The role of orexin in motivated behaviours

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Abstract | Wakefulness and vigilance levels are required for maintaining purposeful activities and motivated behaviours, which are often triggered by sensory information conveying external cues. An increasing body of work has suggested that orexins (also known as hypocretins) — a pair of neuropeptides that are crucial for maintaining wakefulness — are also involved in the regulation of motivated behaviours, including feeding, emotional behaviour and reward seeking, and that these functions are mediated by two subtypes of orexin receptors. Autonomic and endocrine responses, which accompany these motivated behaviours, are also influenced by the orexin system. Orexin-producing neurons act as a hub that links information about the internal and external environments of an animal to vigilance levels and internal bodily functions to support various motivated behaviours.

Orexins are hypothalamic neuropeptides that play a highly important part in the regulation of wakefulness. Orexin A (also known as hypocretin 1) and orexin B (also known as hypocretin 2) are derived from a common precursor peptide, and both bind to the orexin receptor type 1 (OX1R) and orexin receptor type 2 (OX2R) (BOX 1).

Orexins are receiving a lot of attention as endogenous, potent, arousal-promoting peptides, and several orexin receptor antagonists are expected to be clinically available for treating insomnia in the near future. However, an enormous body of work has shown that orexins are involved in the regulation of a wide range of behaviours. Indeed, the orexin system has close functional relationships with systems that regulate emotion and reward. Orexin neurons, which are located in the lateral hypothalamic area (LHA), perifornical area (PFA), dorsomedial hypothalamus (DMH) and posterior hypothalamus, receive and integrate internal and external information and regulate autonomic and neuroendocrine systems, arousal (or vigilance) levels and behaviour accordingly (FIG. 1). Thus, although orexin receptor antagonists are thought to have fewer side effects compared with other sedatives, such as classical benzodiazepine or other $GABA_A$ receptor modulators, it is important to understand the role of orexins in physiological processes other than the regulation of sleep–wakefulness states.

The neuronal pathways and receptors via which orexins are involved in these processes seem to be partly overlapping and partly distinct. For example, several findings have suggested that the arousal-promoting function of orexins is mainly mediated by OX2R[1,2], whereas the role of orexin in regulating reward and feeding is predominantly mediated by OX1R[3–5]. In addition, several studies have suggested that orexin neurons in the LHA interact with the reward system, whereas those in the PFA and DMH are important for arousal and vigilance control[3,6,7]. However, it remains to be seen whether there indeed are disparate functional roles of these orexin neuron clusters, as these findings have not yet been confirmed by other studies.

In this Review, I discuss the multiple physiological roles of these multitasking neuropeptides beyond their role in controlling sleep and wakefulness, as well as the receptors and neuronal pathways involved in these functions. I then consider how the orexin system coordinates vigilance and autonomic and neuroendocrine functions to support motivated behaviour in accordance with the internal and external conditions of the animal.

Feeding behaviour

Feeding behaviour is one typical example of motivated behaviour. It is evoked by food-predictive cues as well as by humoral signals that indicate that energy balance is low. Orexin was initially reported as a factor that regulates feeding behaviour, mainly because neurons that produce orexin (orexin neurons) are bilaterally distributed within the LHA (and adjacent regions), which is known as the classical feeding centre[8]. In rats, lesions in this region cause anorexia, whereas electrical stimulation results in overeating and obesity[9]. An orexigenic effect of intracerebroventricular (ICV) administration of orexin A and orexin B in rats was first reported in 1998 (REF. 8), and this effect was subsequently confirmed

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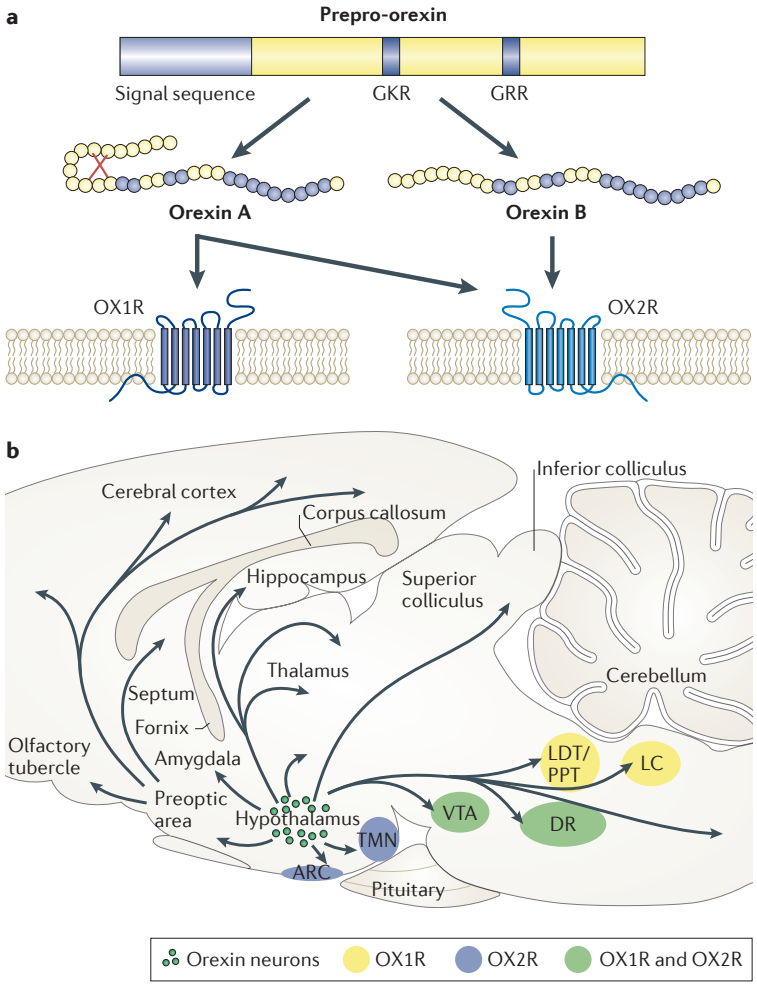
Box 1 | **Orexins and orexin receptors**

Orexin A and orexin B were identified from rat brain extracts as two endogenous ligands for two orphan G protein-coupled receptors (GPCRs) by a method called 'reverse pharmacology' (REF. 8). Molecular cloning studies showed that orexin A and orexin B are derived from a common precursor peptide, prepro-orexin (see the figure, part **a**). An mRNA encoding the same precursor peptide was independently identified by de Lecea *et al.* as a hypothalamus-specific transcript[132]. The authors predicted that the transcript encoded a polypeptide precursor that is cleaved at amino acid residues GKR and GRR (see the figure, part **a**) to form two neuropeptides, hypocretin-1 and hypocretin-2 (which correspond to orexin A and orexin B, respectively). Structural analysis of the purified peptides showed that orexin A is a 33 amino acid peptide with an amino-terminal pyroglutamyl residue, two intra-chain disulphide bonds (indicated by the red lines), and carboxy-terminal amidation. This structure is completely conserved among several mammalian species (human, rat, mouse, cow, sheep, dog and pig). Orexin B is a 28 amino acid, C-terminally amidated linear peptide. Analysis of the amino acid sequence of orexin B has revealed that there are several species differences, although overall orexin B is highly conserved. The C-terminal portion of orexin B is similar to that of orexin A, whereas the N-terminal portion is more variable. Amino-acids with blue colours in part **a** of the figure indicate residues that are conserved between orexin A and orexin B.

The actions of orexins are mediated by two GPCRs: orexin receptor type 1 (OX1R) and orexin receptor type 2 (OX2R) (see the figure, part **a**). OX1R has one order of magnitude greater affinity for orexin A over orexin B, whereas OX2R accepts both ligands with similar affinities[8]. The two orexin receptors exhibit a markedly different and basically complementary distribution, which suggests that they have distinct physiological roles through different neuronal pathways[88] (see the figure, part **b**).

Orexin neurons, of which there are approximately 3,000 in the rat brain and approximately 70,000 in the human brain[51,52], are localized exclusively in the hypothalamus, including the lateral hypothalamic area (LHA), perifornical area and posterior hypothalamus[51,52,133], but project widely in the brain (see the figure, part **b**). Projections to monoaminergic and cholinergic nuclei in the brain stem, where OX1R and OX2R are differentially expressed, are particularly dense (see the figure, part **b**). The signal transduction mechanisms for the two orexin receptors have been reviewed elsewhere[134,135].

The best understood role of orexins is their regulation of sleep and wakefulness. Indeed, as deficiencies in orexin or OX2R cause narcolepsy — an inability to maintain wakefulness — in mice, dogs and humans[136,137], the orexin system is thought to play an important part in the regulation of sleep and wakefulness, especially in the maintenance of long, consolidated awake periods. Nevertheless, sleep-deprivation studies have shown that humans and animals with narcolepsy are capable of maintaining wakefulness (although their sleep–wake pattern is fragmented), which suggests that the role of orexin in arousal is not exclusive and can be compensated for when orexins are absent[138]. It is now thought that the orexin system orchestrates and modulates the control of arousal and vigilance levels in response to external cues. ARC, arcuate nucleus; DR, dorsal raphe nuclei; LC, locus coeruleus; LDT, laterodorsal tegmental nucleus; PPT, pedunculopontine tegmental nucleus; TMN, tuberomamillary nucleus; VTA, ventral tegmental area.



in several species[10]. Furthermore, central administration of an orexin antibody or an OX1R antagonist has been shown to decrease food intake[4,11].

Intraperitoneal administration of the selective OX1R antagonist (1-SORA) SB-334867 (BOX 2) or RNAi-mediated knockdown of the orexin gene reduced food intake in mice exposed to mild food restriction[12], and orexin-deficient mice show decreased food intake[13,14]. Importantly, orexin signalling increases not only food intake but also energy expenditure, and an increase in the overall orexin tone generally results in decreased body weight[15]. The role of orexins in body-weight regulation is discussed in the next section.

The orexin system might contribute to the regulation of energy homeostasis by integrating information regarding metabolic state and regulating sleep–wake state in order to support feeding behaviour[1,16,17]. Indeed, mice lacking orexin neurons do not show an increase in wakefulness or locomotor activity in response to starvation, unlike wild-type mice[16]. Moreover, *prepro-orexin* mRNA is upregulated in fasted animals[8], and several studies report that the firing rates of orexin neurons are influenced by glucose, triglycerides and amino acids[16,18–21]. Furthermore, orexin neurons are innervated by neurons in the arcuate nucleus (which are primary sensors for plasma leptin levels)[22], and they are directly inhibited by leptin and excited by

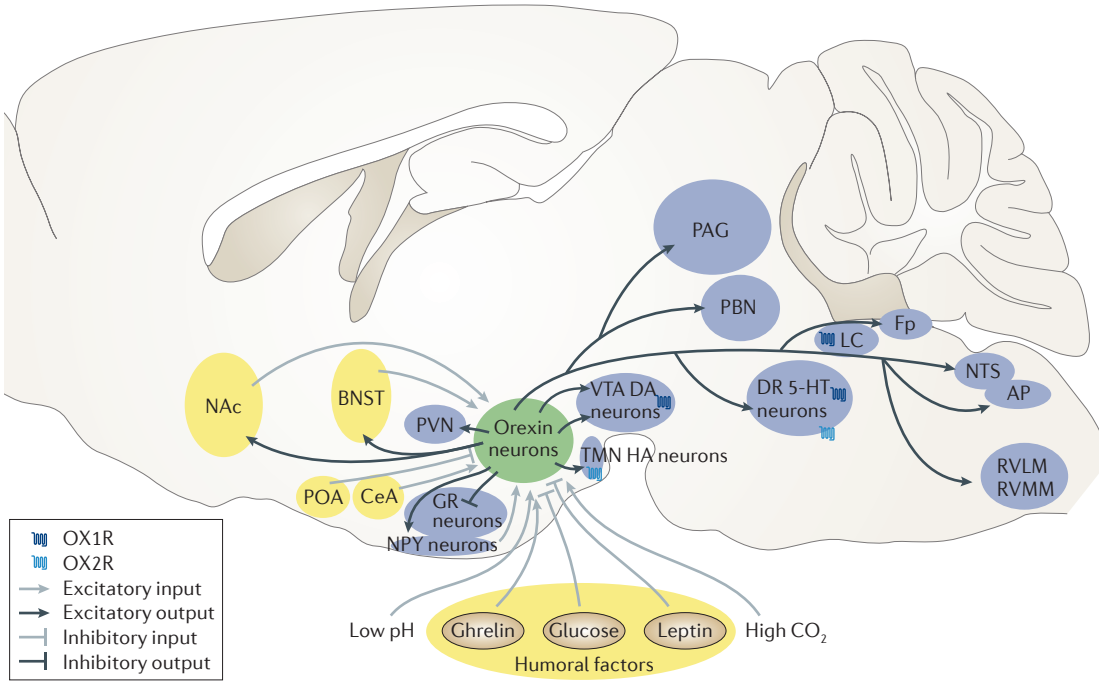


Figure 1 | **Input and output of orexin neurons.** Input areas are shown in yellow, output areas are shown in blue. Orexin neurons respond to salient cues or contexts, which are conveyed by projections from the nucleus accumbens (NAc) and limbic structures, such as the bed nucleus of the stria terminalis (BNST) and the central nucleus of the amygdala (CeA). Orexin neurons also monitor factors that reflect the metabolic state of the body, such as ghrelin, glucose and leptin, and their activity is further affected by CO_2 and pH levels. Orexin neurons send excitatory projections to various regions that are implicated in the regulation of feeding, including the NAc, nucleus of the solitary tract (NTS), paraventricular nucleus of the hypothalamus (PVN), neuropeptide Y (NPY) neurons in the arcuate nucleus and glucoreceptor (GR) neurons in the ventromedial hypothalamus. Orexin neurons also connect with autonomic regulatory regions to increase sympathetic outflow in response to salient cues or contexts. Connections between the NAc, orexin neurons and the ventral tegmental area (VTA) might have a role in the reward system. Perception of cues that predict reward might be conveyed by the connection between the NAc and orexin neurons, which send excitatory signals to dopamine (DA) neurons in the VTA. Orexin neurons also increase arousal to support motivated behaviour through connections between these cells and monoaminergic centres, including the dorsal raphe nuclei (DR), locus coeruleus (LC) and tuberomamillary nucleus (TMN). Note that 'excitatory' and 'inhibitory' do not necessarily indicate direct excitatory and inhibitory connections. 5-HT, 5-hydroxytryptamine (also known as serotonin); AP, area postrema; Fp, folium-p; HA, histamine; OX1R, orexin receptor type 1; OX2R, orexin receptor type 2; PAG, periaqueductal grey; PBN, parabrachial nucleus; POA, preoptic area; RVLM, rostral ventrolateral medulla; RVMM, rostral ventromedial medulla.

ghrelin[16]. Together, these observations suggest that orexin neurons sense the metabolic and nutritional status of the animal and integrate this information to evoke the level of arousal necessary to promote food-seeking behaviour in response to negative energy balance.

OX2R is thought to be a major player in the regulation of wakefulness, whereas the studies using 1-SORA SB-334867 point to the importance of OX1R in the regulation of food seeking[4,12]. This suggests that the orexin system influences food intake and wakefulness through at least partially different receptors and pathways.

Orexin neurons directly affect the neuronal circuits in the hypothalamus that are implicated in the regulation of feeding behaviour. They inhibit glucoreceptor neurons in the ventromedial hypothalamus (VMH) and excite neuropeptide Y (NPY) neurons in the arcuate nucleus (ARC) and melanin-concentrating hormone (MCH) neurons in the LHA[23–25]. Conversely, NPY stimulates feeding in an orexin-dependent manner, and this interaction might be modulated by leptin signalling[26]. Local application of orexin in the paraventricular nucleus of the hypothalamus (PVN), DMH or LHA increases food intake in rats[27–29]. The area postrema and nucleus of the solitary tract (NTS) have also been shown to be involved in orexin-mediated feeding behaviour[30,31]. Together, these studies suggest that orexin promotes feeding behaviour by influencing multiple aspects of the feeding circuitry (FIG. 2).

Orexin is also likely to play an important part in the hedonic and reward aspects of feeding: in rats, ICV administration of orexin can increase the motivation for food seeking, especially for palatable food[32–34]. OX1R is involved in reward regulation by orexin (see below)[3,12,35]. Furthermore, feeding behaviour induced by administration of the μ-opioid receptor agonist DAMGO (D-Ala(2)-N-MePhe(4)-Gly-ol(5)-encephalin) into the shell of the nucleus accumbens (NAc) (a key structure in the reward system) was dependent on OX1R activation[36], and intraperitoneal injection of the 1-SORA SB-334867 reduced high-fat food intake in food-restricted rats[32,35,37].

Box 2 | **Orexin receptor antagonists**

As orexins have been implicated in the maintenance of arousal, several companies have been exploring the possibility of using orexin receptor antagonists as drugs for insomnia treatment. In particular, suvorexant is expected to be clinically available soon. A recent randomized, placebo-controlled, parallel-group trial for primary insomnia showed that suvorexant improved sleep as indicated by subjective measures of sleep onset and maintenance. So far, several orexin receptor antagonists with different pharmacological characteristics have been developed (see the table). These compounds are broadly used for pharmacological studies in order to investigate which receptor (or receptors) is involved in particular physiological functions.

Type	Compound	Affinity (K_i) (nM)		Refs
		For human OX1R	For human OX2R	
DORA	ACT-078573 (almorexant)	13	8	139
DORA	MK-4305 (suvorexant)	0.6	0.4	140
DORA	SB-649868	0.3	0.4	141
1-SORA	SB-410220	8.1 (pK_b)	6.3 (pK_b)	142
1-SORA	SB-334867	28	1,704	143
1-SORA	SB-408124	22	1,405	142
1-SORA	SB-674042	1.1	129	142
1-SORA	ACT-335827	6 (IC_{50})	417 (IC_{50})	72
2-SORA	1-(2-bromo-phenyl)-3-((4S,5S)-2,2-dimethyl-4-phenyl-[1,3] dioxan-5-yl)-urea	5.3–6.1(pK_i)	6.8–7.1(pK_i)	144
2-SORA	JNJ-10397049	1,644	6	144
2-SORA	C1m	27 (IC_{50})	3,000 (IC_{50})	2,145
2-SORA	EMPA	900	1.1	146
2-SORA	TCS-OX2-29	ND	7.4 (pK_i)	147

1-SORA, selective OX1R antagonist; 2-SORA, selective OX2R antagonist; DORA, dual non-selective orexin receptor antagonist; IC_{50}, half-maximal inhibitory concentration; ND, not determined; OX1R, orexin receptor type 1; OX2R, orexin receptor type 2; pK_b, the negative logarithm of the dissociation constant of a competitive agonist (K_b); pK_i, the negative logarithm of the inhibition constant (K_i).

A recent study showed that the number of FOS-immunoreactive orexin neurons in the hypothalamus increased in response to a chow-predictive (that is, conditioned) cue in rats[38]. Similarly, the expectation of receiving a palatable food, such as chocolate, increased the number of FOS-positive hypothalamic orexin neurons[35]. The number of FOS-positive orexin neurons in the LHA also increased after conditioned place preference training for a sweet cereal reward in rats[3]. Together, these findings indicate that orexin has a role in food pursuit — especially when motivation towards food is high (for example, when an animal is food-deprived or when foods are palatable) or when conditioned cues are present. Inputs from the limbic system and NAc — which are thought to process the affective content of the perception of food[39] — are possibly involved in this function, and this information might be passed onto orexin neurons. Interestingly, food-related cues often evoke cataplexy in narcoleptic dogs (which have disrupted orexin signalling)[40]. This suggests that the perception of food normally induces orexin signalling and that this signalling is necessary to elicit feeding behaviour, including the maintenance of motor activity and wakefulness.

Cataplexy
A sudden weakening of muscle tone that often accompanies narcolepsy.

Narcolepsy
A sleep disorder caused by a specific loss of hypothalamic orexin neurons.

In summary, to promote feeding behaviour, orexin neurons are excited by food-related cues and/or a low energy balance through neuronal connections with the limbic system and by factors that indicate a low energy balance (FIG. 2).

Body-weight homeostasis

As orexins regulate feeding behaviour and sense metabolic state, they are likely to influence body-weight homeostasis as well. Indeed, the incidence of obesity among individuals with narcolepsy, who have a reduced number of orexin neurons, is twice as high as in the general population, even though narcoleptic individuals tend to have a lower food intake[41]. This is consistent with animal studies that showed that orexin deficiency results in late-onset obesity in mice[13,14,37,42,43]. Furthermore, people with narcolepsy have a higher incidence of obesity than people with idiopathic hypersomnia, which suggests that the sleepiness and reduced activity associated with narcolepsy are not the sole causes of obesity in these individuals[41].

Interestingly, as mentioned above, orexins increase feeding and energy expense simultaneously. Indeed, acute administration of orexins promotes feeding, and orexin-overexpressing transgenic mice show resistance to high-fat diet-induced obesity and insulin insensitivity — an effect that is predominantly mediated by OX2R signalling[15], although the precise site of action for this effect is not currently known. Furthermore, orexin overexpression enhanced the anorectic–catabolic effects of central leptin administration, whereas orexin overexpression (or OX2R agonist administration) had no effect in obese leptin-deficient mice[15]. These findings suggest that enhanced OX2R signalling confers resistance to diet-induced obesity, at least partly by enhancing the effect of leptin. The idea that orexin signalling confers resistance to obesity is supported by a recent study using naturally occurring variations in spontaneous activity in Sprague–Dawly rats. High-activity rats had higher expression of *prepro-orexin* mRNA and a higher sensitivity to the behavioural effects of orexin injection, as well as higher basal energy expenditure and greater resistance to obesity[44]. Moreover, repeated orexin administration prevented diet-induced obesity in these rats. Consistent with the idea that orexin expression is associated with motor activity, central administration of orexin was shown to potently increase locomotor activity in animals[45], whereas orexin-deficient mice exhibited reduced daily locomotor activity compared with wild-type mice[42]. The orexin-induced stimulation of motor activity might partly contribute to the anti-obesity effect of orexins.

A study in rodents showed that orexin A injections into the VMH increased insulin sensitivity in muscle (an effect that was mediated by OX1R) and enhanced feeding-associated glucose use in skeletal muscle by increasing the activity of the sympathetic nervous system[46]. Thus, orexin neurons also contribute to the regulation of peripheral metabolism. Furthermore, orexins have a role in diet-induced thermogenesis, which might (partially) prevent weight gain when animals are exposed to increased caloric load[47].

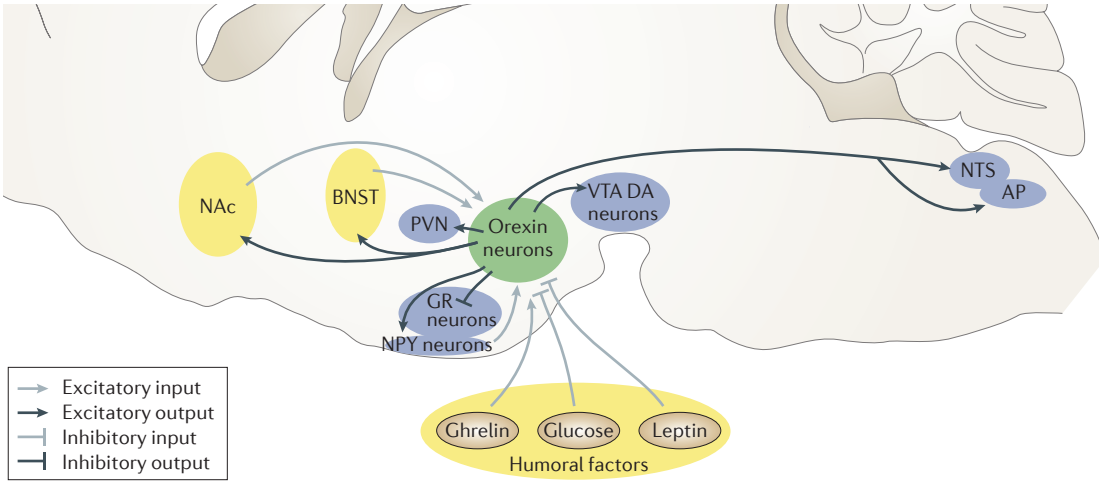


Figure 2 | **Orexin neurons in the regulation of feeding.** Orexin neurons receive input from neuropeptide Y (NPY) neurons in the arcuate nucleus and can sense circulating hormones and nutrients, such as leptin, ghrelin and glucose. Inputs from the limbic system, including the bed nucleus of the stria terminalis (BNST) and nucleus accumbens (NAc) (shown in yellow), might convey emotive components that are associated with food recognition. Orexin neurons project to the paraventricular nucleus of the hypothalamus (PVN), dorsomedial hypothalamus (not shown), nucleus of the solitary tract (NTS), area postrema (AP) and NAc (all shown in blue) to increase food intake. Note that functional excitation might include disinhibition, such as inhibition of GABAergic projections. GR, glucoreceptor; DA, dopamine; VTA, ventral tegmental area.

In summary, orexins simultaneously increase food intake and energy expenditure, but the net effect of increasing orexinergic tone is a decrease in body weight. Orexins enhance leptin sensitivity through OX2R and insulin sensitivity through OX1R. They also increase sympathetic outflow to regulate peripheral metabolic states and thermogenic function. Similar input pathways to those discussed above, including inputs from the limbic system, might be important in these functions (FIG. 2). The efferent pathways have not been established with certainty, but VMH neurons seem to be involved in orexin-mediated regulation of peripheral metabolism.

Autonomic function

The LHA has been recognized as the 'defence area' as electrical stimulation here can evoke aggressive behaviour and the accompanying sympathetic activation[48]. Emotion- or reward-driven behaviours are usually accompanied by activation of autonomic and neuroendocrine systems. Emotional stimuli are thought to evoke autonomic responses and arousal via neural connections between the amygdala and LHA. Indeed, orexin neurons in the LHA receive innervations from limbic regions — including the lateral septum, bed nucleus of the stria terminalis (BNST) and the amygdala[49,50] — and send projections to monoaminergic and cholinergic regions in the brain stem, periaqueductal grey (PAG), parabrachial nucleus, nucleus of the solitary tract (NTS), PVN, rostral ventrolateral medulla (RVLM) and rostral ventromedial medulla (RVMM)[51,52]. Thus, orexin neurons link limbic structures with premotor autonomic centres (FIG. 3). Indeed, several studies have shown that excitation of orexin neurons increases sympathetic outflow in response to various physiological and emotional stimuli[53–56].

In accordance with the above, the orexin system is also involved in the regulation of respiratory and cardiovascular functions. For example, the 1-SORA SB-334867 reduced the hyperventilation response to hypoxia or hypercapnia in rats[57,58], and individuals with narcolepsy have a higher than average incidence of obstructive sleep apnoea syndrome[59]. Furthermore, ICV injection of orexins increased blood pressure and heart rate in rats by increasing sympathetic nerve activity[60,61], whereas orexin-knockout mice showed a reduced cardiovascular response to a psychosocial stressor in the resident–intruder paradigm[53]. This suggests that orexin neurons have a role in evoking the defence response. In pharmacological studies, ICV administration of the 1-SORAs SB-334867 and SB-408124 nearly completely blocked the cardiovascular response induced by ICV administration of orexin A[62,63]. However, the selective OX2R antagonist (2-SORA) TCS-OX229 blocked the pressor response and tachycardia that are induced by orexin A more potently than the 1-SORA SB-334867 when injected in the cisterna magna[64]. Another study showed that intra-RVLM injections of orexin A and the OX2R-selective agonist [Ala11, D-Leu15]-Orexin B had cardiovascular effects of similar potencies when injected into the RVLM[65]. In addition, the 1-SORA SB-334867 only partially blocked the cardiovascular effect that is induced by orexin A[65]. Together, these findings suggest that both OX1R and OX2R mediate orexin-induced sympathetic activation depending on the regions.

Orexin activates the sympathetic nervous system, but it could also stimulate parasympathetic activity in some conditions. For example, microinjection of low doses of orexin A or orexin B in the NTS induced both bradycardia and a depressor response with similar potencies[66–68]. By contrast, intra-NTS injections of high doses

Hypercapnia
Abnormally increased levels of CO_2 in the blood.

Obstructive sleep apnoea syndrome
A condition characterized by repetitive pauses in breathing during sleep (known as apnoeas) caused by obstruction in the upper airway.

Resident–intruder paradigm
An experimental design in which a new ('intruder') mouse is placed in the home cage of another ('resident') mouse. The effect of the stress is then assessed in the resident mouse.

Defence response
The visceral and hormonal changes that accompany fear reactions. They are adaptations that prepare an animal to cope with an emergency, and specifically to perform the extreme muscular exertion of flight or attack.

Pressor response
An increase in arterial blood pressure in response to an internal or external trigger.

Tachycardia
An abnormally high heart rate.

Bradycardia
An abnormally low heart rate.

Depressor response
A decrease in arterial blood pressure in response to an internal or external trigger.

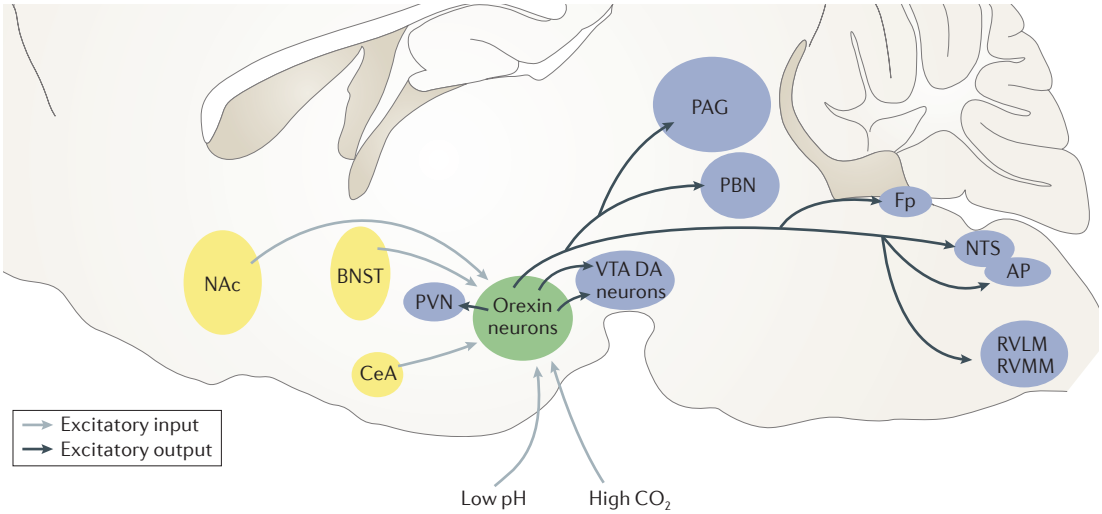


Figure 3 | **Orexin neurons in the regulation of autonomic function.** Input areas are shown in yellow, output areas are shown in blue. Salient emotional stimuli evoke autonomic responses and arousal through neural connections between the amygdala and lateral hypothalamic area (LHA), in which orexin neurons are located. Orexin neurons receive innervations from limbic regions, including the nucleus accumbens (NAc), bed nucleus of the stria terminalis (BNST) and central amygdala (CeA), and send projections to the premotor autonomic centres, including the periaqueductal grey (PAG), parabrachial nucleus (PBN), nucleus of the solitary tract (NTS), paraventricular nucleus of the hypothalamus (PVN), rostral ventrolateral medulla (RVLM) and rostral ventromedial medulla (RVMM). The activity of orexin neurons is further influenced by CO_2 and pH, which also reflect the autonomic state. Note that 'excitatory' does not necessarily indicate direct excitatory connections and includes disinhibition. AP, area postrema; Fp, folium-p; NAc, nucleus accumbens; VTA DA, dopamine neurons in the ventral tegmental area.

of orexins evoked tachycardic and pressor responses, and orexin A is more potent than orexin B[67]. These observations suggest that a low dose of orexins increases parasympathetic tone through OX2R, whereas a high dose increases sympathetic tone through OX1R in the NTS.

A recent pharmacological study using selective orexin receptor antagonists showed the importance of orexin in pressor and tachycardic responses induced by stimulation of the PFA. Almost one-half of the cardiovascular responses evoked by injection of the $GABA_A$ receptor antagonist bicuculline in the PFA in rats were blocked by systemic administration of the dual non-selective orexin receptor antagonist (DORA) almorexant[69]. Systemic administration of the 1-SORAs SB-334867 and SB-408124 also reduced the pressor response that is evoked by electrical stimulation of the dorsal hypothalamus and PAG in rabbits[70], and this effect was mediated by orexin action in a unique microzone of the cerebellum located in folium-p of the flocculus. These observations indicate that orexins evoke pressor responses through actions in multiple brain regions.

Interestingly, oral administration of the DORA almorexant inhibited cardiovascular responses to novelty and contextual fear[71] without affecting responses to cold or restraint stress. Similarly, orexin-knockout mice have a decreased cardiovascular response to social stress but a normal response to a tail pinch[53]. Together with the finding that oral delivery of the 1-SORA ACT-335827 reduced the tachycardic response to social stress[72], these data suggest that orexin more profoundly contributes to autonomic responses to psychological stressors than to physical stressors.

In addition, orexin neurons respond to changes in extracellular pH and CO_2 levels[73], and this response might be involved in the autonomic regulation by orexins. Indeed, the pressor and tachycardic responses evoked by sodium lactate injection in panic-prone rats were reduced by intraperitoneal administration of the 1-SORAs SB-334867 or SB-408124. Moreover, the 1-SORA SB-334867 also reduced the pressor response to hypercapnia[74]. These results suggest that OX1R mediates the orexin-induced sympathetic activation in response to these challenges.

Emotion and emotional memory

In an early study, electrical stimulation of the PFA, around where orexin neurons are localized, elicited defensive or aggressive responses in cats, which implicates this region in the regulation of emotion[75]. Several subsequent papers have provided more direct evidence for an involvement of the orexin system in emotion and emotional memory, and dysregulation of the orexin system has been implicated in anxiety and panic-like behaviour in humans and rats[76]. For example, humans with panic anxiety have higher orexin levels in the cerebrospinal fluid compared to people without panic anxiety[76], as do patients with post-traumatic stress disorder (PTSD)[77]. In the following section, I describe the role of orexins and orexin receptors in emotion and in the establishment and consolidation of emotional memory.

Emotional responses. Motivated behaviours are generally elicited by emotion, which is evoked by external sensory cues. As mentioned above, orexin neurons receive abundant input from the limbic system[49,50,78],

which suggests that orexins might regulate or modulate physiological responses to emotional and stressful stimuli. Indeed, the cardiovascular and locomotor responses that wild-type mice show after exposure to an intruder mouse are diminished in mice lacking orexins[53]. Similarly, cardiovascular responses to air-jet stress were reduced in mice in which orexin neurons were genetically ablated[56]. Disinhibition of the amygdala or BNST using microinjections of a $GABA_A$ receptor antagonist increased FOS immunoreactivity in orexin neurons and induced cardiorespiratory excitation in wild-type mice but not (or did, but to a lesser extent) in mice lacking orexin neurons[54]. Together, these observations indicate that orexin neurons are excited by input from the amygdala and BNST[54]. Interestingly, individuals with narcolepsy show reduced autonomic responses to emotional stimuli, especially aversive ones[79], whereas they have a normal cardiovascular response to basic homeostatic challenges (such as head-up tilt, Valsalva manoeuvre and cold pressor test). This suggests that orexin regulates the sympathetic nervous system primarily in response to salient emotional cues or contexts (see below). Thus, it is possible that activation of orexin neurons by the limbic system maintains wakefulness during emotional arousal by conveying various emotional stimuli to orexin neurons.

Some data suggest that output from the orexin system might itself have a role in regulation of the limbic system. For example, a functional MRI (fMRI) study showed abnormal amygdala activation in patients with narcolepsy[80]. Specifically, these patients showed no amygdala activation (as indicated by the blood oxygen-dependent (BOLD) signal) and no increase in functional coupling between the amygdala and medial prefrontal cortex in response to an aversive conditioned stimulus. A recent microdialysis study in humans showed that levels of orexin A in the amygdala increase during positive emotion, social interaction and anger — behaviours that often induce cataplexy in narcoleptic patients[81].

The regulation of orexin neurons by the limbic system is also implicated in pathophysiology of cataplexy: strong, generally positive emotional stimuli are well known to trigger this phenomenon in patients. Cholinergic neurons in the pedunculopontine tegmental nucleus (PPT) have a role in rapid eye movement (REM)-related atonia[82] and are therefore likely to be implicated in cataplexy as well. Indeed, local injections of orexin into the PPT strongly inhibited REM-related atonia in cats[83]. Thus, excitatory input from the limbic system — conveying emotional stimuli — might increase orexin release in the PPT to sustain muscle tone that may be required to respond to salient situations.

Emotional memory. Noradrenergic neurons (NA neurons) in the locus coeruleus (LC) have been implicated in establishing emotional memory. For example, the activity of LC NA neurons increased after fear conditioning in rats[84], and β-adrenergic blockade has been shown to be effective for treating patients with PTSD[85]. The projection from LC NA neurons to the lateral amygdala might play an important part in the consolidation of fear memory. Indeed, noradrenaline release in the lateral amygdala increased with presentation of stressful stimuli in rats[86]. As orexin neurons send abundant projections to the LC and LC NA neurons strongly express OX1R[87,88], the orexin–LC–lateral amygdala pathway might be involved in the formation of emotional memory. Indeed, the finding that individuals with narcolepsy–cataplexy show reduced amygdala activity during aversive conditioning[80] suggests that orexin deficiency may result in impaired emotional learning.

A recent study provided direct evidence that OX1R in the LC is involved in the establishment of fear memory. Mice lacking this receptor (*Ox1r*$^{-/-}$ mice) displayed reduced freezing (a behavioural expression of fear) and reduced lateral amygdala activation (as measured by expression of the immediate-early gene *Zif268* (also known as *Egr1*)) in response to cued and contextual fear stimuli[89]. Interestingly, re-expression of OX1R in LC NA neurons by an adeno-associated virus-mediated gene transfer in these mice restored both freezing time and lateral amygdala activation in the test phase of the cued fear conditioning procedure but not in the contextual fear procedure. Mice lacking OX2R (*Ox2r*$^{-/-}$ mice) also showed reduced freezing in the contextual fear test but normal freezing in the cued fear test[89]. This study thus suggested that OX1R, but not OX2R, plays a major part in the establishment of explicit cue-dependent emotional memory.

The DORA almorexant (administered orally 30 minutes before the test session) did not reduce freezing in a fear-associated context, nor did it significantly reduce the associated ultrasonic vocalizations in rats[71]. However, in another report, almorexant (administered orally 1 hour before testing) decreased the fear response to a conditioned fear cue[90]. Two other studies reported that acute pharmacological blockade of OX1R and genetic disruption of *Ox1r* impaired freezing in response to cued fear[89,90]. Conversely, focal expression of OX1R in LC NA neurons of *Ox1r*$^{-/-}$ mice increased freezing behaviour to a level comparable with that of wild-type animals in cued fear testing, but did not affect freezing in response to contextual fear[89]. Thus, there seems to be dissociation between the effects of orexin receptor function on contextual versus cued fear.

ICV infusion of the 1-SORA SB-334867 blocked the establishment of long-term fear memory, whereas infusion of the 2-SORA TCS-OX2-29 did not[91]. Furthermore, blockade of OX1R signalling in the LC before conditioning, but not immediately after conditioning, inhibited threat-memory formation. These findings suggest that OX1R signalling is important during the learning phase of fear memory formation.

A recent study using pharmacological MRI in rats found that amphetamine-induced activation in the extended amygdala, BNST and NAc — regions involved in emotion processing and emotional memory formation — was attenuated by administration of the 1-SORA GSK-1059865, whereas activation of the frontal cortex and thalamus — regions that are involved in regulating arousal — was attenuated by the 2-SORA JNJ-1037049 (REF. 92). These findings suggest that OX2R

Head-up tilt
A test to find the autonomic response in humans. The test involves lying quietly on a bed and being tilted at different angles (30 to 60 degrees) for a period of time while blood pressure, heart rates and blood-oxygen level are monitored.

Valsalva manoeuvre
Attempted exhalation against a closed airway: subjects are usually instructed to blow out as if blowing up a balloon while keeping their mouth closed and pinching their nose shut.

Cold pressor test
A test to assess the effect of cold-water immersion (of all or part of the body) on blood pressure.

Atonia
Loss of muscle tone.

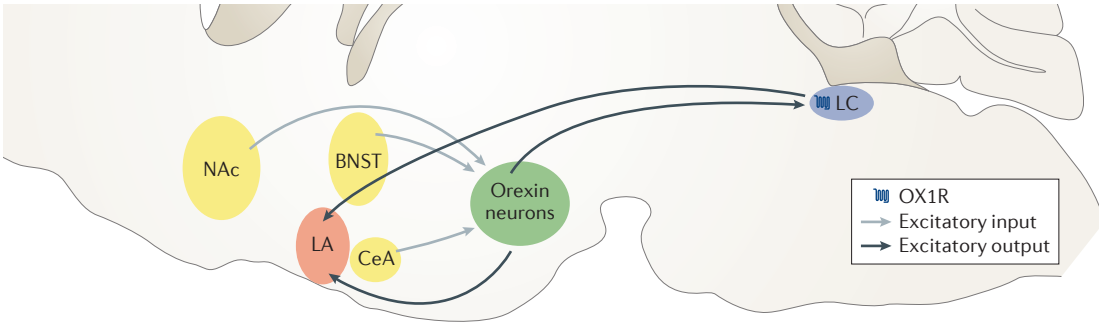


Figure 4 | **Orexin neurons in the consolidation of cue-dependent fear memory.** Input areas are shown in yellow, output area is shown in blue. Orexin neurons receive (functionally) excitatory input from the central amygdala (CeA), nucleus accumbens (NAc) and bed nucleus of the stria terminalis (BNST). These connections are likely to convey salient emotive components to orexin neurons. Noradrenergic neurons in the locus coeruleus (LC) abundantly express orexin receptor type 1 (OX1R) and receive prominent innervation from orexin-producing neurons. These neurons in turn send noradrenergic projections to the lateral amygdala (LA) (shown in orange) to consolidate emotional memory through activation of β-adrenergic receptors.

has a major role in the maintenance of arousal, whereas OX1R is predominantly involved in processing emotive or rewarding information.

Together, these observations suggest that the orexin–LC–lateral amygdala pathway is important in the formation of cued fear memory (FIG. 4). This is consistent with the observation that individuals with narcolepsy show impairments in fear-response acquisition as well as reduced amygdala activity (relative to controls) when exposed to aversively conditioned stimuli[80,93]. Moreover, the findings suggest that OX1R is required for emotional memory formation mediated by orexin.

The reward system

Rewards are closely associated with arousal: cues that predict rewards increase arousal, and reward-seeking behaviour is accompanied by arousal. Accordingly, orexins seem to have a role in the reward system. Indeed, orexin neurons send dense projections to the ventral tegmental area (VTA), in which dopaminergic neurons that send innervations to the NAc are located, and the NAc in turn sends projections to orexin neurons[50] (FIG. 5). Moreover, ICV or local infusions of orexins into the VTA can reinstate previously extinguished drug-seeking or food-seeking behaviour in rodents[3,94], and orexin neurons are activated during the behavioural expression of preferences for cues associated with reward[3].

By what mechanisms might orexins modulate the reward system? The VTA expresses both OX1R and OX2R[88], with dopaminergic neurons predominantly expressing OX1R (T.S., unpublished observations), and orexin signalling in the VTA has been implicated in reinforcement and reward-related processes through actions on VTA dopamine neurons[95]. In addition, a recent study suggested that neurotransmitter release from dorsal raphe serotonergic neurons — which express both OX1R and OX2R[87] — contribute to reward-related behaviour[96].

Cues and contexts associated with rewards, including food, sex and drugs, increase the number of FOS-positive orexin neurons and *prepro-orexin* mRNA levels[3,8,97,98]. Moreover, in one study the number of FOS-positive orexin neurons in the LHA (but not in the DMH or PFA) correlated with the degree of conditioned place preference for morphine, cocaine or food in rats[3].

A role in addiction. An increasing body of work shows that orexin neurons also play a part in the behavioural presentation of addiction to cocaine, amphetamine, morphine, heroin, nicotine, ethanol and cannabinoids[99–101]. Generally, orexin seems to be involved in the modulation of highly motivated reward seeking, especially when this seeking is triggered by external cues. OX1R seems to mainly mediate this function; for example, although the 1-SORA SB-334867 did not affect the expression of cocaine and amphetamine sensitization in animals tested immediately after training[102], it blocked the expression of sensitization after a period of abstinence following amphetamine-sensitization training, as did the DORA almorexant[103,104]. This suggests that OX1R is involved in the acquisition of sensitization. In another example, the 1-SORAs SB-334867 and GSK-1059865 attenuated the expression of cocaine and amphetamine-induced conditioned place preference in rats[92,105,106].

Importantly, neither orexins nor their receptor antagonists affect self-administration of addictive drugs such as cocaine in rodents[99,105,107], which suggests that orexins have no major role in the reinforcing or the priming effects of cocaine. However, orexin A has been shown to promote motivation in a study in rats in which high levels of effort were required for seeking addictive drugs in self-administration protocols[32]. These results suggested that orexins are essential in reward seeking — not by influencing the primary reinforcing or priming effects of rewards, but by supporting motivated behaviour. ICV administration of orexins can reinstate previously extinguished cocaine-seeking behaviour, and administration of a corticotropin-releasing hormone (CRH) receptor antagonist or the α2 receptor agonist clonidine reduced and blocked this effect, respectively, which suggests that the role of orexin signalling in reward-seeking behaviour is mediated by CRH and noradrenergic systems[94]. Intraperitoneal injections or intra-VTA administration of the 1-SORA SB-334867

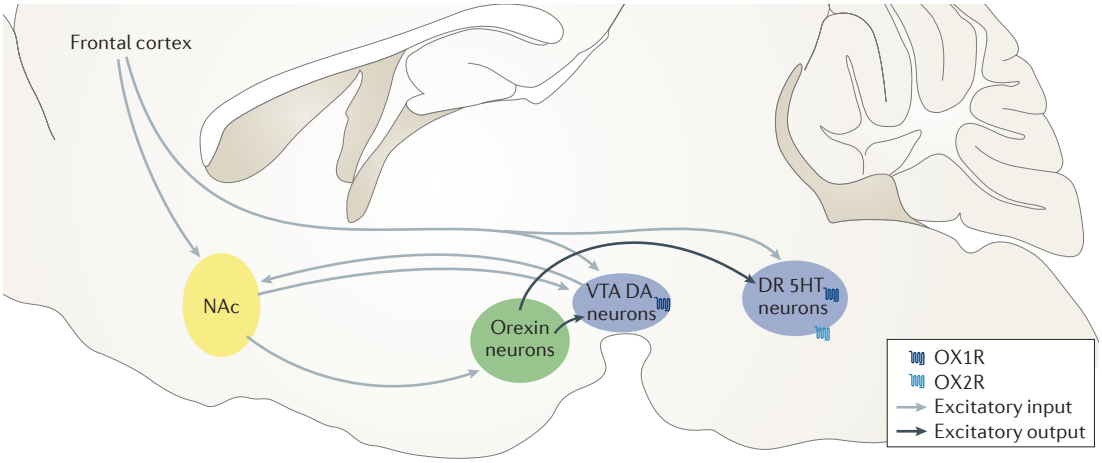


Figure 5 | **Orexin neurons influence the reward-processing system.** Input area is shown in yellow, output areas are shown in blue. The motor, somatosensory and prefrontal cortices send projections to dopamine (DA) neurons in the ventral tegmental area (VTA) and nucleus accumbens (NAc) and to serotonin (5-hydroxy tryptamine (5-HT)) neurons in the dorsal raphe nuclei (DR). The NAc sends innervations both to orexin neurons and VTA DA neurons[148]. Information about the perception of sensory cues that predict reward might be conveyed to orexin neurons through this connection. Orexin neurons send excitatory projections to VTA DA neurons and to DR 5-HT neurons to enhance activation of these neurons.

blocked reinstatement of cocaine seeking elicited by either discrete cues or contextual stimuli in rats[108–110], whereas intraperitoneal injection of the 2-SORA 4-PT did not affect cue-induced reinstatement[108]. These observations suggest that orexin neurons might be activated by external reward-related stimuli and send information to the VTA to induce reinstatement and that OX1R is mainly involved in this pathway.

Orexins also seem to play a part in addiction to drugs other than cocaine and amphetamine. For example, *Fos* expression was increased in orexin neurons in rats following acute nicotine administration or nicotine withdrawal[111,112], and the nicotine withdrawal response was attenuated in orexin knockout mice[112]. Prior intraperitoneal administrations of the 1-SORA SB-334867, but not the 2-SORA TCSOX229, attenuated nicotine withdrawal[112], as did intra-PVN infusion of the 1-SORA SB-334867 (REF. 112). Moreover, systemic administration of the DORA almorexant or the 1-SORA SB-334867 and local infusion of SB-334867 into the insular cortex reduced nicotine self-administration[113,114].

Orexin might also be involved in opiate addiction; for example, orexin-knockout mice and wild-type mice that received the 1-SORA SB-334867 showed reduced morphine withdrawal responses[12,115,116]. Furthermore, intraperitoneal injection of the 1-SORA SB-334867 reduced the expression of morphine-induced conditioned place preference in rats and mice[3,116]. Similarly, orexin-knockout mice did not show morphine-induced conditioned place preference and hyperlocomotion[117], although in another study, orexins did not mediate morphine-induced hyperlocomotion[116]. Orexinergic neurotransmission in the VTA is important for the expression of morphine-induced conditioned place preference[118]. Moreover, in contrast with the lack of effect of the 1-SORA SB-334867 on cocaine self-administration, intraperitoneal delivery of the 1-SORA SB-334867 did reduce heroin self-administration[119].

Several studies have suggested a role for orexin in ethanol consumption and addiction[120]. The 2-SORA JNJ-10397049 decreased the acquisition, expression and reinstatement of ethanol-induced conditioned place preference in mice[121]. By contrast, the 1-SORAs SB-334867 and SB-408142 had little effect on these behaviours[121]. This suggests that OX2R, but not OX1R, is involved in orexin-mediated regulation of ethanol reward. Consistent with this, subcutaneous injections of the 2-SORA JNJ-10397049 decreased ethanol self-administration in rats, whereas the 1-SORA SB-408124 did not[121] (although another 1-SORA, SB-334867, reduced ethanol reinstatement driven by olfactory or light cues[122,123]). Moreover, ethanol seeking or context-induced reinstatement of ethanol consumption increased the number of FOS-positive (that is, active) orexin neurons in the hypothalamus[124]. These findings suggest that orexin signalling through both receptors is involved in the regulation of ethanol consumption and addiction and that the OX1R pathway specifically is required for cue-induced ethanol seeking, which — like the reward seeking associated with other drugs — is a highly-motivated behaviour.

Stress responses

Early studies showed that ICV administration of orexin results in increased CRH levels in the hypothalamus and activation of the hypothalamus–pituitary–adrenal (HPA) axis[125,126] and, conversely, that orexin neurons are activated by CRH[78,127]. Indeed, in an *in vitro* study, application of CRH depolarized the membrane potential and increased the firing rate in a subpopulation of orexin neurons by activating CRH receptor 1 on these neurons[78]. These findings are in accordance with the reciprocal connections between CRH neurons in the PVN and orexin neurons in the LHA[78] and suggest that orexin might play some part in the hormonal response to stress. Indeed, forced-swim stress caused orexin neuron

activation (as assessed by FOS-immunostaining) and increased plasma levels of adrenocorticotropic hormone (ACTH)[128], and ICV administration of a 2-SORA reduced this ACTH response. Considering that the PVN expresses abundant *Ox2r* mRNA[88], these results suggest that stress increases orexin neuron firing to stimulate CRH neurons in the PVN via activation of OX2R.

The number of FOS-positive orexin neurons was reported to be increased by a fear-conditioned cue, but not by restraint stress[71]. These observations again suggest that orexin neurons are activated by psychological stressors — an appropriate response to which requires a proper vigilance level and attention to environmental cues — but not by physical stressors.

Interestingly, orexin neurons also express dynorphins — a class of opioid peptides[129]. Dynorphins preferably bind to κ-opioid receptors and have been hypothesized to mediate negative emotional states, such as depression[130]. In mice, forced-swim stress and inescapable footshock produced place aversion, and this effect was blocked by administration of a κ-opioid receptor antagonist[131]. These observations suggest that psychological stressor-induced activation of orexin neurons results in the release of dynorphins, which might evoke depressive or dysphoric-like effects so as to modify the emotional state of the animal. As dynorphins are also implicated in reward and addiction, the colocalization of orexins and dynorphins might also be relevant for these processes[130].

Conclusions and future directions

Any purposeful behaviours that are regulated by emotion, reward and energy balance require wakefulness and certain internal body states, including an appropriate tone of the autonomic nervous system and the HPA axis. Appropriate arousal levels are especially necessary for executing any purposeful behaviours that require high motivation. OX2R has been thought to have a major role in maintaining wakefulness, and recent studies using selective antagonists suggest that OX1R is involved in a broad range of functions, including emotion, reward and autonomic regulation. As discussed in this article, these functions are closely related and are interconnected via the orexin system. Orexin-producing neurons, which reside in the LHA, link forebrain structures such as the amygdala, BNST and NAc — which are implicated in the processing of emotion and motivation — with brain-stem regions, which regulate wakefulness and reward (FIG. 1). Orexin neurons thus have an important role in translating sensory cues to a diverse range of motivated behaviours.

Many orexin receptor antagonists have recently been developed (BOX 2). These agents are useful in pharmacological experiments aimed at identifying the physiological roles of orexins. As many studies cited in this review used ICV administration of these antagonists and/or orexin peptides, one should be careful when interpreting these results, as the diffusion of these factors from the cerebrospinal fluid into tissues might vary among regions. Thus, these studies might not necessarily inform us about how orexin acts in the normal functioning brain. Intravenous or intraperitoneal injection of these agents might result in a relatively homogenous distribution of these compounds in the relevant tissues, but because the currently available compounds are competitive antagonists, the blockade of receptors by these agents is not complete. Likewise, overdosing might affect the receptor selectivity of subtype-selective antagonists. A caveat of using transgenic mice to study the orexin system is that orexin receptor-deficient mice will have undergone compensatory changes in orexin-target regions in response to chronic deficiency in orexin signalling, and thus the phenotypes of these animals might not necessarily reflect the physiological effects of a change in the level of orexin signalling. Nevertheless, considering the findings from studies using these different methodologies together should provide valuable insights into the physiological functions of the orexin system.

Several orexin receptor antagonists (DORAs and SORAs) are expected to become next-generation drugs for insomnia, and orexin agonists might be available for narcolepsy treatment in the future. Given the broad range of functions of the orexin system, these drugs might also be beneficial for treating various conditions other than sleep disorders. Antagonists are indeed expected to be used in the treatment of addictive disorders. Such drugs may also prove to be useful for mood disorders as orexins have important roles in reward, hedonia and motivation and orexins act on monoaminergic and cholinergic systems. Finally, orexin system-based interventions might also be beneficial for eating disorders, such as obesity and anorexia nervosa.

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Competing interests statement
The author declares no competing interests.