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REVIEW

The hypocretins/orexins: integrators of multiple physiological functions

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The hypocretins (Hcrts), also known as orexins, are two peptides derived from a single precursor produced in the posterior lateral hypothalamus. Over the past decade, the orexin system has been associated with numerous physiological functions, including sleep/arousal, energy homeostasis, endocrine, visceral functions and pathological states, such as narcolepsy and drug abuse. Here, we review the discovery of Hcrt/orexins and their receptors and propose a hypothesis as to how the orexin system orchestrates these multifaceted physiological functions.

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Abbreviations
CRF, corticotrophin releasing factor; Hcrt, hypocretin; LH, lateral hypothalamus; NREM, non-rapid eye movement sleep; OX, orexin; PVN, paraventricular nucleus

Introduction: discovery

Gautvik *et al*. (1996) used subtractive hybridization to generate a library of cDNAs representing the most prevalent mRNAs present in the hypothalamus. This list, which contained 43 different sequences, revealed that the hypothalamus specializes in making intercellular signalling molecules, as 40% of the clones encoded neuropeptide transmitters. One of the most frequent cDNA clones encoding the precursor of one such peptide transmitter was called preproorexin (preprohypocretin).

In situ hybridization studies showed that preproorexin mRNA is produced by a discrete group of neurons (~5000 in rodents; 20–50 000 in humans) bilaterally distributed in the lateral posterior hypothalamus (Gautvik *et al*., 1996; de Lecea *et al*., 1998). These cells produce a single polypeptide that is processed into two peptides orexin-A (Hcrt-1) and orexin-B (Hcrt-2) of 33 and 28 amino acid residues, which show a seven out of seven sequence match with secretin. Orexin neurons are excitatory, express the vesicular glutamate transporter VGLUT2 (Rosin *et al*., 2003), and also produce dynorphin (Chou *et al*., 2001), neuronal activity related pentraxin (NARP; Reti *et al*., 2002) and protein delta-like 1 homologue (DLK-1; Meister *et al*., 2013).

Sakurai *et al*. (1998) identified the same peptides, which they named orexins, as ligands of two orphan GPCRs and determined the chemical sequence of the processed peptides. As per journal guidelines, we refer to the peptides as orexins, but it is clear that the main function of the peptides is not appetite, and we personally prefer the original nomenclature of Hcrt. These two receptors, Hcrt receptor type 1 and Hcrt receptor type 2, also known as OX_1 (orexin 1) and OX_2 receptors, have broad and partially overlapping but a distinct distribution throughout the brain. Orexin-B binds preferentially to OX_2 receptors whereas orexin-A binds with equal affinity to OX_1 and OX_2 receptors. An excellent review of the cell signalling properties of OX (Hcrt) receptors has been recently published elsewhere (Kukkonen, 2013). Drug/molecular target nomenclature throughout this manuscript conforms to BJP's Concise Guide to PHARMACOLOGY (Alexander *et al*., 2013).

Several OX receptor antagonists have been described (Smart *et al*., 2001; Gunthorpe *et al*., 2004). SB-334867 was the first and most widely used compound, and although

initially was claimed to be selective for OX_1 receptors, it blocks binding to both OX receptors at the concentrations used in most studies (3–30 mg·kg^{-1}). Studies on this compound were followed by investigations into several orally bioavailable molecules with high selectivity for OX receptors (McAtee *et al.*, 2004; Brisbare-Roch *et al.*, 2007). More recently, suvorexant, a non-selective OX receptor antagonist, has been successfully used in clinical trials to treat primary insomnia (Winrow *et al.*, 2011).

Despite their restricted location in the lateral hypothalamus (LH), orexin neurons project broadly throughout the whole brain, and can be modulated by multiple humoral signals and neuronal inputs, such as innervation from other hypothalamic areas and the limbic system. These properties suggest that the orexin system may sense the fluctuation of both internal and external environments to orchestrate an appropriate response.

The main inputs to and regulators of orexinergic neurons originate from structures in the limbic system (Sakurai *et al.*, 2005; Yoshida *et al.*, 2006); in addition, humoral signals make the orexin system susceptible to the effects of environmental stimuli and homeostatic states. Based on these inputs, which reflect the needs of an organism in different conditions, the orexin system can act as an integrator of numerous physiological functions, including sleep/arousal states, sensory, locomotion, cognition, energy homeostasis, endocrine and visceral functions.

Physiological roles

Arousal

Soon after the identification of orexins in 1998, two groups demonstrated an association between orexin deficiency and the sleep disorder narcolepsy (Chemelli *et al.*, 1999; Lin *et al.*, 1999; Nishino *et al.*, 2000; 2001; Peyron *et al.*, 2000; Thannickal *et al.*, 2000; Hara *et al.*, 2001). In addition, several studies have shown that OX receptor knockout (KO) mice and mice deficient in OX_2 receptors (Mochizuki *et al.*, 2011) have normal amounts of sleep and wakefulness across the light/dark cycle (Mochizuki *et al.*, 2004; Anaclet *et al.*, 2009) but the stability of these behavioural states is considerably reduced. Dogs with mutations in OX_2 receptors exhibit narcolepsy with cataplexy (Lin *et al.*, 1999). Patients that suffer from narcolepsy with cataplexy have very low levels of orexin-A in their CSF (Nishino *et al.*, 2000; Peyron *et al.*, 2000; Thannickal *et al.*, 2000). These deficits are probably caused by the selective degeneration of orexin cells (rather than a down-regulation of the orexin gene) because other markers that colocalize with orexin are also reduced in narcoleptic patients (Crocker *et al.*, 2005). All of these data clearly demonstrate that orexin signalling is essential for the stability of the arousal state.

The first recordings of orexin neurons *in vitro* indicated that these cells are spontaneously active and respond to numerous stimuli. Studies by Fujiki *et al.* (2001) using microdialysis and Estabrooke *et al.* (2001) using c-Fos mapping revealed a circadian modulation of orexin peptide concentration in the CSF and orexin cell activity respectively. However, due to their very low temporal resolution, these methods did not enable these circadian changes to be monitored precisely. Parallel studies using juxtacellular recordings in head-fixed or freely moving animals showed that, surprisingly, orexin activity is mostly phasic and precedes sleep to wake transitions (Lee *et al.*, 2005; Mileykovskiy *et al.*, 2005). The question remained as to whether this phasic activity of orexin neurons was permissive or instructive for awakenings. In the first *in vivo* study to investigate the effect of optogenetic photostimulation of orexinergic neurons on the behaviour of animals (Adamantidis *et al.* (2007), it was found that activation of these neurons specifically increases the probability of transitions from sleep to wakefulness This effect was frequency-dependent as only frequencies >5 Hz increased the probability of the animal waking up. Semi-chronic stimulation of orexin neurons did not significantly increase the amount of non-rapid eye movement (NREM) sleep suggesting that phasic activation of orexin cells is involved in the transition to wakefulness, but not in the maintenance of this state. Optogenetic silencing of orexin neurons induced sleep during the light phase, but not during the dark phase (Tsunematsu *et al.*, 2011). These findings were further validated using a newly developed pharmacogenetic technique (designer receptors exclusively activated by designer drugs; Sasaki *et al.*, 2011) that allows the modulation of neural activity with temporal resolution for several hours. Hence, it is thought that the orexin system acts as a regulator of behaviour states by modulating the arousal threshold (Sutcliffe and de Lecea, 2002), so that a mammal or human can maintain appropriate and adequate wakefulness to cope with fluctuations in the external and internal environments (Figure 1).

The next question we asked was does the existence of two subtypes of receptor account for different aspects of the functions of orexin? Mice deficient in OX_2 receptors display fragmented wakefulness similar to the narcoleptic phenotype, whereas OX_1 receptor-KO mice only show a mild sleep disorder (Willie *et al.*, 2001). However, double, OX_1 and OX_2 receptor-KO mice were found to have more severe deficits in their sleep-wake cycle than OX_2 receptor-KO mice, which exhibited a low level of cataplexy and disruption of REM sleep (Chemelli *et al.*, 1999; Willie *et al.*, 2003). Therefore, it was concluded that both OX_1 and OX_2 receptor are essential for maintaining a stable sleep/wakefulness cycle, with the role of OX_2 receptors being more important (Sakurai, 2007). However, in a recent study it was found that the effects of orexin-A on wakefulness and NREM were attenuated in both OX_1 and OX_2 receptor-KO mice (Mieda *et al.*, 2011). Furthermore, in a recent functional magnetic resonance imaging study, it was shown that an antagonist of OX_2 receptors, but not OX_1 receptors, increased REM, NREM and total sleep time, suggesting that the two receptors have distinct roles in regulating sleep and wakefulness (Gozzi *et al.*, 2011). Also, the recent development of OX receptor selective antagonists showed that blocking OX_1 receptors attenuates the effects of an OX_2 receptor antagonist and revealed a complex interaction between these two receptors (Dugovic *et al.*, 2009). Selective and non-selective OX receptor antagonists have recently completed phase III clinical trials for the treatment of insomnia (Herring *et al.*, 2012), a remarkable achievement for a gene product that was only discovered 15 years ago.

Effectors of orexinergic neurons: the monoamines. Monoaminergic neurons constitute one side in the flip/flop model of

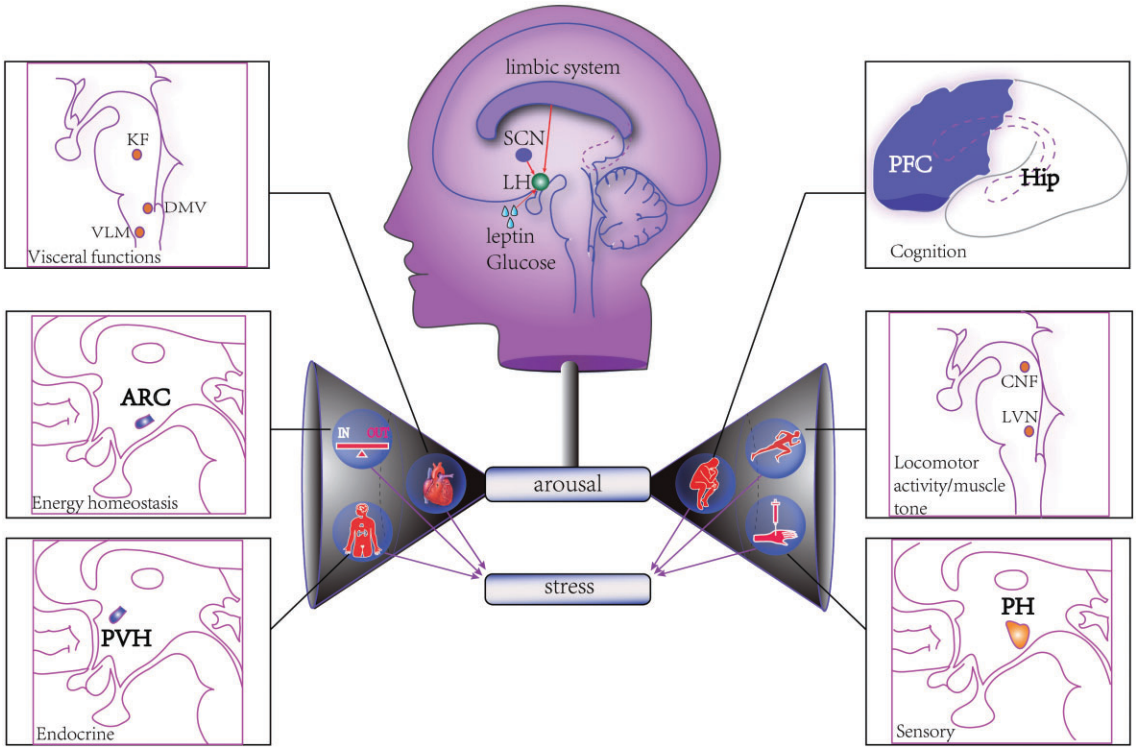

Figure 1

The six schematic drawings show the representative nuclei (or nucleus) involved in the corresponding functions, namely, the structure involved in regulation of sensory information [the posterior hypothalamic area, (PH)], in locomotion [the cuneiform nucleus (CNF) and lateral vestibular nucleus (LVN)], in cognition [the prefrontal cortex (PFC) and hippocampus (Hip)], in energy homeostasis [the arcuate nucleus (ARC)], in endocrine [the paraventricular nucleus of hypothalamus (PVH)] and in visceral functions [the ventrolateral medulla (VLM), pontine Kölliker-Fuse nucleus (KF) and dorsal motor nucleus of the vagus (DMV)].

the sleep-wake cycle (Saper *et al.*, 2010); they excite the neocortex but inhibit sleep centres to promote wakefulness. Importantly, these monoaminergic neurons present in the tuberomammillary nucleus (TMN, histaminergic), locus coeruleus (LC, noradrenergic), dorsal raphe nuclei (DRN, 5-hydroxytryptaminergic) and ventral periaqueductal gray matter (vPAG, dopaminergic) receive dense projections of orexinergic neurons (Peyron *et al.*, 1998; Saper *et al.*, 2005) and, consistent with the various roles of orexin (Marcus *et al.*, 2001), the LC mainly expresses OX_1 receptors, the TMN mainly OX_2 receptors and the DRN expresses both OX_1 and OX_2 receptors. Mieda *et al.* (2011) showed that the effects of i.c.v. orexin-A (Hcrt-1) on wakefulness and NREM sleep are significantly attenuated in both KO mice as compared with wild-type mice, with the effect being substantially larger in OX_2 receptor-KO mice than in OX_1 receptor-KO mice. These results suggest that although the OX_2 receptor-mediated pathway has a pivotal role in the promotion of wakefulness, the OX_1 receptor is also involved in promoting arousal. However, both receptors appear to be similarly involved in REM sleep suppression.

Orexinergic neurons exhibit parallel firing patterns with monoaminergic neurons that exhibit tonic firing during wakefulness especially during active wakefulness, mild firing during slow-wave sleep, and then are silent during REM sleep (Estabrooke *et al.*, 2001; Lee *et al.*, 2005; Mileykovskiy *et al.*, 2005), except during the transition to wakefulness, when intensive firing is observed (Sakurai, 2007). These data are also consistent with the oscillation of extracellular orexin-A levels that peak during the waking state and decrease to about half their maximum levels during sleep (Yoshida *et al.*, 2001; Zeitzer *et al.*, 2003). These observations suggest that the orexin system stabilizes the state of wakefulness by driving the arousal system (Saper *et al.*, 2010).

Indeed, *in vitro* electrophysiological studies showed that orexin activates the TMN histaminergic (Bayer *et al.*, 2001; Eriksson *et al.*, 2001; Huang *et al.*, 2001), LC noradrenergic (Hagan *et al.*, 1999) and DRN 5-hydroxytryptaminergic (Liu *et al.*, 2002) neurons. Furthermore, *in vivo* experiments revealed the involvement of the LC and OX_1 receptors in the LC (Bourgin *et al.*, 2000), as well as the histamine H_1 receptor (Huang *et al.*, 2001) and OX_2 receptor signalling in the TMN (Mochizuki *et al.*, 2011), in orexin- induced arousal. Although, in contrast, recent studies have shown that the orexin-mediated sleep-to-wake transition in mice is not dependent on the histaminergic system (Carter *et al.*, 2009a) and mice deficient in both H_1 receptors and OX_1 receptors display normal sleep/wakefulness patterns (Hondo *et al.*, 2010).

Moreover, Lu and Greco (2006) demonstrated that loss of dopaminergic neurons in vPAG results in a 20% reduction in wakefulness accompanied by an increase in NREM, REM sleep. This finding is supported by a recent report (Kaur

et al., 2009) that demonstrated activation of the orexin-vPAG circuit suppresses REM sleep but not non-REM sleep. Orexinergic neurons also receive inhibitory innervation from noradrenergic (Li *et al.*, 2002), 5-hydroxytrptaminergic (Yamanaka *et al.*, 2003b; Kumar *et al.*, 2007) and dopaminergic (Yamanaka *et al.*, 2006) inputs, whereas histamine has little, if any, effect (Yamanaka *et al.*, 2003b). However, the effect of noradrenergic innervation on the activity of orexinergic neurons remains controversial, as some reports show excitatory effects in rats and others demonstrate an inhibitory action (Grivel *et al.*, 2005).

Orexin activates the monoaminergic neurons (Carter *et al.*, 2012), which project to the LH, basal forebrain, as well as cerebral cortex to exert its arousal effect. This notion is supported by a recent study, which showed that mice deficient in the histamine synthesizing enzyme histidine decarboxylase (HDC -/-mice) but not OX receptor -/-mice exhibit abnormal cortical electroencephalographic activity (Anaclet *et al.*, 2009). Interestingly, optogenetic activation (photostimulation) of orexin neurons is not affected in HDC KO mice (Carter *et al.*, 2009a), suggesting that histamine is not essential for orexin-mediated awakenings.

Furthermore, the cholinergic neurons in the pedunculopontine tegmental nucleus/laterodorsal tegmental nucleus (PPT/LDT) fire rapidly during wakefulness and REM sleep but slowly during NREM sleep (Saper *et al.*, 2005), suggesting that they help to maintain cortical activation in the states of wakefulness and REM sleep. Injecting orexin-A into the laterodorsal tegmental nucleus (LDT) results in a significant increase in wakefulness but a decrease of the amount rather than the duration of REM sleep (Xi *et al.*, 2001). In addition, orexin-A has been shown to elicit long-lasting excitation in both cholinergic and non-cholinergic neurons of the LDT (Takahashi *et al.*, 2002). Interestingly, the cholinergic synaptic transmission of LDT neurons is augmented in OX receptor-KO mice, which may induce cataplexy (Kalogiannis *et al.*, 2010). In addition, the (pedunculopontine tegmental nucleus) PPT may also be involved in orexin-induced modulation of wakefulness, as orexin-A has been shown to inhibit the activation of PPT cholinergic neurons, which induces REM sleep onset and atonia (Takakusaki *et al.*, 2004) via GABAergic neurons in substantia nigra pars reticulata and the PPT itself (Takakusaki *et al.*, 2005).

In vitro studies have shown that carbachol, a cholinergic agonist, excites orexinergic neurons (Bayer *et al.*, 2005). In addition, i.c.v. administration of orexin-A (Piper *et al.*, 2000) or its local application into the LC (Bourgin *et al.*, 2000), basal forebrain (Espana *et al.*, 2001; Thakkar *et al.*, 2001) or lateral preoptic area (Methippara *et al.*, 2000) increases the waking time at the expense of sleep. In summary, orexin-induced arousal is modulated not only by monoaminergic neurons, but also by cholinergic neurons in the PPT/ LDT and basal forebrain.

Importantly, the orexin system may be modulated by the circadian clock and homeostatic states (Deboer *et al.*, 2004; Carter *et al.*, 2009a; Appelbaum *et al.*, 2010). Even though there is no evidence of a direct synaptic connection between the suprachiasmatic nucleus (SCN) and orexin cells, the circadian clock drives the orexin system through the output circuits of the SCN (Deurveilher and Semba, 2005). Additionally, local modulation of orexinergic neurons induced by orexin release (Li *et al.*, 2002; Yamanaka *et al.*, 2010), melanin-concentrating hormone (MCH) (Rao *et al.*, 2008; Hassani *et al.*, 2009) or neurons expressing an active form of the leptin receptor (Leinninger *et al.*, 2011) may also be important in the circadian stabilization of a proper sleep-wake cycle. Modulation of orexin activity by energy homeostasis will be expanded in the Energy homeostasis section. Intrinsic plasticity mechanisms may regulate the firing probability of orexin cells during the day and at night (Appelbaum *et al.*, 2010). During the wakefulness period, tonic excitation of orexin neurons may be enhanced when certain stressors are present, like emotional stimulation, which involves the limbic input (Tsujino and Sakurai, 2009). Adamantidis *et al.* (Adamantidis and de Lecea, 2008a,b) suggested a dual mode of action of orexin: phasic activity lasting 1–10 s that is mostly responsible for the state transitions, and a circadially-regulated oscillation that encodes superimposed information about metabolic and circadian state.

Sensory

So far, the involvement of orexin in sensory modulation has focused on its role in nociception, in addition to an emerging role in olfaction.

Pain. The analgesic properties of orexin peptides have been well-established with i.v., intrathecal or i.c.v. injection approaches in mouse and rat models of thermal (hot-plate, tail-flick, paw-withdrawal), mechanical (tail pressure, partial sciatic nerve ligation), chemical (formalin, carrageenan, capsaicin and abdominal stretch) nociception and (or) hyperalgesia (Bingham *et al.*, 2001; Yamamoto *et al.*, 2002; 2003a,b). In all of those models, the orexin-induced analgesic effects were suppressed by the OX_1 receptor antagonist SB-334867 but not by naloxone, an opioid receptor inverse agonist, suggesting that regulation of nociception by orexin is independent of the opiate system (Figure 2). In addition to the direct projection of orexin neurons to the spinal cord (van den Pol, 1999), orexin signalling in the posterior hypothalamic area (Bartsch *et al.*, 2004), the pontine reticular nucleus, oral part (Watson *et al.*, 2010), and periaqueductal gray matter have been shown to be important for its antinociceptive effects. Moreover, orexin plays a significant role in the regulation of stress-induced analgesia (SIA), coordinating with the nociceptin/orphanin FQ systems (Xie *et al.*, 2008; Gerashchenko *et al.*, 2011). Consistent with these data, preproorexin KO mice exhibit hyperalgesia and less SIA (Watanabe *et al.*, 2005). The involvement of orexin in pain is also supported by clinical observations, which have shown there is an association between changes in the Ox receptors and headaches (see below) (Rainero *et al.*, 2004) and a recent multicentre case-control study revealed that chronic pain is more common in patients with narcolepsy with cataplexy than in the controls (Dauvilliers *et al.*, 2011).

Olfaction. The participation of orexin in olfactory function is suggested by the presence of orexin neurons and receptors at all levels of the olfactory system, and their ability to modulate the excitability of olfactory sensory and relay neurons (Caillol *et al.*, 2003; Gorojankina *et al.*, 2007). Indeed, i.c.v. injection of orexin-A increases the olfactory sensitivity to

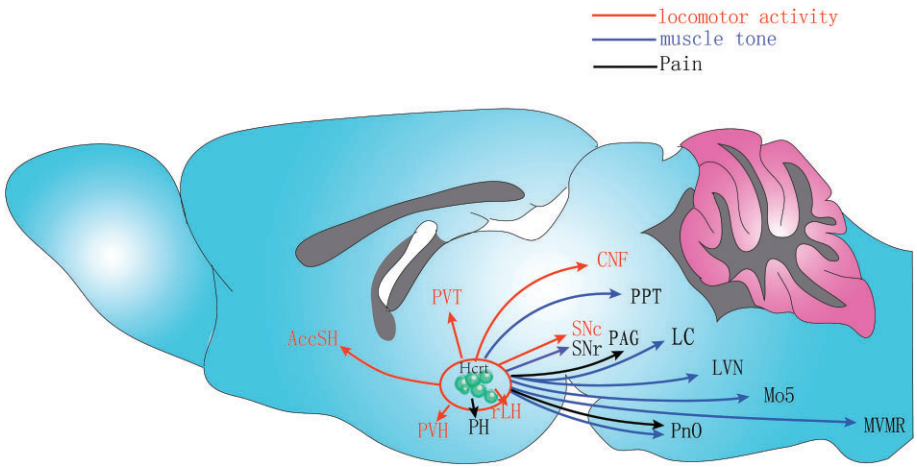

Figure 2

Effect of orexins (Hcrt) on structures involved in locomotion and sensory systems (pain). This diagram summarizes the effects of orexin (Hcrt) on structures involved in locomotion and pain. Orexin regulates locomotor activities and muscle tone. Notably, especially in terms of muscle tone, these regulations are bidirectional, namely, orexin facilitates muscle tone or inhibits it depending on the region. We consider regulation of pain as representative of sensory systems. Both the cuneiform nucleus (CNF) and pedunculopontine tegmental nucleus (PPT) are parts of the so called mesencephalic locomotor region (MLR), and nucleus pontis oralis (PnO) contains the alleged pontine inhibitory area. Abbreviations: AccSh, nucleus accumbens shell; LC, locus coeruleus; LVN, lateral vestibular nucleus; Mo5, motor trigeminal nucleus; MVMR, medioventral medullary region; PAG, periaqueductal gray matter; PH, posterior hypothalamic area; PVH, paraventricular nucleus of hypothalamus; PVT, paraventricular nucleus of thalamus; rLH, rostrolateral hypothalamus; SNc, substantia nigra pars compact; SNr, substantia nigra pars reticulate.

isoamyl acetate (Julliard *et al.*, 2007) and food odour (Prud'homme *et al.*, 2009), although it is unclear whether these increases in sensory perception are related to modulation of brain reward function. Importantly, in studies in humans, it has been established that olfactory dysfunction is a feature of narcolepsy with or without cataplexy (Stiasny-Kolster *et al.*, 2007; Buskova *et al.*, 2010), and that this state could be reversed by intranasal orexin-A (Baier *et al.*, 2008).

Locomotion

The relationship between the orexin system and locomotion was highlighted initially in behavioural tests; i.c.v. administration of orexin was shown to enhance locomotor activity (Hagan *et al.*, 1999; Ida *et al.*, 1999), which involves dopamine D_1 and D_2 receptors (Nakamura *et al.*, 2000), 5-HT (Duxon *et al.*, 2001; Matsuzaki *et al.*, 2002) and central α_1-adrenoceptors (Stone *et al.*, 2005), whereas the selective OX_1 receptor antagonist SB-334867 reversed this effect of orexin (Duxon *et al.*, 2001). Orexin-A injected into multiple structures stimulates locomotor activity, but the effect of orexin-A in the LH is independent of its feeding effect (Kotz *et al.*, 2002; Kotz, 2006). Moreover, both orexin-A and -B, injected into the nucleus accumbens shell (AccSh), potentiated the dopamine-dependent pivoting in rats (Kotani *et al.*, 2008). However, the OX_1 receptor antagonist SB-334867 decreased the orexin-A-induced spontaneous physical activity when injected into the paraventricular nucleus (PVN) (Kiwaki *et al.*, 2004), but did not have any effects when injected into the AccSh (Thorpe and Kotz, 2005). Consistent with its motor stimulating effect, an injection of orexin into the medioventral medullary alpha parts, LC (Kiyashchenko *et al.*, 2001; Mileykovskiy *et al.*, 2002), pedunculopontine nuclei (PPN), substantia nigra pars reticulata (Takakusaki *et al.*, 2005) or trigeminal motor nucleus (Peever *et al.*, 2003) increased muscle tone, but inhibited muscle tone when injected in the gigantocellular nucleus sites, dorsal paragigantocellular nucleus and nucleus pontis oralis (Kiyashchenko *et al.*, 2001; Mileykovskiy *et al.*, 2002).

Recently, Zhang *et al.* (2011) demonstrated that orexin-A signalling in the rat lateral vestibular nucleus (LVN) is involved in the vestibular-mediated motor, postural control and negative geotaxis, and intriguingly, whereas microinjection of SB-334867 into the LVN usually has no effect on this condition, it makes a difference when the rat is facing a major motor challenge. These results suggest that the motor effects of orexin are independent of its function in arousal and emotion, and provide a possible mechanism for the loss of muscle tone in cataplexy attacks. Interestingly, administration of orexin-A into the paraventricular nucleus of the midline thalamus reduces distance travelled, yet enhances the grooming and freezing behaviours in animals, whereas the OX receptor antagonist SB-334867 has no effects on these variables (Li *et al.*, 2009; 2010b). Consistent with the elevated levels of activity of the orexin system during active wakefulness being associated with high muscle tone and stirring movements (Kiyashchenko *et al.*, 2002; Torterolo *et al.*, 2003), the orexin system has been shown to be involved in the maintenance of food anticipatory activity (Akiyama *et al.*, 2004).

Cognition

Results from *in vitro* studies have shown that orexin augments excitatory activity in the prefrontal cortex (PFC) through both pre- (Fetissov *et al.*, 2004; Huang *et al.*, 2006) and postsynaptic (Song *et al.*, 2005; Xia *et al.*, 2005; 2009; Li *et al.*,

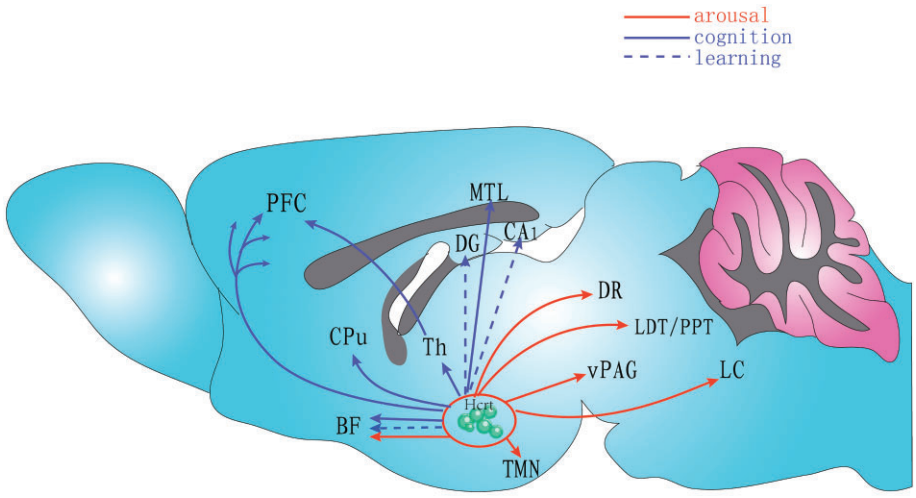

Figure 3

Effects of orexin (Hcrt) on arousal and cognition areas. This diagram summarizes the effects of orexin on nuclei involved in arousal and cognition (including learning). It should be noted that a role for the medial temporal lobe (MTL) and striatum [i.e. caudate putamen (CPu)] in these effects of orexin were suggested by a human study, in addition to the prefrontal cortex (PFC) and thalamus suggested from animal and human studies. The medial septum and diagonal band of Broca (MSDM) is included in the basal forebrain (BF); the MTL consists of the hippocampus, entorhinal cortex (EC), peri- and postrhinal cortex, subiculum, and pre- and parasubiculum (Pr-PaS). Abbreviations: CA1, Cornu Ammonis area 1; DG, dentate gyrus; DR, dorsal raphe nuclei; LC, locus coeruleus; LDT/PPT, laterodorsal tegmental nucleus/pedunculopontine tegmental nucleus; Th, thalamus; TMN, tuberomammillary nucleus; vPAG, ventral periaqueductal gray matter.

2010a; Yan *et al.*, 2012) mechanisms, which implies a potential role for orexin in cognition.

This involvement of orexin in cognition *in vivo* was first demonstrated by Lambe *et al.* (2005); they showed that an intra-PFC injection of orexin-B improved attentional processes in rats. Also i.v. injections and nasal delivery of orexin-A have been found to reduce the deleterious effects of sleep deprivation on cognitive performance in non-human primates. This effect was attributed to an increase in the activity of the dorsolateral prefrontal cortex, striatum and thalamus and an attenuation of the activation of the medial temporal lobe (Deadwyler *et al.*, 2007). Moreover, an injection of orexin-A into the rostral intralaminar thalamic nuclei has been found to promote working memory (Mair and Hembrook, 2008), whereas, administration of OX_1 receptor antagonist SB-334867 disrupts attentional performance in rats (Boschen *et al.*, 2009). More recently, it was observed that the ability of narcoleptic patients to make decisions under ambiguity conditions is impaired compared to their performance under explicit conditions (Bayard *et al.*, 2011).

More interestingly, i.c.v. injection of orexin-A has been shown to improve memory in both an active and passive avoidance paradigm (Jaeger *et al.*, 2002; Telegdy and Adamik, 2002). In contrast, i.c.v. administration of the OX_1 receptor antagonist SB-334867 attenuated taste preference learning in rats (Mediavilla *et al.*, 2011). Furthermore, antagonizing OX_1 receptors with SB-334867 in the CA1 or dentate gyrus impairs acquisition, consolidation and retrieval in the Morris water maze (Akbari *et al.*, 2006; 2007) and passive avoidance tasks (Akbari *et al.*, 2008). Further, in support of a role for orexin in memory, the spatial memory of rats with orexin- saporin lesions in the medial septum and diagonal band of Broca was impaired (Smith and Pang, 2005). Consistent with these observations, orexin-A has been shown to activate noradrenaline-induced long-term potentiation (LTP) in the dentate gyrus (Walling *et al.*, 2004), and to modulate long-term synaptic plasticity in CA1 region in an age-dependent manner (Selbach *et al.*, 2004; 2010). However, the exact role of orexin in learning and memory is still unclear. Although there is evidence that orexin-A impairs Morris water maze performance of rats and suppresses the LTP in hippocampal CA1 neurons (Aou *et al.*, 2003), it has also been found that administration of almorexant (p.o.), a dual OX receptor antagonist, has no effect on the learning and memory of rats (Dietrich and Jenck, 2010). However, the neuroexcitatory activity of the orexin peptides is consistent with an increase in excitability of postsynaptic dendrites in hippocampal neurons and enhanced LTP and memory, but the slow dynamics and the diversity of hyperpolarizing conductances activated by OX receptors may also explain the opposite results obtained (see Figure 3).

The reward system

The LH has long been known to have a prominent role in reward. Olds and Milner (Olds and Milner, 1954; Olds, 1962) showed that rats would self-stimulate current when an electrode was placed in the lateral hypothalamic region, in close proximity to orexin neurons. This effect was thought to be mediated by the medial forebrain bundle, which contains fibres of passage to the dopaminergic mesencephalic neurons from several neighbouring nuclei. Indeed, anatomical data have shown that orexin cells project to dopaminergic neurons in the VTA (Balcita-Pedicino and Sesack, 2007), although few synapses have been observed and many of these projections are fibres of passage.

The functional role of orexin in the reward process was demonstrated by Boutrel *et al*. (2005), who showed that i.c.v. infusion of orexin-A elevated intracranial self-stimulation thresholds in rats, which suggests that it decreases brain reward function by an action different from dopamine excitation. Indeed, there is much evidence connecting orexin with the effects of opioids and corticotrophin-releasing factor (CRF). Furthermore, orexin-A has been shown to facilitate glutamatergic synapses in dopaminergic neurons in the ventral tegmental area (VTA), providing a cellular basis for its behavioural effects (Borgland *et al*., 2006). Many other authors have now shown a direct role for orexin in the re-instatement of opioid- (Harris *et al*., 2005), cocaine- (Aston-Jones *et al*., 2008), alcohol- (Lawrence *et al*., 2006) and nicotine-seeking (Plaza-Zabala *et al*., 2010) behaviour. The orexin system may be differentially involved in stress- compared to cue-induced re-instatement of drug seeking behaviour. Interactions with non-dopaminergic systems such as CRF (see Stress section) or noradrenergic signalling may account for these neuromodulatory effects on drug-seeking behaviour following its eradication.

Also, it has been hypothesized that orexins have a role in sexual behaviour (Muschamp *et al*., 2007; Bai *et al*., 2009). Although the mechanisms of increased sexual drive may include the circuits involved in natural reward, there is no data directly linking orexin-induced hypersexuality and dopaminergic transmission. Detailed reviews on the role of orexin in brain reward and addiction have been recently published elsewhere (Mahler *et al*., 2012).

Energy homeostasis

Sakurai *et al*. (1998) delineated the orexigenic effect of the peptide in his landmark paper, which was validated by subsequent studies (Yamanaka *et al*., 1999); they showed that i.c.v. administration of pharmacological doses of orexin increases food intake, whereas administration of an antibody or OX receptor antagonist reduces food consumption in rats (Haynes *et al*., 2000). Yamanaka *et al*. (2003a) further demonstrated in an elegant study that one possible function of the orexin system is to integrate metabolic state into locomotor activity. Thus, they showed that mice lacking orexinergic neurons do not show an increase in locomotor activity induced by starvation. Other groups have shown that orexin-induced feeding is modulated by caloric challenge (Thorpe *et al*., 2005b). Orexinergic neurons are inhibited by glucose (Yamanaka *et al*., 2003a; Burdakov, 2004; Burdakov and Alexopoulos, 2005), triglycerides (Chang *et al*., 2004) and amino acids (Karnani *et al*., 2011). Moreover, the orexigenic peptide ghrelin activates orexinergic neurons whereas leptin, a hormone from adipose tissue, inhibits orexin cells (Yamanaka *et al*., 2003a,b). There is substantial evidence suggesting that orexinergic neurons in the LH have a prominent role in sensing the steady-state levels of physiologically relevant metabolites and integrate this information into a coherent value that is conveyed to arousal centres (Adamantidis and de Lecea, 2008b; 2009; Carter *et al*., 2009b).

With regard to the local hypothalamic circuit that regulates feeding, orexin excites MCH neurons (van den Pol *et al*., 2004) and inhibits ventral medial hypothalamic (VMH) glucoreceptors to enhance feeding behaviours (Shiraishi *et al*., 2000). Moreover, a recent study demonstrated that the area postrema and nucleus of the tractus solitarius (NTS) are necessary for orexin-mediated hyperphagia (Baird *et al*., 2009). More recently, another elegant study suggested that orexin neurons belong to the higher-order brain neurons that regulate the feeding-related motor and autonomic end organs (Perez *et al*., 2011). Furthermore, administration of orexin into the accumbens shell augments feeding behaviours (Thorpe and Kotz, 2005). DAMGO (a μ-opioid receptor agonist) injected into the core of the nucleus accumbens, mediated feeding behaviour by affecting OX_1 receptor signalling in the VTA (Zheng *et al*., 2007).

In addition to a direct interaction with feeding circuits and with humoral signals affecting feeding, orexinergic neurons have a prominent regulatory role in the brain reward system, and particularly for palatable food (Thorpe *et al*., 2005a; Borgland *et al*., 2009; 2010). The OX_1 receptor has been shown to mediate food motivation and reward-based feeding behaviour in mice (Sharf *et al*., 2010) and rats (Choi *et al*., 2010).

Importantly, orexins also modulate energy homeostasis by coordinating humoral factors. Administration of orexin-A s.c. increases the blood concentration of two adiposity signals: insulin (Nowak *et al*., 2000) and leptin (Switonska *et al*., 2002), which may explain why orexin signalling is enhanced in obesity-resistant rats (Mavanji *et al*., 2010). The relationship between orexin and leptin has been demonstrated at the anatomical level by Myers and colleagues (Louis *et al*., 2010; Leinninger *et al*., 2011); they identified a population of MCH-GABA + neurons in the LH that contain leptin receptors and make dense synaptic contacts with orexin cells. The specific role of these neurons in mediating food reward and integrating limbic signals remains to be determined. Funato *et al*. (2009) showed that augmented OX_2 receptor signalling improves insulin sensitivity and protects the mouse from diet-induced obesity, probably through improving leptin sensitivity. Following this study, Shiuchi *et al*. (2009) demonstrated that administration of orexin-A into the VMH also improves insulin sensitivity and enhances feeding-associated glucose utilization in skeletal muscle by enhancing the sympathetic tone and β-adrenoceptor-mediated signalling.

All these findings suggest that orexinergic neurons maintain a proper balance between energy intake or storage and expenditure initially by monitoring the nutritional state of the body in real time. Orexins also regulate energy intake or storage and expenditure by modulating the feeding and energy homeostasis-related circuits. It has been shown that the effect of orexin on appetite requires intact arcuate nucleus activity (Moreno *et al*., 2005), partially due to its dependence on activation of the neuropeptide Y (NPY) pathway (Yamanaka *et al*., 2000). Also in further support of a role for orexin in the modulation of local feeding circuits, it was found that administration of orexin into the paraventricular hypothalamus, dorsomedial hypothalamus, perifornical area or LH increases food intake (Dube *et al*., 1999; Thorpe *et al*., 2003). Like ghrelin, orexin facilitates the pacemaker activities of NPY/AgRP neurons but inhibits the effects of the proopiomelanacortin (POMC) neurons that suppress appetite (Muroya *et al*., 2004; Ma *et al*., 2007).

Endocrine

During the past decade, as discussed in detail in other reviews (Ferguson and Samson, 2003; Lopez *et al.*, 2010), anatomical data have suggested that orexin plays an important role in the regulation of endocrine function, including the hypothalamic-pituitary-adrenal (HPA), hypothalamic-pituitary-gonad (HPG), hypothalamic-pituitary-thyroid (HPT) systems, growth hormone (GH) and prolactin axes.

These data have now been supported by several physiological studies as detailed below.

HPA axis. Administration of orexin-A, i.c.v., increases plasma adrenocorticotropic hormone (ACTH), corticosterone concentrations, as well as CRF mRNA levels and fos expression in the parvocellular cells of PVN (Al-Barazanji *et al.*, 2001). This orexin-induced activation of the HPA axis involves both OX receptors (Samson and Taylor, 2001; Samson *et al.*, 2002; Ferguson and Samson, 2003), and NPY-dependent (Jaszberenyi *et al.*, 2001) and NPY-independent (Moreno *et al.*, 2005) pathways. Interestingly, these responses are attenuated by pregnancy or CRF antagonists (Jaszberenyi *et al.*, 2000). Moreover, ACTH production is reduced in narcoleptic patients (Kok *et al.*, 2002).

However, orexin-A suppresses the CRF-stimulated ACTH secretion (Samson and Taylor, 2001). Moreover, orexin-A directly stimulates corticosterone secretion (Malendowicz *et al.*, 1999; 2001; Ziolkowska *et al.*, 2005), and stimulates (Kawada *et al.*, 2003), inhibits (Nanmoku *et al.*, 2000), or has no effect (Mazzocchi *et al.*, 2001) on catecholamine release from the adrenal medulla or phaeochromocytoma cells. In addition, orexin also modulates the proliferation of normal adrenocortical cells or adenomatous cells (Spinazzi *et al.*, 2005a,b).

Whether orexin peptides can be produced by extrahypothalamic sources is still not known. A significant body of literature indicates peptide activity and receptor expression in peripheral organs including the adrenal gland and the gonads. However, there is still no consensus about the origin and the quality of orexin immunoreactive signals in plasma (Arihara *et al.*, 2001; Dalal *et al.*, 2001; Adam *et al.*, 2002; Higuchi *et al.*, 2002; Igarashi *et al.*, 2003; Busquets *et al.*, 2004; Baranowska *et al.*, 2005).

Taken together, the existing data suggest that the orexinergic system at least centrally, is an important modulator of CRF responses and, by extension, of the HPA axis, with potential implications in stress, anxiety and panic disorders.

HPG axis. Administration of orexins, i.c.v., increases LH secretion in ovariectomized (OVX) female rats in the presence of oestradiol and progesterone (Brunton and Russell, 2003), consistent with the observation from orexin-deficient narcolepsy patients exhibit diminished LH release (Kok *et al.*, 2004). However, in the absence of oestradiol or progesterone, orexins suppressed LH release in OVX rats (Pu *et al.*, 2000). Potential mechanisms for this effect may involve NPY (Kiyokawa *et al.*, 2011), CRF (Iwasa *et al.*, 2007), β-endorphin (Irahara *et al.*, 2001) and oestrogen (Furuta *et al.*, 2002). In addition to its ovarian steroid-dependent actions, orexin-A has region-dependent effects on LH release; it increases LH release when microinjected into the rostral preoptic area but reduces LH release when microinjected into the medial preoptic area or arcuate/median eminence, an effect which can be inhibited by the selective OX_1 receptor antagonist SB-334867 (Small *et al.*, 2003).

Notably, orexins also exert their endocrine effects at the testicular level, as orexin-A directly stimulates testosterone secretion in rat testis. In contrast, expressions of key Sertoli cell genes and DNA synthesis in specific stages of the seminiferous epithelium are inhibited by orexin-A (Barreiro *et al.*, 2004; 2005). In addition, i.p. injection of an OX_1 receptor antagonist (SB-334867) and/or a selective OX_2 receptor antagonist (JNJ-10397049) decreased pro-oestrus gonadotropins and ova number, accompanied by haematological (hyperaemic and/or haemorrhagic) reaction of ovaries with more preovulatory follicles and less corpora lutea (Silveyra *et al.*, 2007). Among the peripheral actions of the orexin peptides, those reported in the gonads seem to have the strongest and most reliable responses. No reports of decreased fertility in narcoleptics have been published, but whether orexin-deficiency in narcolepsy also applies to peripheral sources is unclear.

HPT axis. Orexin-A, i.c.v., inhibits the release of hypothalamic thyrotropin-releasing hormone and subsequently reduces the plasma levels of thyrotropin (TSH). However, orexin-A failed to affect TSH release at the adenohypophysis level, and the plasma thyroid hormone levels showed no changes after peripheral or intra-PVN administration of orexin-A (Mitsuma *et al.*, 1999; Samson and Taylor, 2001; Russell *et al.*, 2002). In addition, narcoleptic patients exhibit decreased plasma TSH concentrations (Kok *et al.*, 2005) but normal plasma thyroid levels (Chabas *et al.*, 2007). In contrast to orexin-A, i.c.v. administration of orexin-B increases the plasma TSH level (Jones *et al.*, 2001). It is possible that imbalances in TSH levels are the cause of metabolic alterations and obesity in adolescent narcoleptics.

GH. In early studies, orexin-A was reported not to stimulate GH release directly (Xu *et al.*, 2002) but to enhance GH-releasing hormone (GHRH)-stimulated GH secretion (Xu *et al.*, 2002) in primary cultured ovine somatotrophs, whereas orexin-B directly increased GH secretion. Furthermore, both orexin-A (Seoane *et al.*, 2004) and orexin-B (Barb and Matteri, 2005) failed to affect the GH secretion from pituitary cells. Administration of orexin-A, i.c.v., was found to reduce the plasma GH levels (Hagan *et al.*, 1999) by inhibiting spontaneous GH secretion (Seoane *et al.*, 2004). Moreover, orexin-A stimulates somatostatin release (Russell *et al.*, 2000) and increases the somatostatin mRNA content in the hypothalamic periventricular nucleus in a GH-dependent manner (Lopez *et al.*, 2004) but selectively decreases the GHRH mRNA in PVN. In addition, orexin-A markedly blunted the GH responses to ghrelin but not GHRH (Seoane *et al.*, 2004). Finally, orexin-deficient narcoleptic patients exhibit normal rates of basal and pulsatile GH secretion (Overeem *et al.*, 2003).

Prolactin. Orexins decrease basal plasma prolactin levels (Jones *et al.*, 2001), and reduce the domperidone-induced plasma prolactin concentrations (Russell *et al.*, 2000), probably through a mechanism involving NPY (Hsueh *et al.*, 2002). In addition, orexin-A restores the fasting-induced abo-

lition of prolactin and LH surges, and anti-orexin-A antisera also abolish these surges in normally fed rats (Kohsaka *et al.*, 2001). Interestingly, the study on the pituitary explants showed that the regulation of prolactin secretion by orexin-A is day-length dependent, namely, orexin-A increases prolactin secretion during the long days (May) but decreases it during the short days (December) (Molik *et al.*, 2008). However, a recent clinical study showed that prolactin secretion is no different in narcolepsy patients than in matched controls (Donjacour *et al.*, 2011), consistent with a lack of effect of both orexin-A and orexin-B on prolactin secretion at the pituitary level (Russell *et al.*, 2000; Russell *et al.*, 2001; Samson and Taylor, 2001).

Stress

The terminology of 'stress' means a subjective state perceiving or anticipating the adverse disturbances in surroundings, which further activates various stress mediators to elicit proper responses (Joels, 2009). The role of the orexinergic system in stress responses has been well established on the basis of three kinds of evidence. Firstly, many stressors, including immobilization, footshock, cold exposure, conditioned fear, food and neonatal maternal deprivation are able to activate the orexinergic system (Ida *et al.*, 2000; Sakamoto *et al.*, 2004; Winsky-Sommerer *et al.*, 2004; 2005; Furlong *et al.*, 2009) (Horvath and Gao, 2005). Secondly, some stress-induced responses, such as stress-induced analgesia (Xie *et al.*, 2008), footshock-induced re-instatement of cocaine seeking (Boutrel *et al.*, 2005; Boutrel and de Lecea, 2008; Boutrel *et al.*, 2009) as well as stress-induced ACTH and cardiovascular responses (Samson and Taylor, 2001; Kayaba *et al.*, 2003; Chang *et al.*, 2007), induce activation of the orexin system. It is noteworthy that orexin-A is not involved in stress-induced thermogenesis (Zhang *et al.*, 2010) or cardiovascular responses to cold exposure (Furlong *et al.*, 2009), even though it is able to inhibit stress-induced delayed increase in the amount of REM sleep (Rachalski *et al.*, 2009). Finally, activation of orexinergic neurons results in some stress-like effects. As discussed earlier, orexin can activate the HPA axis including CRF, ACTH and corticosterone, stimulate stress-related behaviours like grooming and chewing of inedible material, and enhance the activation of the monoamine system in a stress-like manner (Berridge *et al.*, 2010).

A link between orexin and stress-related pathologies, such as panic disorder, has recently been established (Johnson *et al.*, 2010; Lungwitz *et al.*, 2012). Panic attacks involve activation of the HPA axis and the autonomic system. Intrahypothalamic administration of an RNAi to orexin or an OX_1 receptor antagonist has been shown to block these panic responses in rats injected with sodium lactate, and elevated levels of orexin-A have been detected in humans with panic disorder (Johnson *et al.*, 2010).

Visceral functions

The orexin system has also been found to effect visceral functions, in addition to its roles in energy homeostasis and endocrine function, mentioned previously.

Circulatory system. Orexin-deficient mice exhibit lower arterial blood pressure, heart rate and sympathetic tone (Kayaba *et al.*, 2003). Furthermore, i.c.v., intracisternally or intrathecally applied orexin increases the mean arterial pressure (MAP), heart rate (HR), renal sympathetic nerve activity and plasma catecholamine or vasopressin levels (Samson *et al.*, 1999; 2005; Shirasaka *et al.*, 1999; 2002; Matsumura *et al.*, 2001; Hirota *et al.*, 2003), effects that are blocked or attenuated by the OX_1 receptor antagonist SB-334867 (Hirota *et al.*, 2003; Shahid *et al.*, 2011). However, i.v. injections of orexin-A have no effect on sympathetic activity (Matsumura *et al.*, 2001), suggesting that the cardiac effects of orexin are mediated centrally. Consistently, microinjections of orexin-A into the rostral ventrolateral medulla (Huang *et al.*, 2010) or rostral ventromedial medulla (Ciriello and de Oliveira, 2003) elicit cardiovascular excitatory responses through the activation of both OX_1 and OX_2 receptors (Huang *et al.*, 2010).

However, orexin-A signalling in the nucleus ambiguus (NA) (de Oliveira and Ciriello, 2003) and subfornical organ (Smith *et al.*, 2007) has been shown to produce bradycardia responses, which are, respectively, mediated by an elevation of vagal excitation and a reduction of sympathetic tone. In contrast, it has also been found that orexin-A enhances the inhibitory input and attenuates excitatory synapses to vagal neurons in the NA (Dergacheva *et al.*, 2005). Moreover, the cardiac effects of orexin in the NTS are both dose- and site-dependent, as orexin increases MAP and HR at higher doses (>20 pmol) but reduces these variables at a lower dose (5 pmol). Furthermore, microinjections of orexin into the caudal lateral and medial subnuclei of the NTS decrease both the MAP and HR (de Oliveira *et al.*, 2003b), whereas pressor and tachycardiac effects were obtained when orexin was injected into the commissural nucleus of NTS (Smith *et al.*, 2002).

Respiratory effects. Several studies with preproorexin-KO and orexinergic neuron-ablated mice have demonstrated the essential role orexin plays in the hypercapnic chemoreflex response, as well as in phrenic and ventilatory long-term facilitation (Deng *et al.*, 2007; Nakamura *et al.*, 2007; Terada *et al.*, 2008). Also, pharmacological experiments have shown that i.c.v., intracisternal or intrathecal administration of orexin has the ability to elevate respiratory frequency, tidal volume and minute ventilation (Shahid *et al.*, 2011). Furthermore, orexin signalling on OX_1 receptors in the retrotrapezoid nucleus contributes to the control of the hypercapnic chemoreflex (Dias *et al.*, 2009). In addition, when applied to the pre-Botzinger region and phrenic nuclei, orexin augments the phrenic nerve discharge and, subsequently, the electromyographic activity of the diaphragm (Liu *et al.*, 2010). Moreover, injection of orexin-B into pontine Kölliker-Fuse nucleus results in an increase in respiratory frequency and facilitation of upper airway patency (Dutschmann *et al.*, 2007). In parallel, it may be worth noting that orexinergic neurons are strongly inhibited by anaesthetics and orexin-KO mice show delayed emergence from isofluorane anaesthesia (Kelz *et al.*, 2008). However, ambient levels of H^+ and CO_2 can significantly enhance the activation of orexinergic neurons (Williams *et al.*, 2007; Williams and Burdakov, 2008). Unlike in mice, chemoresponsiveness in humans is independent of OX_1 receptors, as the different ventilatory responses to hypoxia observed in humans with narcolepsy-cataplexy have

been associated with the HLA-DQB1*0602 allele that segregates with narcolepsy, but not an orexin deficiency (Han, 2012). Therefore, in humans ventilatory responses to hypoxia may be mediated by other factors or immune components independent of orexin.

It has been suggested that orexinergic neurons are involved in sleep apnoea syndrome, as patients show increased plasma levels of orexin-A (Igarashi *et al.*, 2003; Busquets *et al.*, 2004). Hypercapnia and associated reflexes may increase the activity of orexinergic neurons during sleep, facilitating microarousals and a cascade of sympathetic activity that results in elevated blood pressure during the night. Thus, OX receptor antagonists could be used to prevent these peaks of blood pressure in mild sleep apnoea.

Regulation of digestive activity. Early work showed that orexin immunoreactivity is present in intestinal tissue (Kirchgessner and Liu, 1999). Intracisternal or intraventromedial hypothalamus, but not i.p., injections of orexin-A stimulate gastric acid secretion by activating the vagal system through OX_1 receptors (Takahashi *et al.*, 1999; Yamada *et al.*, 2005; Eliassi *et al.*, 2009; Kermani and Eliassi, 2012). Moreover, activation of OX_1 receptors in the dorsal motor nucleus of the vagus results in facilitation of vagal pancreatic efferent nerve activities (Wu *et al.*, 2004), stimulating pancreatic exocrine secretion (Miyasaka *et al.*, 2002). Administration of orexin-A, i.a., increases duodenal secretion in normal fed but not in fasted animals, by an effect that is independent of cholinergic pathways (Flemstrom *et al.*, 2003; Bengtsson *et al.*, 2007). In addition, orexin-A can modify gastrointestinal motility, including gastric emptying, gastric interdigestive motility (Naslund *et al.*, 2002; Ehrstrom *et al.*, 2005a,b; Bulbul *et al.*, 2010), and enteric peristalsis (Satoh *et al.*, 2006), as well as colonic motility (Kirchgessner and Liu, 1999; Nozu *et al.*, 2011). It is noteworthy that orexin exerts region-specific effects on gastric contractility and relaxation both at the central and peripheral levels, by mechanisms involving ACh and NO respectively (Kobashi *et al.*, 2002; Krowicki *et al.*, 2002; Baccari *et al.*, 2009; Baccari, 2010). Furthermore, orexin-A shows gastroprotective effects against stress-induced (Brzozowski *et al.*, 2008), ischaemia-reperfusion-induced (Bulbul *et al.*, 2008) or ethanol-induced (Yamada *et al.*, 2007) gastric damage.

Urinary activity. The presence of orexin-A and its receptors has been shown in human kidneys and urine (Takahashi *et al.*, 2006), as well as in the bovine urethroprostatic complex (Russo *et al.*, 2008). These findings are supported by results from physiological studies, which demonstrated that orexin-A is involved in the pelvic-urethral reflex (Peng *et al.*, 2008) and the micturition reflex (Kobayashi *et al.*, 2009).

Overall perspective

The remarkable pleiotropic role of a relatively small neuronal system such as the one formed by orexin cells can be explained in the context of an integrating system that controls some key features of a homeostatic output. Indeed, it is hard to imagine a central role in such diverse functions as endocrine, pain or respiration for such a relatively recent peptide, evolutionary speaking. The ubiquitous distribution of the axonal processes of orexin, the phasic nature of its activity (possibly superimposed on a circadially regulated endocrine release), and the very slow action on at least some postsynaptic sites suggests it has a broad modulatory role. Orexinergic neurons may thus integrate activity from diverse inputs from limbic structures over windows of 1–10 s and, depending on the state of membrane depolarization, may fire phasically to induce postsynaptic depolarizations in many structures. These depolarizations may be subthreshold or elicit action potentials, depending on the state of depolarization of their postsynaptic targets. Failure to integrate critical information at precise times of awakening causes the behavioural instability associated with narcolepsy/cataplexy. As reviewed here, the orexins are also involved in many peripheral actions, including endocrine, respiration, cardiovascular and gastrointestinal effects, but the mechanisms of such effects are still poorly understood. As drugs that affect orexin signalling enter the market, more attention will be devoted to the myriad possible functions of these hormones. Moreover, these advances may also lead to the development of small molecule orexin ligands that could serve as an effective treatment for narcolepsy/cataplexy and other sleep disorders.

Conflict of interest

None.

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