Lett 1998, 438: 71–75.

[13] Hervieu GJ, Cluderay JE, Harrison DC, Roberts JC, Leslie RA. Gene expression and protein distribution of the orexin-1 receptor in the rat brain and spinal cord. Neuroscience 2001, 103: 777–797.

[14] Cluderay JE, Harrison DC, Hervieu GJ. Protein distribution of the orexin-2 receptor in the rat central nervous system. Regul Pept 2002, 104: 131–144.

[15] Marcus JN, Aschkenasi CJ, Lee CE, Chemelli RM, Saper CB, Yanagisawa M, *et al*. Differential expression of orexin receptors 1 and 2 in the rat brain. J Comp Neurol 2001, 435: 6–25.

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

[17] Lund PE, Shariatmadari R, Uustare A, Detheux M, Parmentier M, Kukkonen JP, *et al*. The orexin OX1 receptor activates a novel Ca^{2+} influx pathway necessary for coupling to phospholipase C. J Biol Chem 2000, 275: 30806–30812.

[18] Zhu Y, Miwa Y, Yamanaka A, Yada T, Shibahara M, Abe Y, *et al*. 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 2003, 92: 259–266.

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

[20] Chen XW, Mu Y, Huang HP, Guo N, Zhang B, Fan SY, *et al*.

Hypocretin-1 potentiates NMDA receptor-mediated somatodendritic secretion from locus ceruleus neurons. J Neurosci 2008, 28: 3202–3208.

[21] Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, *et al*. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 1999, 98: 437–451.

[22] Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, *et al*. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 1999, 98: 365–376.

[23] Hara J, Beuckmann CT, Nambu T, Willie JT, Chemelli RM, Sinton CM, *et al*. Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. Neuron 2001, 30: 345–354.

[24] Willie JT, Chemelli RM, Sinton CM, Tokita S, Williams SC, Kisanuki YY, *et al*. Distinct narcolepsy syndromes in orexin receptor-2 and orexin null mice: molecular genetics dissection of Non-REM and REM sleep regulatory processes. Neuron 2003, 38: 715–730.

[25] Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, *et al*. 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.

[26] Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, *et al*. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000, 27: 469–474.

[27] Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000, 355: 39–40.

[28] Hagan JJ, Leslie RA, Patel S, Evans ML, Wattam TA, Holmes S, *et al*. 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] Bourgin P, Huitrón-Reséndiz S, Spier AD, Fabre V, Morte B, Criado JR, *et al*. Hypocretin-1 modulates rapid eye movement sleep through activation of locus coeruleus neurons. J Neurosci 2000, 20: 7760–7765.

[30] Estabrooke IV, McCarthy MT, Ko E, Chou TC, Chemelli RM, Yanagisawa M, *et al*. Fos expression in orexin neurons varies with behavioral state. J Neurosci 2001, 21: 1656–1662.

[31] Yoshida Y, Fujiki N, Nakajima T, Ripley B, Matsumura H, Yoneda H, *et al*. Fluctuation of extracellular hypocretin-1 (orexin A) levels in the rat in relation to the light-dark cycle and sleep-wake activities. Eur J Neurosci 2001, 14: 1075–1081.

[32] Lee MG, Hassani OK, Jones BE. Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. J Neurosci 2005, 25: 6716–6720.

[33] Nakamura T, Uramura K, Nambu T, Yada T, Goto K, Yanagisawa M, *et al*. Orexin-induced hyperlocomotion and stereotypy are mediated by the dopaminergic system. Brain Res 2000, 873: 181–187.

[34] 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.

[35] 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.

[36] Yamanaka A, Tsujino N, Funahashi H, Honda K, Guan JL, Wang QP, *et al*. Orexins activate histaminergic neurons via the orexin 2 receptor. Biochem Biophys Res Commun 2002, 290: 1237–1245.

[37] Mieda M, Yanagisawa M. Sleep, feeding, and neuropeptides: roles of orexins and orexin receptors. Curr Opin Neurobiol 2002, 12: 339–345.

[38] Eggermann E, Serafin M, Bayer L, Machard D, Saint-Mleux B, Jones BE, *et al*. Orexins/hypocretins excite basal forebrain cholinergic neurons. Neuroscience 2001, 108: 177–181.

[39] Eriksson KS, Sergeeva O, Brown RE, Haas HL. Orexin/hypocretin excites the histaminergic neurons of the tuberomammillary nucleus. J Neurosci 2001, 21: 9273–9279.

[40] Huang ZL, Qu WM, Li WD, Mochizuki T, Eguchi N, Watanabe T, *et al*. Arousal effect of orexin A depends on activation of the histaminergic system. Proc Natl Acad Sci U S A 2001, 98: 9965–9970.

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

[42] 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.

[43] Matsuki T, Nomiyama M, Takahira H, Hirashima N, Kunita S, Takahashi S, *et al*. Selective loss of $GABA_B$ receptors in orexin-producing neurons results in disrupted sleep/wakefulness architecture. Proc Natl Acad Sci U S A 2009, 106: 4459–4464.

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

[45] Kiyashchenko LI, Mileykovskiy BY, Maidment N, Lam HA, Wu MF, John J, *et al*. Release of hypocretin (orexin) during waking and sleep states. J Neurosci 2002, 22: 5282–5286.

[46] Takakusaki K, Takahashi K, Saitoh K, Harada H, Okumura T, Kayama Y, *et al*. Orexinergic projections to the midbrain mediate alternation of emotional behavioral states from locomotion to cataplexy. J Physiol 2005, 568: 1003–1020.

[47] Korotkova TM, Eriksson KS, Haas HL, Brown RE. Selective excitation of GABAergic neurons in the substantia nigra of the rat by orexin/hypocretin *in vitro*. Regul Pept 2002, 104:

83–89.

[48] Thorpe AJ, Kotz CM. Orexin A in the nucleus accumbens stimulates feeding and locomotor activity. Brain Res 2005, 1050: 156–162.

[49] Yu L, Zhang XY, Zhang J, Zhu JN, Wang JJ. Orexins excite neurons of the rat cerebellar nucleus interpositus via orexin 2 receptors *in vitro*. Cerebellum 2010, 9: 88–95.

[50] Zhang J, Li B, Yu L, He YC, Li HZ, Zhu JN, *et al*. A role for orexin in central vestibular motor control. Neuron 2011, 69: 793–804.

[51] Yamanaka A, Sakurai T, Katsumoto T, Yanagisawa M, Goto K. Chronic intracerebroventricular administration of orexin-A to rats increases food intake in daytime, but has no effect on body weight. Brain Res 1999, 849: 248–252.

[52] Kotz CM. Integration of feeding and spontaneous physical activity: role for orexin. Physiol Behav 2006, 88: 294–301.

[53] Haynes AC, Jackson B, Chapman H, Tadayyon M, Johns A, Porter RA, *et al*. A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. Regul Pept 2000, 96: 45–51.

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

[55] Yamanaka A, Kunii K, Nambu T, Tsujino N, Sakai A, Matsuzaki I, *et al*. Orexin-induced food intake involves neuropeptide Y pathway. Brain Res 2000, 859: 404–409.

[56] van den Top M, Nolan MF, Lee K, Richardson PJ, Buijs RM, Davies CH, *et al*. Orexins induce increased excitability and synchronization of rat sympathetic preganglionic neurones. J Physiol 2003, 549: 809–821.

[57] Li Y, van den Pol AN. Differential target-dependent actions of coexpressed inhibitory dynorphin and excitatory hypocretin/orexin neuropeptides. J Neurosci 2006, 26: 13037–13047.

[58] Muroya S, Funahashi H, Yamanaka A, Kohno D, Uramura K, Nambu T, *et al*. 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 2004, 19: 1524–1534.

[59] Ma X, Zubcevic L, Bruning JC, Ashcroft FM, Burdakov D. Electrical inhibition of identified anorexigenic POMC neurons by orexin/hypocretin. J Neurosci 2007, 27: 1529–1533.

[60] Berthoud HR. Mind versus metabolism in the control of food intake and energy balance. Physiol Behav 2004, 81: 781–793.

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

[62] Novak CM, Levine JA. Daily intraparaventricular orexin-A treatment induces weight loss in rats. Obesity 2009, 178: 1493–1498.

[63] Zhang S, Zeitzer JM, Sakurai T, Nishino S, Mignot E. Sleep/wake fragmentation disrupts metabolism in a mouse model of narcolepsy. J Physiol 2007, 581: 649–663.

[64] Yamanaka A, Beuckmann CT, Willie JT, Hara J, Tsujino N, Mieda M, *et al*. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. Neuron 2003, 38: 701–713.

[65] Sellayah D, Bharaj P, Sikder D. Orexin is required for brown adipose tissue development, differentiation, and function. Cell Metab 2011, 14: 478–490.

[66] Akimoto H, Honda Y, Takahashi Y. Pharmacotherapy in narcolepsy. Dis Nerv Syst 1960, 21: 704–706.

[67] Georgescu D, Zachariou V, Barrot M, Mieda M, Willie JT, Eisch AJ, *et al*. Involvement of the lateral hypothalamic peptide orexin in morphine dependence and withdrawal. J Neurosci 2003, 23: 3106–3111.

[68] Narita M, Nagumo Y, Hashimoto S, Narita M, Khotib J, Miyatake M, *et al*. Direct involvement of orexinergic systems in the activation of the mesolimbic dopamine pathway and related behaviors induced by morphine. J Neurosci 2006, 26: 398–405.

[69] Fadel J, Deutch AY. Anatomical substrates of orexin-dopamine interactions: lateral hypothalamic projections to the ventral tegmental area. Neuroscience 2002, 111: 379–387.

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

[71] Boutrel B, Kenny PJ, Specio SE, Fardon RM, Markou A, Koob GF, *et al*. Role for hypocretin in mediating stress-induced reinstatement of cocaine-seeking behavior. Proc Natl Acad Sci U S A 2005, 102: 19168–19173.

[72] Harris GC, Wimmer M, Aston-Jones G. A role for lateral hypothalamic orexin neurons in reward seeking. Nature 2005, 437: 556–559.

[73] Lawrence AJ, Cowen MS, Yang HJ, Chen F, Oldfield B. The orexin system regulates alcohol-seeking in rats. Br J Pharmacol 2006, 148: 752–759.

[74] Kuru M, Ueta Y, Serino R, Nakazato M, Yamamoto Y, Shibuya I, *et al*. Centrally administered orexin/hypocretin activates HPA axis in rats. Neuroreport 2000, 11: 1977–1980.

[75] Lopez M, Seoane LM, Tovar S, Nogueiras R, Dieguez C, Senaris R. Orexin-A regulates growth hormone-releasing hormone mRNA content in a nucleus-specific manner and somatostatin mRNA content in a growth hormone-dependent

fashion in the rat hypothalamus. Eur J Neurosci 2004, 19: 2080–2088.

[76] Seoane LM, Tovar SA, Perez D, Mallo F, Lopez M, Senaris R, *et al*. Orexin A suppresses *in vivo* GH secretion. Eur J Endocrinol 2004, 150: 731–736.

[77] Campbell RE, Grove KL, Smith MS. Gonadotropin-releasing hormone neurons coexpress receptor immunoreactivity immunoreactivity and receive direct contacts by orexin fibers. Endocrinology 2003, 144: 1542–1548.

[78] Small CJ, Goubillon ML, Murray JF, Siddiqui A, Grimshaw SE, Young H, *et al*. Central orexin A has site-specific effects on luteinizing hormone release in female rats. Endocrinology 2003, 144: 3225–3236.

[79] Kok SW, Roelfsema F, Overeem S, Lammers GJ, Frolich M, Meinders AE, *et al*. Pulsatile LH release is diminished, while FSH secretion is normal in hypocretin deficient narcoleptic men. Am J Physiol Endocrinol Metab 2004, 287: E630–E636.

[80] Katafuchi T, Puthuraya KP, Yoshimatsu H, Oomura Y. Responses of rat lateral hypothalamic neuron activity to vestibular nuclei stimulation. Brain Res 1987, 400: 62–69.