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REVIEW

Activation of the basal forebrain by the orexin/hypocretin neurones

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Abstract

The orexin neurones play an essential role in driving arousal and in maintaining normal wakefulness. Lack of orexin neurotransmission produces a chronic state of hypoarousal characterized by excessive sleepiness, frequent transitions between wake and sleep, and episodes of cataplexy. A growing body of research now suggests that the basal forebrain (BF) may be a key site through which the orexin-producing neurones promote arousal. Here we review anatomical, pharmacological and electrophysiological studies on how the orexin neurones may promote arousal by exciting cortically projecting neurones of the BF. Orexin fibres synapse on BF cholinergic neurones and orexin-A is released in the BF during waking. Local application of orexins excites BF cholinergic neurones, induces cortical release of acetylcholine and promotes wakefulness. The orexin neurones also contain and probably co-release the inhibitory neuropeptide dynorphin. We found that orexin-A and dynorphin have specific effects on different classes of BF neurones that project to the cortex. Cholinergic neurones were directly excited by orexin-A, but did not respond to dynorphin. Non-cholinergic BF neurones that project to the cortex seem to comprise at least two populations with some directly excited by orexin-A that may represent wake-active, GABAergic neurones, whereas others did not respond to orexin-A but were inhibited by dynorphin and may be sleep-active, GABAergic neurones. This evidence suggests that the BF is a key site through which orexins activate the cortex and promote behavioural arousal. In addition, orexins and dynorphin may act synergistically in the BF to promote arousal and improve cognitive performance.
Keywords basal forebrain, dynorphin, orexin/hypocretin.

Orexin-A and -B (also known as hypocretin-1 and -2) are two neuropeptides produced by a cluster of wake-active neurones in the lateral hypothalamus (de Lecea *et al.* 1998, Sakurai *et al.* 1998, Lee *et al.* 2005b, Mileykovskiy *et al.* 2005). The orexin neurones heavily innervate brain regions involved in arousal and excite post-synaptic neurones through the two orexin receptors Ox1R and Ox2R (hypocretin-1 and -2 receptors) (Peyron *et al.* 1998, Sakurai *et al.* 1998). Over 90% of people with narcolepsy with cataplexy have very low or undetectable orexin levels in their cerebrospinal fluid, likely from an autoimmune attack on the orexin-producing neurones (Peyron *et al.* 2000, Thannickal *et al.* 2000, Mignot *et al.* 2002, Crocker *et al.* 2005). Dogs lacking Ox2R and mice lacking orexin peptides or the orexin receptors have a phenotype strongly resembling human narcolepsy, with an inability to remain awake for long periods and sudden episodes of muscle atonia known as cataplexy in the midst of active wake (Chemelli *et al.* 1999, Lin *et al.* 1999, Willie *et al.* 2003, Mochizuki *et al.* 2004). The sleepiness of narcolepsy clearly demonstrates that the orexin neurones are necessary for normal arousal, but the specific brain regions through which orexins promote arousal remain unknown.

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A growing body of evidence suggests that the basal forebrain (BF) is a key site through which the orexin neurones promote arousal. This paper comprehensively reviews the anatomical, pharmacological and electrophysiological studies, including data from our own *in vitro* recordings on how the orexin neurones can promote arousal by exciting BF neurones that activate the cortex. A better understanding of how orexins act through the BF should provide novel insights into the neurobiology of arousal and may also lead to a better understanding of disorders of cognition.

Role of the BF in cortical activation and behavioural arousal

The BF is an essential wake-promoting region that extends from the septum back to the substantia innominata (SI) and is roughly defined by the presence of magnocellular cholinergic neurones (Szymusiak 1995, Semba 2000, Jones 2004). In conjunction with monoaminergic and cholinergic projections from more caudal regions, the BF is considered a key extrathalamic relay to the cerebral cortex from the brainstem reticular activating system initially proposed by Moruzzi & Magoun (1949) (Fig. 1). BF neurones project to the cortical mantel in a topographical pattern in which the medial septum and other rostral-medial regions mainly project to the hippocampus and cingulate cortex, whereas the SI, magnocellular preoptic nucleus (MCPO) and other caudal-lateral regions project to the amygdala, medial prefrontal and most other cortical areas (Saper 1984). In addition to ascending projections to the cortex, BF neurones also project caudally to state-regulatory regions in the lateral hypothalamus and brainstem (Swanson *et al.* 1984, Semba *et al.* 1989, Gritti *et al.* 1994, Semba 2000) (Fig. 1).

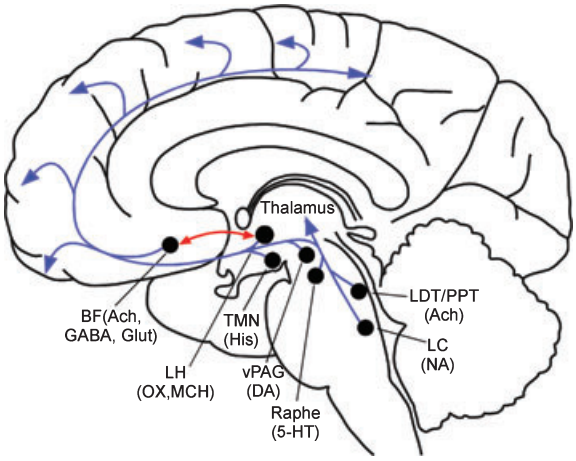

Figure 1 The ascending arousal systems are diffusely projecting neurones (blue) that use acetylcholine, monoamines or neuropeptides to produce broad changes in neuronal activity. The pedunculopontine (PPT) and laterodorsal tegmental (LDT) nuclei are the major cholinergic inputs to the thalamus. The key monoaminergic nuclei include the locus coeruleus (LC) which is a major source of noradrenaline (NA) to the hypothalamus and cortex, the dorsal and median raphe nuclei which produce serotonin (5-HT), the A10 cell group of the ventral periaqueductal grey matter (vPAG) which produces dopamine (DA), and the tuberomammillary nucleus (TMN) which produces histamine. In addition, peptidergic neurones in the lateral hypothalamus (LH) produce orexins and melanin-concentrating hormone (MCH). All these regions innervate the basal forebrain (BF), and BF neurones send descending projections back to the lateral hypothalamus (red), thalamus and brainstem.

The BF is the major source of cholinergic input to the cortex (Woolf 1991). During wakefulness and rapid eye movement (REM) sleep, cholinergic neurones of the MCPO and SI fire most rapidly and acetylcholine release in the cortex is maximal (Jasper & Tessier 1971, Marrosu *et al.* 1995). During non-REM sleep, the cholinergic neurones are relatively silent and acetylcholine levels are low (Duque *et al.* 2000, Jones 2004, Lee *et al.* 2005a).

An additional and large population of cortically projecting BF neurones produce GABA and a smaller number produce glutamate (Freund & Gulyas 1991, Gritti *et al.* 1997, Hur & Zaborszky 2005, Henny & Jones 2008). GABAergic neurones account for about one-third of the MCPO/SI cortically projecting neurones, and they are co-distributed with the cholinergic population (Gritti *et al.* 1997). In the MCPO/SI there are two physiologically distinct groups of GABAergic neurones that can be antidromically activated from the cortex; one is active during cortical arousal, and a second group discharges in association with cortical slow wave activity and may express α_{2A}-adrenergic receptors and/or contains neuropeptide Y (NPY) (Duque *et al.* 2000, Manns *et al.* 2000, Modirrousta *et al.* 2004).

Activation of BF neurones with glutamate agonists increases wake (Manfridi *et al.* 1999, Cape & Jones 2000, Wigren *et al.* 2007). Conversely, selective lesions of the cholinergic population can transiently reduce wake, whereas excitotoxic lesions that kill both cholinergic and non-cholinergic neurones increase EEG delta activity (Kaur *et al.* 2008). Even larger lesions that encompass most of the BF markedly reduce wake (Buzsaki *et al.* 1988). Furthermore, inhibition of BF neurones with an adenosine A1 receptor agonist promotes sleep, even after lesioning of the cholinergic population (Portas *et al.* 1997, Blanco-Centurion *et al.* 2006a). These results demonstrate the importance of the BF in promoting wake and suggest that cholinergic and non-cholinergic neurones across much of the BF act synergistically to promote wake (Szymusiak *et al.* 2000, Jones 2005).

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Anatomical studies

Although the orexin peptides are produced by a relatively small number of neurones in the perifornical region of the lateral hypothalamus, these neurones project widely and orexin receptors are distributed through much of the brain (Peyron *et al.* 1998, Sakurai *et al.* 1998, Nambu *et al.* 1999, Hervieu *et al.* 2001, Marcus *et al.* 2001). A robust projection from the lateral hypothalamus to the BF was described even before the discovery of the orexin peptides (Zaborszky & Cullinan 1989, Cullinan & Zaborszky 1991). More recently, studies have shown that projections from orexin neurones make a substantial contribution to this pathway (Fig. 1), and orexin terminals innervate the BF from the medial septum back to the MCPO/SI region (Peyron *et al.* 1998, Wu *et al.* 2004, Espana *et al.* 2005, Fadel & Frederick-Duus 2008). The orexin projections to the BF are predominantly ipsilateral, show no apparent topographic organization and target multiple BF regions and send collateral projections to the brainstem (Espana *et al.* 2005). In addition, orexin fibres closely appose and synapse on cholinergic neurones of the BF (Wu *et al.* 2004, Espana *et al.* 2005, Fadel *et al.* 2005, Fadel & Frederick-Duus 2008). An ultrastructural study reveals that 70% of the cholinergic neurones of the medial septum receive at least one orexin immunoreactive bouton on their cell body or proximal dendrites (Wu *et al.* 2004). With light microscopy, orexin immunoreactive appositions are common on SI cholinergic cell bodies and dendrites, suggesting direct activation of BF cholinergic neurones by the orexin neurones (Fadel *et al.* 2005, Fadel & Frederick-Duus 2008).

In addition, BF neurones send reciprocal connections back to the orexin neurones (Henny & Jones 2006a,b) (Fig. 1). Most of these descending projections to the orexin neurones use GABA and glutamate and only 4% are cholinergic (Henny & Jones 2006b). However, the orexin neurones are strongly excited by acetylcholine, though the major cholinergic input probably comes from the cholinergic neurones of the pedunculopontine (PPT) and laterodorsal tegmental (LDT) nuclei (Ford *et al.* 1995, Bayer *et al.* 1999, 2005, Sakurai *et al.* 2005). The BF glutamatergic input to the orexin neurones may originate from wake-promoting neurones that discharge in association with high muscle tone (Henny & Jones 2006a). Indeed many non-cholinergic BF neurones discharge during waking and are quiet during non-REM and REM sleep (Szymusiak & McGinty 1986, Lee *et al.* 2004). On the other hand, the GABAergic input from the BF may originate from sleep-active neurones (Duque *et al.* 2000, Modirrousta *et al.* 2004) and may help inhibit the orexin neurones during non-REM and REM sleep.

The BF neurones express both Ox1 and Ox2 receptors. In the medial septum, Ox2R mRNA levels and protein are expressed at high levels but Ox1R mRNA is sparse (Trivedi *et al.* 1998, Hervieu *et al.* 2001, Marcus *et al.* 2001). Neurones of the vertical and horizontal limbs of the diagonal band show higher levels of Ox1R mRNA compared to the medial septum, but still Ox2R mRNA is more abundant (Marcus *et al.* 2001). No data yet exist concerning the distribution of orexin receptor subtypes in more caudal BF regions including the MCPO/SI. In addition, pharmacological studies have produced conflicting results, with some reporting that BF neurones are more responsive to orexin-B suggesting an Ox2R effect, whereas others conclude that orexin-A signalling is more important (Eggermann *et al.* 2001, Espana *et al.* 2001, Dong *et al.* 2006, Frederick-Duus *et al.* 2007). Lack of selective orexin receptor antagonists has made it difficult to firmly establish the relative roles of Ox1 and Ox2 receptors using pharmacological approaches. Future studies using mice lacking Ox1 or Ox2 receptors and especially mice lacking orexin receptors in specific neuronal populations should help determine which orexin receptor subtypes are necessary to mediate wake-promoting effects of orexins in the BF and in which BF neuronal types.

Measurement and manipulation of orexins in the BF using microdialysis

Microdialysis is a very helpful method for measuring orexin concentrations across sleep/wake states. The orexin neurones are active during wake (Estabrooke *et al.* 2001, Lee *et al.* 2005b), and a small study in cats showed that orexin-A levels are high in the BF during wake (Kiyashchenko *et al.* 2002). As expected, orexin concentrations were lower during non-REM sleep but surprisingly, orexin levels were high during REM sleep (Kiyashchenko *et al.* 2002). This apparent release of orexin-A in REM sleep was unexpected as the orexin neurones are generally silent during REM sleep, except for transient bursts of activity during phasic REM sleep and just prior to awakening (Lee *et al.* 2005b, Mileykovskiy *et al.* 2005). Optogenetic activation of the orexin neurones can trigger awakenings from sleep (Adamantidis *et al.* 2007), and it is possible that in addition to promoting wakefulness, the orexin neurones help drive awakenings from sleep.

Local application of orexins to the BF promotes wakefulness and improves cognitive performance. Infusion of orexins into the BF induces acetylcholine release in the cortex and strongly promotes wake for several hours (Eggermann *et al.* 2001, Espana *et al.* 2001, Thakkar *et al.* 2001, Fadel *et al.* 2005). In rats conditioned to anticipate food, acetylcholine is released in the cortex just before the expected arrival of food, but the

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behavioural response and the rise in acetylcholine is blunted in rats with lesions of the orexin neurones and adjacent cells in the lateral hypothalamus (Frederick-Duus *et al.* 2007). This observation suggests that orexins are necessary for the activation of BF cholinergic neurones, though it should be interpreted cautiously as this type of lesion kills much more than just the orexin neurones (Gerashchenko *et al.* 2001). Orexins can also have direct effects in the cortex to improve performance on an attention task by exciting the same thalamocortical synapses that are activated by acetylcholine from the BF (Lambe *et al.* 2005). Thus orexins may promote cortical activation and attention by increasing cortical acetylcholine release and by directly acting on thalamocortical projections.

Orexins may also act through non-cholinergic neurones of the BF. Orexin-B excites GABAergic neurones of the medial septum that project to the hippocampus (Wu *et al.* 2002), and we have found similar effects of orexin-A in cortically projecting, GABAergic neurones of the MCPO/SI region (see below). In fact, microinjection of orexin-A into the BF still promotes arousal after selective lesioning of the BF cholinergic neurones (Blanco-Centurion *et al.* 2006b). Altogether these pharmacological studies strongly support the hypothesis that orexin stimulation of the BF is able to promote cortical activation and behavioural arousal by acting on cholinergic and non-cholinergic neurones.

Electrophysiological responses to orexins

Several studies using *in vitro* slice recordings have shed light on how the orexin neurones activate the BF (Eggermann *et al.* 2001, Wu *et al.* 2002, 2004). Most of these studies focused on the effects of orexins on medial septum neurones that project to the hippocampus (Wu *et al.* 2002, 2004), and so far, the cortically projecting neurones of the caudal BF have received less attention. Eggermann *et al.* (2001) reported early on that orexins directly excites MCPO cholinergic neurones. They also compared the effect of orexin-A and orexin-B and concluded that because orexin-B had a stronger effect, Ox2R and not Ox1R were responsible for orexin response in the MCPO cholinergic neurones.

Much more is known about the responses of neurones in the medial septum. Wu *et al.* (2004) found that orexins directly excite septohippocampal cholinergic neurones by two underlying ionic mechanisms: the inhibition of a K^+ conductance, presumably an inwardly rectifying potassium current, and the activation of a Na^+/Ca^{2+} exchanger. Similar effects of orexin-A on a constitutively active, inwardly rectifying potassium current were also reported in cultured BF neurones of the nucleus basalis (Hoang *et al.* 2004). In about 80% of septohippocampal cholinergic neurones, these two effects co-exist, whereas orexins only reduce a K^+ current in the locus coeruleus, central amygdala and thalamic neurones (Ivanov & Aston-Jones 2000, Bayer *et al.* 2002, 2004, Bisetti *et al.* 2006) and only activates a Na^+/Ca^{2+} exchanger in neurones of the arcuate nucleus and tuberomammillary nucleus (TMN) (Eriksson *et al.* 2001, Burdakov *et al.* 2003). Wu *et al.* (2004) also found that cholinergic septohippocampal neurones had similar EC_{50} values for orexin-A and orexin-B, suggesting that Ox2Rs are responsible for the orexin responses as suggested by the high levels of Ox2R mRNA and protein in the medial septum (Trivedi *et al.* 1998, Hervieu *et al.* 2001, Marcus *et al.* 2001). Orexins also directly excite GABAergic septohippocampal neurones by activation of a Na^+/Ca^{2+} exchanger, and the dose–response curve for the two peptides suggests an Ox2R-mediated effect as well (Wu *et al.* 2002). In addition, orexins increase GABA release onto the GABAergic septohippocampal neurones, and this effect was spike-dependent, suggesting that it was mediated by the activation of local GABAergic neurones within the slice preparation (Wu *et al.* 2002).

To better understand how orexins promote cortical activation, we examined the responses of cortically projecting MCPO/SI neurones to orexins and dynorphin, another neuropeptide synthesized in the orexin neurones (Chou *et al.* 2001, Crocker *et al.* 2005). We identified cortically projecting MCPO/SI neurones by injecting fluorescent latex beads (green) into the medial prefrontal cortex (mPFC) that are retrogradely transported back to the BF. We also injected Cy3-p75-IgG into the lateral ventricle (red) which immunolabels cholinergic neurones in the BF as nearly all express the p75 receptor (Hartig *et al.* 1998, Wu *et al.* 2000, Arrigoni *et al.* 2006). Thus, cholinergic neurones projecting to mPFC were recognized by the presence of both green beads and red Cy3-p75-IgG (Fig. 2). Non-cholinergic, cortically projecting neurones contained green beads but lacked red Cy3-p75-IgG (Fig. 3).

We found that SI cholinergic neurones were directly excited by orexin-A but did not respond to dynorphin-A. In addition, orexin-A increased the amplitude of evoked glutamatergic excitatory post-synaptic currents (EPSCs) in cholinergic MCPO/SI neurones (Fig. 2). We found two populations of non-cholinergic MCPO/SI neurones that project to the mPFC. In one cell type, orexin-A was excitatory whereas dynorphin had no direct effect but showed a slight inhibition of the evoked glutamatergic EPSCs. These neurones showed the same electrophysiological properties previously reported in GABAergic neurones of the medial septum that project to the hippocampus (Wu *et al.* 2000). These may be GABAergic, cortically projecting neurones (Fig. 3). An additional class of non-cholinergic cortically projecting neurones that display different firing properties, including the

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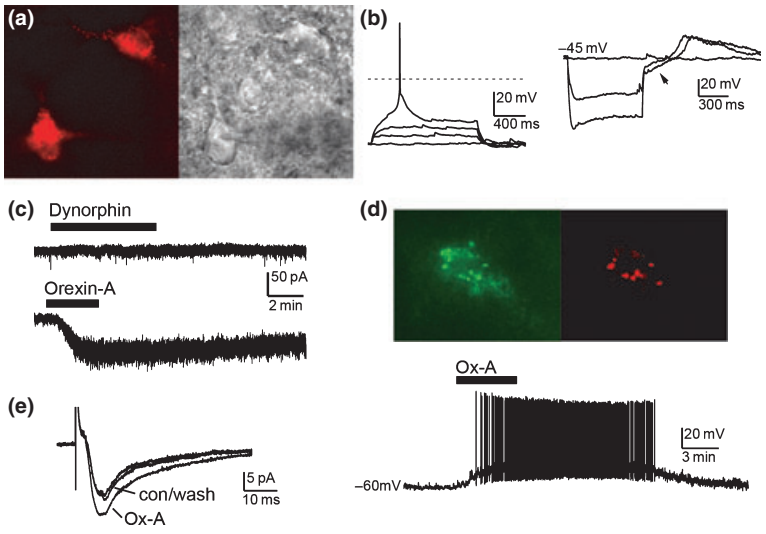

Figure 2 Cholinergic neurones of the magnocellular preoptic nucleus (MCPO) and substantia innominata (SI) are excited by orexin-A but do not respond to dynorphin. (a) Two SI cholinergic neurones labelled with Cy3-p75-IgG (left) and the same neurones under infrared differential interference contrast (IR-DIC) visualization. (b) Firing properties of MCPO/SI neurones during depolarizing (left) and hyperpolarizing current pulses [in tetrodo toxin (TTX) 1 μM, right], showing low threshold Ca^{2+}, delayed firing followed by hyperpolarizing potentials due to activation of $I_{K(A)}$ (arrowhead) and a small I_h. (c) MCPO/SI neurones do not respond to dynorphin (10 μM), but orexin-A (300 nM) activates an inward current (V_h = −60 mV). (d) An SI cholinergic neurone that projects to the medial prefrontal cortex is double labelled with retrograde fluorescent beads (green) and Cy3-p75-IgG (red) and has a sustained increased in firing with orexin-A (trace below). (e) Orexin-A potentiates excitatory post-synaptic currents evoked by local electrical stimulation (V_h = −60 mV).

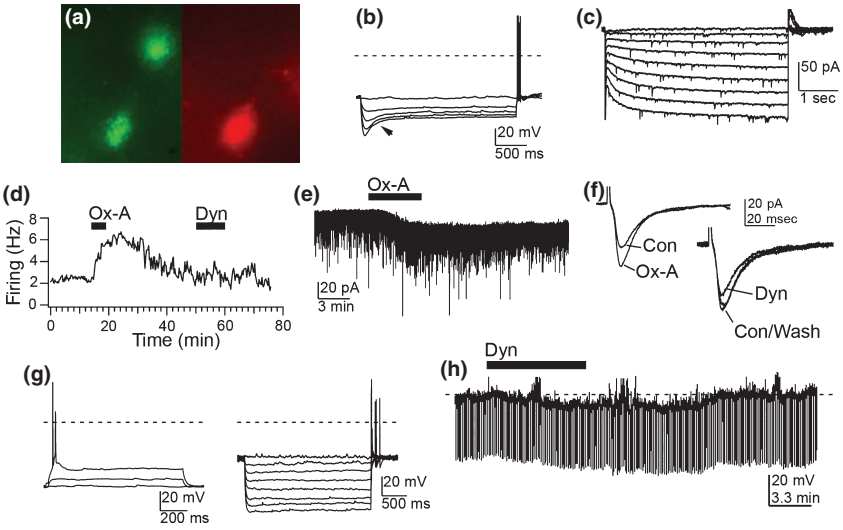

Figure 3 Non-cholinergic, cortically projecting neurones in the magnocellular preoptic nucleus (MCPO) and substantia innominata (SI) have two types of responses to orexin-A and dynorphin. (a) Two SI neurones retrogradely labelled with green fluorescent beads from the medial prefrontal cortex. The lower cell is also labelled with red Cy3-p75-IgG, a marker for the basal forebrain cholinergic neurones; the upper cell is a non-cholinergic. (b) A subset of these neurones has pronounced depolarizing sags during negative current pulses (arrowhead) due to the activation of I_h. (c) I_h recorded in voltage clamp mode (V_h = −50 mV; −10 mV pulses). (d) Spontaneous firing is increased by orexin-A (300 nM) but is unaffected by dynorphin (10 μM). (e) Inward current activated by orexin-A (V_h = −60 mV). (f) Evoked excitatory post-synaptic currents are potentiated by orexin-A and inhibited by dynorphin (V_h = −60 mV). (g) A second subset of non-cholinergic cortically projecting neurones in the MCPO/SI have burst discharges, no I_h and no $I_{K(A)}$. (h) This type of neurone is inhibited by dynorphin (dotted line = −60 mV).

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lack of both I_h and $I_{K(A)}$, and that fire in short bursts when depolarized from hyperpolarizing potentials showed no response to orexin-A but was directly inhibited by dynorphin. These cells may be sleep-active, GABAergic neurones (Duque *et al.* 2000, Manns *et al.* 2000, Modirrousta *et al.* 2004). These results show that orexins and dynorphin have specific effects on different classes of BF neurones. These responses may provide a synergistic mechanism by which the co-release of orexins and dynorphin can activate cholinergic and non-cholinergic wake-active neurones and can inhibit non-cholinergic sleep-active neurones to promote wakefulness and improve cognitive performance.

Dynorphin and glutamate may act synergistically to excite BF neurones

In addition to the orexin peptides, the orexin-producing neurones contain other neurotransmitters. In rats, mice and humans, essentially all orexin-producing neurones also make the endogenous opiate dynorphin (Chou *et al.* 2001, Crocker *et al.* 2005). At the ultrastructural level it remains to be determined whether orexins and dynorphin are co-stored in the same pre-synaptic vesicles, but if they are, it is reasonable to assume that they are released together (Salio *et al.* 2006). In addition, the BF and nearly all brain regions innervated by the orexin neurones express κ opiate receptors, the main receptor for dynorphin (DePaoli *et al.* 1994, Mansour *et al.* 1994, Marcus *et al.* 2001). This is remarkable because orexin-A and orexin-B excite their target neurones, but dynorphin has inhibitory effects.

Possibly, orexin and κ receptors reside on different target neurones or are located on different parts of the target neurones. For example while orexins directly excites TMN neurones and NPY neurones of the arcuate nucleus (Eriksson *et al.* 2001, van den Top *et al.* 2004, Acuna-Goycolea & van den Pol 2005), dynorphin has no post-synaptic effects but reduces GABAergic synaptic input to these neurones (Eriksson *et al.* 2004, Li & van den Pol 2006). Thus in these two nuclei, co-release of orexins and dynorphin should produce synergistic effects that increase activity in the target cell. Another mechanism is that orexins and dynorphin may have effects that differ over time. For example, the melanin-concentrating hormone (MCH) neurones are initially inhibited by dynorphin when orexins and dynorphin are co-applied, but this response desensitizes quickly, and over time, the excitatory effect of orexins dominates (Li & van den Pol 2006). Perhaps this same phenomenon occurs in neurones of the locus coeruleus and dorsal raphe in which orexins and dynorphin seem to act in opposition (McFadzean *et al.* 1987, Pinnock 1992, Hagan *et al.* 1999, Ivanov & Aston-Jones 2000, Brown *et al.* 2001, 2002, Hoang *et al.* 2003, Kohlmeier *et al.* 2008, Kreibich *et al.* 2008). This finding has interesting implications, as one could speculate that during a brief arousal from sleep, the excitatory effects of orexins could be initially damped by the inhibitory effects of dynorphin, but if the orexin neurones remain active, dynorphin signalling would desensitize and the excitatory effects of orexins would then help sustain wakefulness.

In addition to dynorphin, the orexin neurones also produce and probably release glutamate (Abrahamson *et al.* 2001, Torrealba *et al.* 2003). Orexins and glutamate localize at the same terminals but in different vesicles. Glutamate is stored in small clear vesicles in the active zones while orexin peptides is confined in large dense core vesicles (Torrealba *et al.* 2003). If co-released, orexins and glutamate should act synergistically to excite BF and other target neurones. As the release of neuropeptides may require a higher firing frequency than the release of glutamate (De Camilli & Jahn 1990), it is conceivable that low frequency firing of the orexin neurones may release predominantly glutamate but higher frequency firing may promote the additional release of orexins from dense core vesicles.

Another molecular marker found to colocalize with orexins is the neuronal activity-regulated pentraxin (NARP), a secreted immediate early gene product. NARP is a synaptic signalling protein that stimulates clustering of glutamatergic AMPA receptors (Tsui *et al.* 1996, Fong & Craig 1999, O'Brien *et al.* 1999). The orexin neurones of mice and humans express NARP (Reti *et al.* 2002, Blouin *et al.* 2005, Crocker *et al.* 2005), and it is possible that NARP itself potentiates pre- or post-synaptic responses to glutamate.

Much remains to be learned about the functional roles of dynorphin, glutamate and NARP in the orexin neurones. However, mice lacking the orexin neurones seem to have a slightly different narcolepsy phenotype and a greater tendency towards obesity than mice simply lacking orexins (Chemelli *et al.* 1999, Hara *et al.* 2001, 2005, Kantor *et al.* 2009), perhaps due to loss of the other signalling molecules.

Role of the MCH neurones

In addition to the orexin neurones, the lateral hypothalamus also contains neurones that produce the inhibitory peptide MCH. Their firing pattern is roughly opposite to the orexin neurones; MCH neurones are silent during wake, fire occasionally during non-REM sleep and fire maximally during REM sleep (Hassani *et al.* 2009). Pharmacological studies and recordings of MCH knockout mice suggest that the MCH system promotes sleep, perhaps especially REM sleep (Verret *et al.* 2003, Adamantidis & de Lecea 2008, Willie *et al.* 2008). MCH neurones contain GABA, they project to

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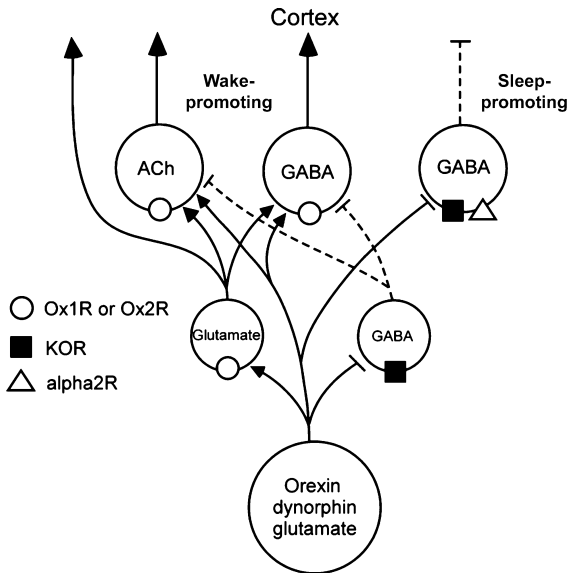

Figure 4 Pathways through which the orexin neurones may activate the basal forebrain (BF) to promote wakefulness. Orexins excite wake-promoting cholinergic and non-cholinergic neurones (most of which probably contain GABA). Orexins also enhance release of glutamate in the BF. In contrast, dynorphin released from the orexin neurones acts through κ opiate receptors (KOR) to inhibit sleep-active cells, including GABAergic interneurones. Solid lines indicate pathways active during wake; dashed lines indicate pathways active during sleep. Arrows indicate excitatory inputs; bars indicate inhibitory inputs. Not shown are the descending projections to the thalamus, hypothalamus and brainstem.

the BF and MCH-R1 are expressed in the BF (Bittencourt & Elias 1998, Hervieu *et al.* 2000, Elias *et al.* 2001). Thus, during sleep, the release of MCH and GABA could inhibit cholinergic and non-cholinergic wake-active BF neurones, but this has not yet been tested directly.

A model of how the orexin neurones mediate arousal through the BF

Considerable evidence suggests that the BF is a key site through which the orexin neurones promote the maintenance of wakefulness as well as arousals from sleep. Here we present a testable model of how this may occur (Fig. 4).

First, orexins may directly excite cortically projecting, wake-promoting cholinergic neurones of the BF (Eggermann *et al.* 2001, Espana *et al.* 2001, Thakkar *et al.* 2001, Fadel *et al.* 2005). We have found that MCPO/SI cholinergic neurones that project to the cortex are excited by orexins, but do not respond to dynorphin and thus probably lack κ receptors (Fig. 2).

Second, orexins may directly excite cortically projecting, wake-promoting non-cholinergic neurones. Most likely these cells produce GABA (Duque *et al.* 2000, Manns *et al.* 2000) and reduce the activity of inhibitory cortical interneurones (Freund & Gulyas 1991, Semba 2000). We found that non-cholinergic cortically projecting MCPO/SI neurones that display the electrophysiological characteristics of GABAergic neurones are strongly excited by orexin-A with no direct response to dynorphin except for slight inhibition of excitatory input (Fig. 3).

Third, orexin may enhance glutamate release in the BF by acting on terminals or soma of glutamatergic neurones. In support of this mechanism, dialysis of orexin-A into the BF increases local release of glutamate (Fadel & Frederick-Duus 2008). Furthermore, we have found that orexin-A increases evoked EPSCs in cholinergic and non-cholinergic (putative GABAergic) cortically projecting neurones. In BF, the source of this glutamate is currently unknown; it may be released from the terminals of BF neurones (Manns *et al.* 2001, Hur & Zaborszky 2005, Henny & Jones 2008, Wu *et al.* 2009), orexin neurones, or inputs from the cortex, midline thalamus or PPT tegmental nucleus (Grove 1988, Carnes *et al.* 1990, Zaborszky *et al.* 1997).

Fourth, release of dynorphin from orexin nerve terminals may inhibit the activity of sleep-promoting neurones in the BF and GABAergic neurones that inhibit the wake-promoting neurones. These sleep-active neurones may produce GABA and NPY, and during wake they may be inhibited by noradrenaline via α2 receptors (Duque *et al.* 2000, Manns *et al.* 2000, 2003a,b, Zaborszky & Duque 2003, Lee *et al.* 2004, Modirrousta *et al.* 2004).

This model encompasses many aspects of BF neurobiology, but it is still a simplification. The model does not include the descending projections from the BF to state-regulatory regions in the lateral hypothalamus and brainstem (Swanson *et al.* 1984, Semba *et al.* 1989, Gritti *et al.* 1994) that may play important roles in sustaining wakefulness. Instead, this model concentrates on the ascending signals from the BF that provide the most direct route for cortical activation.

How might intermittent activity in the orexin neurones produce sustained periods of wakefulness? The orexin neurones fire mainly during active wake (Lee *et al.* 2005b, Mileykovskiy *et al.* 2005, Takahashi *et al.* 2008), yet the sleepiness of narcolepsy is most apparent during quiet wake when an individual is sedentary (Scammell 2003). This paradoxical pattern may be explained by recent *in vitro* studies showing that orexins produce long-lasting effects that persist even after their washout, suggesting that the effects of orexins may last longer than the firing of the orexin neurones (Selbach *et al.* 2004, Borgland *et al.* 2006). Orexin-A, probably through Ox1 receptors, produces sustained potentiation of glutamatergic synaptic transmission in

the hippocampus (Schaffer collateral CA3 → CA1) and in ventral tegmental area (VTA) neurones (Selbach *et al.* 2004, Borgland *et al.* 2006). In the VTA, this long-term potentiation is mediated by an increase in the expression of NMDA receptors that lasts for several hours. Orexins may similarly increase glutamatergic signalling in neurones of the BF through a pre-synaptic mechanism or by up-regulation of post-synaptic glutamatergic receptors. This would make wake-promoting BF neurones more excitable, resulting in more potent and persistent activation of the cortex. This mechanism would also help explain how even intermittent activity in the orexin neurones helps sustain long periods of wakefulness.

Alternative mechanisms

Our model focuses on the BF, but the orexin neurones may promote arousal through other pathways. One possibility is that orexins stabilize wake through monoaminergic neurones such as the TMN, locus coeruleus, raphe nuclei or cholinergic neurones of the PPT and LDT nuclei because microinjections of orexin-A into these and other regions increase neuronal firing and produce arousal (Bourgin *et al.* 2000, Brown *et al.* 2001, 2002, Huang *et al.* 2001, Xi *et al.* 2001, Burlet *et al.* 2002, Saper *et al.* 2005).

Another hypothesis is that orexins directly excite cortical neurones. However, only neurones in lamina 6b directly respond to orexin-B (Bayer *et al.* 2004). These cells might help coordinate activity across cortical regions, but it seems unlikely that this limited population promotes generalized arousal. Orexins also has been hypothesized to indirectly excite the cortex by acting on neurones of the midline and intralaminar thalamic nuclei (Bayer *et al.* 2002, Ishibashi *et al.* 2005, Govindaiah & Cox 2006, Huang *et al.* 2006, Kolaj *et al.* 2007) and on their cortical inputs (Lambe & Aghajanian 2003, Lambe *et al.* 2005). These 'non-specific' nuclei project to widespread regions of the cortex (Van der Werf *et al.* 2000), but a direct wake-promoting role seems unlikely as lesions of the midline thalamus have little impact on the amounts of wake (Buzsaki *et al.* 1988). Thus, in addition to the BF, orexins can activate other arousal systems that may help promote and maintain waking and behavioural arousal.

Future directions

We have reviewed evidence suggesting that the BF is a key target through which the orexin neurones promote wake, yet many fundamental questions remain unanswered. Is orexin signalling in the BF necessary or sufficient to maintain normal wakefulness? Which BF neurones mediate orexin responses and through which electrophysiological and neurochemical mechanisms do orexins and dynorphin promote wake? Defining these mechanisms should provide many novel insights into how the orexin neurones sustain arousal, improve alertness and regulate other key functions of the BF.

Conflict of interest

There is no conflict of interest in this study.

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References

Abrahamson, E.E., Leak, R.K. & Moore, R.Y. 2001. The suprachiasmatic nucleus projects to posterior hypothalamic arousal systems. *Neuroreport* **12**, 435–440.

Acuna-Goycolea, C. & van den Pol, A.N. 2005. Peptide YY(3-36) inhibits both anorexigenic proopiomelanocortin and orexigenic neuropeptide Y neurons: implications for hypothalamic regulation of energy homeostasis. *J Neurosci* **25**, 10510–10519.

Adamantidis, A. & de Lecea, L. 2008. Physiological arousal: a role for hypothalamic systems. *Cell Mol Life Sci* **65**, 1475–1488.

Adamantidis, A.R., Zhang, F., Aravanis, A.M., Deisseroth, K. & de Lecea, L. 2007. Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature* **450**, 420–424.

Arrigoni, E., Chamberlin, N.L., Saper, C.B. & McCarley, R.W. 2006. Adenosine inhibits basal forebrain cholinergic and noncholinergic neurons in vitro. *Neuroscience* **140**, 403–413.

Bayer, L., Risold, P.Y., Griffond, B. & Fellmann, D. 1999. Rat diencephalic neurons producing melanin-concentrating hormone are influenced by ascending cholinergic projections. *Neuroscience* **91**, 1087–1101.

Bayer, L., Eggermann, E., Saint-Mleux, B., Machard, D., Jones, B.E., Muhlethaler, M. & Serafin, M. 2002. Selective action of orexin (hypocretin) on nonspecific thalamocortical projection neurons. *J Neurosci* **22**, 7835–7839.

Bayer, L., Serafin, M., Eggermann, E., Saint-Mleux, B., Machard, D., Jones, B.E. & Muhlethaler, M. 2004. Exclusive postsynaptic action of hypocretin-orexin on sublayer 6b cortical neurons. *J Neurosci* **24**, 6760–6764.

Bayer, L., Eggermann, E., Serafin, M., Grivel, J., Machard, D., Muhlethaler, M. & Jones, B.E. 2005. Opposite effects of noradrenaline and acetylcholine upon hypocretin/orexin versus melanin concentrating hormone neurons in rat hypothalamic slices. *Neuroscience* **130**, 807–811.

Bisetti, A., Cvetkovic, V., Serafin, M., Bayer, L., Machard, D., Jones, B.E. & Muhlethaler, M. 2006. Excitatory action of hypocretin/orexin on neurons of the central medial amygdala. *Neuroscience* **142**, 999–1004.

Bittencourt, J.C. & Elias, C.F. 1998. Melanin-concentrating hormone and neuropeptide EI projections from the lateral hypothalamic area and zona incerta to the medial septal nucleus and spinal cord: a study using multiple neuronal tracers. *Brain Res* **805**, 1–19.

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Blanco-Centurion, C., Xu, M., Murillo-Rodriguez, E., Gerashchenko, D., Shiromani, A.M., Salin-Pascual, R.J., Hof, P.R. & Shiromani, P.J. 2006a. Adenosine and sleep homeostasis in the Basal forebrain. *J Neurosci* **26**, 8092–8100.

Blanco-Centurion, C.A., Shiromani, A., Winston, E. & Shiromani, P.J. 2006b. Effects of hypocretin-1 in 192-IgG-saporin-lesioned rats. *Eur J Neurosci* **24**, 2084–2088.

Blouin, A.M., Thannickal, T.C., Worley, P.F., Baraban, J.M., Reti, I.M. & Siegel, J.M. 2005. Narp immunostaining of human hypocretin (orexin) neurons: loss in narcolepsy. *Neurology* **65**, 1189–1192.

Borgland, S.L., Taha, S.A., Sarti, F., Fields, H.L. & Bonci, A. 2006. Orexin A in the VTA is critical for the induction of synaptic plasticity and behavioral sensitization to cocaine. *Neuron* **49**, 589–601.

Bourgin, P., Huitron-Resendiz, S., Spier, A.D., Fabre, V., Morte, B., Criado, J.R., Sutcliffe, J.G., Henriksen, S.J. & de Lecea, L. 2000. Hypocretin-1 modulates rapid eye movement sleep through activation of locus coeruleus neurons. *J Neurosci* **20**, 7760–7765.

Brown, R.E., Sergeeva, O., Eriksson, K.S. & Haas, H.L. 2001. Orexin A excites serotonergic neurons in the dorsal raphe nucleus of the rat. *Neuropharmacology* **40**, 457–459.

Brown, R.E., Sergeeva, O.A., Eriksson, K.S. & Haas, H.L. 2002. Convergent excitation of dorsal raphe serotonin neurons by multiple arousal systems (orexin/hypocretin, histamine and noradrenaline). *J Neurosci* **22**, 8850–8859.

Burdakov, D., Liss, B. & Ashcroft, F.M. 2003. Orexin excites GABAergic neurons of the arcuate nucleus by activating the sodium–calcium exchanger. *J Neurosci* **23**, 4951–4957.

Burlet, S., Tyler, C.J. & Leonard, C.S. 2002. Direct and indirect excitation of laterodorsal tegmental neurons by hypocretin/orexin peptides: implications for wakefulness and narcolepsy. *J Neurosci* **22**, 2862–2872.

Buzsaki, G., Bickford, R.G., Ponomareff, G., Thal, L.J., Mandel, R. & Gage, F.H. 1988. Nucleus basalis and thalamic control of neocortical activity in the freely moving rat. *J Neurosci* **8**, 4007–4026.

Cape, E.G. & Jones, B.E. 2000. Effects of glutamate agonist versus procaine microinjections into the basal forebrain cholinergic cell area upon gamma and theta EEG activity and sleep-wake state. *Eur J Neurosci* **12**, 2166–2184.

Carnes, K.M., Fuller, T.A. & Price, J.L. 1990. Sources of presumptive glutamatergic/aspartatergic afferents to the magnocellular basal forebrain in the rat. *J Comp Neurol* **302**, 824–852.

Chemelli, R.M., Willie, J.T., Sinton, C.M., Elmquist, J.K., Scammell, T., Lee, C., Richardson, J.A., Williams, S.C., Xiong, Y., Kisanuki, Y., Fitch, T.E., Nakazato, M., Hammer, R.E., Saper, C.B. & Yanagisawa, M. 1999. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* **98**, 437–451.

Chou, T.C., Lee, C.E., Lu, J., Elmquist, J.K., Hara, J., Willie, J.T., Beuckmann, C.T., Chemelli, R.M., Sakurai, T., Yanagisawa, M., Saper, C.B. & Scammell, T.E.. 2001. Orexin (hypocretin) neurons contain dynorphin. *J Neurosci* **21**, RC168.

Crocker, A., Espana, R.A., Papadopoulou, M., Saper, C.B., Faraco, J., Sakurai, T., Honda, M., Mignot, E. & Scammell, T.E. 2005. Concomitant loss of dynorphin, NARP, and orexin in narcolepsy. *Neurology* **65**, 1184–1188.

Cullinan, W.E. & Zaborszky, L. 1991. Organization of ascending hypothalamic projections to the rostral forebrain with special reference to the innervation of cholinergic projection neurons. *J Comp Neurol* **306**, 631–667.

De Camilli, P. & Jahn, R. 1990. Pathways to regulated exocytosis in neurons. *Annu Rev Physiol* **52**, 625–645.

DePaoli, A.M., Hurley, K.M., Yasada, K., Reisine, T. & Bell, G. 1994. Distribution of kappa opioid receptor mRNA in adult mouse brain: an in situ hybridization histochemistry study. *Mol Cell Neurosci* **5**, 327–335.

Dong, H.L., Fukuda, S., Murata, E., Zhu, Z. & Higuchi, T. 2006. Orexins increase cortical acetylcholine release and electroencephalographic activation through orexin-1 receptor in the rat basal forebrain during isoflurane anesthesia. *Anesthesiology* **104**, 1023–1032.

Duque, A., Balatoni, B., Detari, L. & Zaborszky, L. 2000. EEG correlation of the discharge properties of identified neurons in the basal forebrain. *J Neurophysiol* **84**, 1627–1635.

Eggermann, E., Serafin, M., Bayer, L., Machard, D., Saint-Mleux, B., Jones, B.E. & Muhlethaler, M. 2001. Orexins/hypocretins excite basal forebrain cholinergic neurones. *Neuroscience* **108**, 177–181.

Elias, C.F., Lee, C.E., Kelly, J.F., Ahima, R.S., Kuhar, M., Saper, C.B. & Elmquist, J.K. 2001. Characterization of CART neurons in the rat and human hypothalamus. *J Comp Neurol* **432**, 1–19.

Eriksson, K.S., Sergeeva, O., Brown, R.E. & Haas, H.L. 2001. Orexin/hypocretin excites the histaminergic neurons of the tuberomammillary nucleus. *J Neurosci* **21**, 9273–9279.

Eriksson, K.S., Sergeeva, O.A., Selbach, O. & Haas, H.L. 2004. Orexin (hypocretin)/dynorphin neurons control GABAergic inputs to tuberomammillary neurons. *Eur J Neurosci* **19**, 1278–1284.

Espana, R.A., Baldo, B.A., Kelley, A.E. & Berridge, C.W. 2001. Wake-promoting and sleep-suppressing actions of hypocretin (orexin): basal forebrain sites of action. *Neuroscience* **106**, 699–715.

Espana, R.A., Reis, K.M., Valentino, R.J. & Berridge, C.W. 2005. Organization of hypocretin/orexin efferents to locus coeruleus and basal forebrain arousal-related structures. *J Comp Neurol* **481**, 160–178.

Estabrooke, I.V., McCarthy, M.T., Ko, E., Chou, T.C., Chemelli, R.M., Yanagisawa, M., Saper, C.B. & Scammell, T.E. 2001. Fos expression in orexin neurons varies with behavioral state. *J Neurosci* **21**, 1656–1662.

Fadel, J. & Frederick-Duus, D. 2008. Orexin/hypocretin modulation of the basal forebrain cholinergic system: insights from in vivo microdialysis studies. *Pharmacol Biochem Behav* **90**, 156–162.

Fadel, J., Pasumarthi, R. & Reznikov, L.R. 2005. Stimulation of cortical acetylcholine release by orexin A. *Neuroscience* **130**, 541–547.

Fong, D.K. & Craig, A.M. 1999. The Narp hypothesis? *Neuron* **23**, 195–197.

Ford, B., Holmes, C.J., Mainville, L. & Jones, B.E. 1995. GABAergic neurons in the rat pontomesencephalic tegmentum: codistribution with cholinergic and other tegmental neurons projecting to the posterior lateral hypothalamus. *J Comp Neurol* **363**, 177–196.

Frederick-Duus, D., Guyton, M.F. & Fadel, J. 2007. Food-elicited increases in cortical acetylcholine release require orexin transmission. *Neuroscience* **149**, 499–507.

Freund, T.F. & Gulyas, A.I. 1991. GABAergic interneurons containing calbindin D28K or somatostatin are major targets of GABAergic basal forebrain afferents in the rat neocortex. *J Comp Neurol* **314**, 187–199.

Gerashchenko, D., Kohls, M.D., Greco, M., Waleh, N.S., Salin-Pascual, R., Kilduff, T.S., Lappi, D.A. & Shiromani, P.J. 2001. Hypocretin-2-saporin lesions of the lateral hypothalamus produce narcoleptic-like sleep behavior in the rat. *J Neurosci* **21**, 7273–7283.

Govindaiah, G. & Cox, C.L. 2006. Modulation of thalamic neuron excitability by orexins. *Neuropharmacology* **51**, 414–425.

Gritti, I., Mainville, L. & Jones, B.E. 1994. Projections of GABAergic and cholinergic basal forebrain and GABAergic preoptic-anterior hypothalamic neurons to the posterior lateral hypothalamus of the rat. *J Comp Neurol* **339**, 251–268.

Gritti, I., Mainville, L., Mancia, M. & Jones, B.E. 1997. GABAergic and other noncholinergic basal forebrain neurons, together with cholinergic neurons, project to the mesocortex and isocortex in the rat. *J Comp Neurol* **383**, 163–177.

Grove, E.A. 1988. Neural associations of the substantia innominata in the rat: afferent connections. *J Comp Neurol* **277**, 315–346.

Hagan, J.J., Leslie, R.A., Patel, S., Evans, M.L., Wattam, T.A., Holmes, S., Benham, C.D., Taylor, S.G., Routledge, C., Hemmati, P. *et al.* 1999. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. *Proc Natl Acad Sci USA* **96**, 10911–10916.

Hara, J., Beuckmann, C.T., Nambu, T., Willie, J.T., Chemelli, R.M., Sinton, C.M., Sugiyama, F., Yagami, K., Goto, K., Yanagisawa, M. & Sakurai, T. 2001. Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron* **30**, 345–354.

Hara, J., Yanagisawa, M. & Sakurai, T. 2005. Difference in obesity phenotype between orexin-knockout mice and orexin neuron-deficient mice with same genetic background and environmental conditions. *Neurosci Lett* **380**, 239–242.

Hartig, W., Seeger, J., Naumann, T., Brauer, K. & Bruckner, G. 1998. Selective in vivo fluorescence labelling of cholinergic neurons containing p75(NTR) in the rat basal forebrain. *Brain Res* **808**, 155–165.

Hassani, O.K., Lee, M.G. & Jones, B.E. 2009. Melanin-concentrating hormone neurons discharge in a reciprocal manner to orexin neurons across the sleep-wake cycle. *Proc Natl Acad Sci USA* **106**, 2418–2422.

Henny, P. & Jones, B.E. 2006a. Innervation of orexin/hypocretin neurons by GABAergic, glutamatergic or cholinergic basal forebrain terminals evidenced by immunostaining for presynaptic vesicular transporter and postsynaptic scaffolding proteins. *J Comp Neurol* **499**, 645–661.

Henny, P. & Jones, B.E. 2006b. Vesicular glutamate (VGlut), GABA (VGAT), and acetylcholine (VACht) transporters in basal forebrain axon terminals innervating the lateral hypothalamus. *J Comp Neurol* **496**, 453–467.

Henny, P. & Jones, B.E. 2008. Projections from basal forebrain to prefrontal cortex comprise cholinergic, GABAergic and glutamatergic inputs to pyramidal cells or interneurons. *Eur J Neurosci* **27**, 654–670.

Hervieu, G.J., Cluderay, J.E., Harrison, D., Meakin, J., Maycox, P., Nasir, S. & Leslie, R.A. 2000. The distribution of the mRNA and protein products of the melanin-concentrating hormone (MCH) receptor gene, slc-1, in the central nervous system of the rat. *Eur J Neurosci* **12**, 1194–1216.

Hervieu, G.J., Cluderay, J.E., Harrison, D.C., Roberts, J.C. & Leslie, R.A. 2001. Gene expression and protein distribution of the orexin-1 receptor in the rat brain and spinal cord. *Neuroscience* **103**, 777–797.

Hoang, Q.V., Bajic, D., Yanagisawa, M., Nakajima, S. & Nakajima, Y. 2003. Effects of orexin (hypocretin) on GIRK channels. *J Neurophysiol* **90**, 693–702.

Hoang, Q.V., Zhao, P., Nakajima, S. & Nakajima, Y. 2004. Orexin (hypocretin) effects on constitutively active inward rectifier K+ channels in cultured nucleus basalis neurons. *J Neurophysiol* **92**, 3183–3191.

Huang, Z.L., Qu, W.M., Li, W.D., Mochizuki, T., Eguchi, N., Watanabe, T., Urade, Y. & Hayaishi, O. 2001. Arousal effect of orexin A depends on activation of the histaminergic system. *Proc Natl Acad Sci USA* **98**, 9965–9970.

Huang, H., Ghosh, P. & van den Pol, A.N. 2006. Prefrontal cortex-projecting glutamatergic thalamic paraventricular nucleus-excited by hypocretin: a feedforward circuit that may enhance cognitive arousal. *J Neurophysiol* **95**, 1656–1668.

Hur, E.E. & Zaborszky, L. 2005. Vglut2 afferents to the medial prefrontal and primary somatosensory cortices: a combined retrograde tracing in situ hybridization study [corrected]. *J Comp Neurol* **483**, 351–373.

Ishibashi, M., Takano, S., Yanagida, H., Takatsuna, M., Nakajima, K., Oomura, Y., Wayner, M.J. & Sasaki, K. 2005. Effects of orexins/hypocretins on neuronal activity in the paraventricular nucleus of the thalamus in rats in vitro. *Peptides* **26**, 471–481.

Ivanov, A. & Aston-Jones, G. 2000. Hypocretin/orexin depolarizes and decreases potassium conductance in locus coeruleus neurons. *Neuroreport* **11**, 1755–1758.

Jasper, H.H. & Tessier, J. 1971. Acetylcholine liberation from cerebral cortex during paradoxical (REM) sleep. *Science* **172**, 601–602.

Jones, B.E. 2004. Activity, modulation and role of basal forebrain cholinergic neurons innervating the cerebral cortex. *Prog Brain Res* **145**, 157–169.

Jones, B.E. 2005. From waking to sleeping: neuronal and chemical substrates. *Trends Pharmacol Sci* **26**, 578–586.

Kantor, S., Mochizuki, T., Janisiewicz, A.M., Nishino, S., Clarck, E.L. & Scammell, T. 2009. Orexin neurons are necessary for the circadian control of REM sleep. *Sleep*, **32**, 1127–1134.

Kaur, S., Junek, A., Black, M.A. & Semba, K. 2008. Effects of ibotenate and 192IgG-saporin lesions of the nucleus basalis magnocellularis/substantia innominata on spontaneous sleep and wake states and on recovery sleep after sleep deprivation in rats. *J Neurosci* **28**, 491–504.

Kiyashchenko, L.I., Mileykovskiy, B.Y., Maidment, N., Lam, H.A., Wu, M.F., John, J., Peever, J. & Siegel, J.M. 2002. Release of hypocretin (orexin) during waking and sleep states. *J Neurosci* **22**, 5282–5286.

Kohlmeier, K.A., Watanabe, S., Tyler, C.J., Burlet, S. & Leonard, C.S. 2008. Dual orexin actions on dorsal raphe and laterodorsal tegmentum neurons: noisy cation current activation and selective enhancement of Ca^{2+} transients mediated by L-type calcium channels. *J Neurophysiol* **100**, 2265–2281.

Kolaj, M., Doroshenko, P., Yan Cao, X., Coderre, E. & Renaud, L.P. 2007. Orexin-induced modulation of state-dependent intrinsic properties in thalamic paraventricular nucleus neurons attenuates action potential patterning and frequency. *Neuroscience* **147**, 1066–1075.

Kreibich, A., Reyes, B.A., Curtis, A.L., Ecke, L., Chavkin, C., Van Bockstaele, E.J. & Valentino, R.J. 2008. Presynaptic inhibition of diverse afferents to the locus coeruleus by kappa-opiate receptors: a novel mechanism for regulating the central norepinephrine system. *J Neurosci* **28**, 6516–6525.

Lambe, E.K. & Aghajanian, G.K. 2003. Hypocretin (orexin) induces calcium transients in single spines postsynaptic to identified thalamocortical boutons in prefrontal slice. *Neuron* **40**, 139–150.

Lambe, E.K., Olausson, P., Horst, N.K., Taylor, J.R. & Aghajanian, G.K. 2005. Hypocretin and nicotine excite the same thalamocortical synapses in prefrontal cortex: correlation with improved attention in rat. *J Neurosci* **25**, 5225–5229.

de Lecea, L., Kilduff, T.S., Peyron, C., Gao, X., Foye, P.E., Danielson, P.E., Fukuhara, C., Battenberg, E.L., Gautvik, V.T., Bartlett, F.S., 2nd, Frankel, W.N., van den Pol, A.N., Bloom, F.E., Gautvik, K.M. & Sutcliffe, J.G. 1998. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci USA* **95**, 322–327.

Lee, M.G., Manns, I.D., Alonso, A. & Jones, B.E. 2004. Sleep-wake related discharge properties of basal forebrain neurons recorded with micropipettes in head-fixed rats. *J Neurophysiol* **92**, 1182–1198.

Lee, M.G., Hassani, O.K., Alonso, A. & Jones, B.E. 2005a. Cholinergic basal forebrain neurons burst with theta during waking and paradoxical sleep. *J Neurosci* **25**, 4365–4369.

Lee, M.G., Hassani, O.K. & Jones, B.E. 2005b. Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. *J Neurosci* **25**, 6716–6720.

Li, Y. & van den Pol, A.N. 2006. Differential target-dependent actions of coexpressed inhibitory dynorphin and excitatory hypocretin/orexin neuropeptides. *J Neurosci* **26**, 13037–13047.

Lin, L., Faraco, J., Li, R., Kadotani, H., Rogers, W., Lin, X., Qiu, X., de Jong, P.J., Nishino, S. & Mignot, E. 1999. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* **98**, 365–376.

Manfridi, A., Brambilla, D. & Mancia, M. 1999. Stimulation of NMDA and AMPA receptors in the rat nucleus basalis of Meynert affects sleep. *Am J Physiol* **277**, R1488–R1492.

Manns, I.D., Alonso, A. & Jones, B.E. 2000. Discharge profiles of juxtacellularly labeled and immunohistochemically identified GABAergic basal forebrain neurons recorded in association with the electroencephalogram in anesthetized rats. *J Neurosci* **20**, 9252–9263.

Manns, I.D., Mainville, L. & Jones, B.E. 2001. Evidence for glutamate, in addition to acetylcholine and GABA, neurotransmitter synthesis in basal forebrain neurons projecting to the entorhinal cortex. *Neuroscience* **107**, 249–263.

Manns, I.D., Alonso, A. & Jones, B.E. 2003a. Rhythmically discharging basal forebrain units comprise cholinergic, GABAergic, and putative glutamatergic cells. *J Neurophysiol* **89**, 1057–1066.

Manns, I.D., Lee, M.G., Modirrousta, M., Hou, Y.P. & Jones, B.E. 2003b. Alpha 2 adrenergic receptors on GABAergic, putative sleep-promoting basal forebrain neurons. *Eur J Neurosci* **18**, 723–727.

Mansour, A., Fox, C.A., Burke, S., Meng, F., Thompson, R.C., Akil, H. & Watson, S.J. 1994. Mu, delta, and kappa opioid receptor mRNA expression in the rat CNS: an in situ hybridization study. *J Comp Neurol* **350**, 412–438.

Marcus, J.N., Aschkenasi, C.J., Lee, C.E., Chemelli, R.M., Saper, C.B., Yanagisawa, M. & Elmquist, J.K. 2001. Differential expression of orexin receptors 1 and 2 in the rat brain. *J Comp Neurol* **435**, 6–25.

Marrosu, F., Portas, C., Mascia, M.S., Casu, M.A., Fà, M., Giagheddu, M., Imperato, A. & Gessa, G.L. 1995. Microdialysis measurement of cortical and hippocampal acetylcholine release during sleep-wake cycle in freely moving cats. *Brain Res* **671**, 329–332.

McFadzean, I., Lacey, M.G., Hill, R.G. & Henderson, G. 1987. Kappa opioid receptor activation depresses excitatory synaptic input to rat locus coeruleus neurons in vitro. *Neuroscience* **20**, 231–239.

Mignot, E., Lammers, G.J., Ripley, B., Okun, M., Nevsimalova, S., Overeem, S., Vankova, J., Black, J., Harsh, J., Bassetti, C., Schrader, H. & Nishino, S. 2002. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. *Arch Neurol* **59**, 1553–1562.

Mileykovskiy, B.Y., Kiyashchenko, L.I. & Siegel, J.M. 2005. Behavioral correlates of activity in identified hypocretin/orexin neurons. *Neuron* **46**, 787–798.

Mochizuki, T., Crocker, A., McCormack, S., Yanagisawa, M., Sakurai, T. & Scammell, T.E. 2004. Behavioral state instability in orexin knock-out mice. *J Neurosci* **24**, 6291–6300.

Modirrousta, M., Mainville, L. & Jones, B.E. 2004. Gabaergic neurons with alpha2-adrenergic receptors in basal forebrain and preoptic area express c-Fos during sleep. *Neuroscience* **129**, 803–810.

Moruzzi, G. & Magoun, H.W. 1949. Brain stem reticular formation and activation of the EEG. *Electroencephalogr Clin Neurophysiol* **1**, 455–473.

Nambu, T., Sakurai, T., Mizukami, K., Hosoya, Y., Yanagisawa, M. & Goto, K. 1999. Distribution of orexin neurons in the adult rat brain. *Brain Res* **827**, 243–260.

 doi: 10.1111/j.1748-1716.2009.02036.x

O'Brien, R.J., Xu, D., Petralia, R.S., Steward, O., Huganir, R.L. & Worley, P. 1999. Synaptic clustering of AMPA receptors by the extracellular immediate-early gene product Narp. *Neuron* **23**, 309–323.

Peyron, C., Tighe, D.K., van den Pol, A.N., de Lecea, L., Heller, H.C., Sutcliffe, J.G. & Kilduff, T.S. 1998. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci* **18**, 9996–10015.

Peyron, C., Faraco, J., Rogers, W., Ripley, B., Overeem, S., Charnay, Y., Nevsimalova, S., Aldrich, M., Reynolds, D., Albin, R. *et al.* 2000. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat Med* **6**, 991–997.

Pinnock, R.D. 1992. Activation of kappa-opioid receptors depresses electrically evoked excitatory postsynaptic potentials on 5-HT-sensitive neurones in the rat dorsal raphe nucleus in vitro. *Brain Res* **583**, 237–246.

Portas, C.M., Thakkar, M., Rainnie, D.G., Greene, R.W. & McCarley, R.W. 1997. Role of adenosine in behavioral state modulation: a microdialysis study in the freely moving cat. *Neuroscience* **79**, 225–235.

Reti, I.M., Reddy, R., Worley, P.F. & Baraban, J.M. 2002. Selective expression of Narp, a secreted neuronal pentraxin, in orexin neurons. *J Neurochem* **82**, 1561–1565.

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R.M., Tanaka, H., Williams, S.C., Richardson, J.A., Kozlowski, G.P., Wilson, S. *et al.* 1998. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* **92**, 573–585.

Sakurai, T., Nagata, R., Yamanaka, A., Kawamura, H., Tsujino, N., Muraki, Y., Kageyama, H., Kunita, S., Takahashi, S., Goto, K., Koyama, Y., Shioda, S. & Yanagisawa, M. 2005. Input of orexin/hypocretin neurons revealed by a genetically encoded tracer in mice. *Neuron* **46**, 297–308.

Salio, C., Lossi, L., Ferrini, F. & Merighi, A. 2006. Neuropeptides as synaptic transmitters. *Cell Tissue Res* **326**, 583–598.

Saper, C.B. 1984. Organization of cerebral cortical afferent systems in the rat II. Magnocellular basal nucleus. *J Comp Neurol* **222**, 313–342.

Saper, C.B., Scammell, T.E. & Lu, J. 2005. Hypothalamic regulation of sleep and circadian rhythms. *Nature* **437**, 1257–1263.

Scammell, T.E. 2003. The neurobiology, diagnosis, and treatment of narcolepsy. *Ann Neurol* **53**, 154–166.

Selbach, O., Doreulee, N., Bohla, C., Eriksson, K.S., Sergeeva, O.A., Poelchen, W., Brown, R.E. & Haas, H.L. 2004. Orexins/hypocretins cause sharp wave- and theta-related synaptic plasticity in the hippocampus via glutamatergic, gabaergic, noradrenergic, and cholinergic signaling. *Neuroscience* **127**, 519–528.

Semba, K. 2000. Multiple output pathways of the basal forebrain: organization, chemical heterogeneity, and roles in vigilance. *Behav Brain Res* **115**, 117–141.

Semba, K., Reiner, P.B., McGeer, E.G. & Fibiger, H.C. 1989. Brainstem projecting neurons in the rat basal forebrain: neurochemical, topographical, and physiological distinctions from cortically projecting cholinergic neurons. *Brain Res Bull* **22**, 501–509.

Swanson, L.W., Mogenson, G.J., Gerfen, C.R. & Robinson, P. 1984. Evidence for a projection from the lateral preoptic area and substantia innominata to the 'mesencephalic locomotor region' in the rat. *Brain Res* **295**, 161–178.

Szymusiak, R. 1995. Magnocellular nuclei of the basal forebrain: substrates of sleep and arousal regulation. *Sleep* **18**, 478–500.

Szymusiak, R. & McGinty, D. 1986. Sleep-related neuronal discharge in the basal forebrain of cats. *Brain Res* **370**, 82–92.

Szymusiak, R., Alam, N. & McGinty, D. 2000. Discharge patterns of neurons in cholinergic regions of the basal forebrain during waking and sleep. *Behav Brain Res* **115**, 171–182.

Takahashi, K., Lin, J.S. & Sakai, K. 2008. Neuronal activity of orexin and non-orexin waking-active neurons during wake-sleep states in the mouse. *Neuroscience* **153**, 860–870.

Thakkar, M.M., Ramesh, V., Strecker, R.E. & McCarley, R.W. 2001. Microdialysis perfusion of orexin-A in the basal forebrain increases wakefulness in freely behaving rats. *Arch Ital Biol* **139**, 313–328.

Thannickal, T.C., Moore, R.Y., Nienhuis, R., Ramanathan, L., Gulyani, S., Aldrich, M., Cornford, M. & Siegel, J.M. 2000. Reduced number of hypocretin neurons in human narcolepsy. *Neuron* **27**, 469–474.

van den Top, M., Lee, K., Whyment, A.D., Blanks, A.M. & Spanswick, D. 2004. Orexigen-sensitive NPY/AgRP pacemaker neurons in the hypothalamic arcuate nucleus. *Nat Neurosci* **7**, 493–494.

Torrealba, F., Yanagisawa, M. & Saper, C.B. 2003. Colocalization of orexin a and glutamate immunoreactivity in axon terminals in the tuberomammillary nucleus in rats. *Neuroscience* **119**, 1033–1044.

Trivedi, P., Yu, H., MacNeil, D.J., Van der Ploeg, L.H. & Guan, X.M. 1998. Distribution of orexin receptor mRNA in the rat brain. *FEBS Lett* **438**, 71–75.

Tsui, C.C., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Barnes, C. & Worley, P.F. 1996. Narp, a novel member of the pentraxin family, promotes neurite outgrowth and is dynamically regulated by neuronal activity. *J Neurosci* **16**, 2463–2478.

Van der Werf, Y.D., Witter, M.P., Uylings, H.B. & Jolles, J. 2000. Neuropsychology of infarctions in the thalamus: a review. *Neuropsychologia* **38**, 613–627.

Verret, L., Goutagny, R., Fort, P., Cagnon, L., Salvert, D., Leger, L., Boissard, R., Salin, P., Peyron, C. & Luppi, P.H. 2003. A role of melanin-concentrating hormone producing neurons in the central regulation of paradoxical sleep. *BMC Neurosci* **4**, 19.

Wigren, H.K., Schepens, M., Matto, V., Stenberg, D. & Porkka-Heiskanen, T. 2007. Glutamatergic stimulation of the basal forebrain elevates extracellular adenosine and increases the subsequent sleep. *Neuroscience* **147**, 811–823.

Willie, J.T., Chemelli, R.M., Sinton, C.M., Tokita, S., Williams, S.C., Kisanuki, Y.Y., Marcus, J.N., Lee, C., Elmquist, J.K., Kohlmeier, K.A., Leonard, C.S., Richardson, J.A., Hammer, R.E. & Yanagisawa, M. 2003. Distinct narcolepsy syndromes in orexin receptor-2 and orexin null mice:

Journal compilation © 2009 Scandinavian Physiological Society, doi: 10.1111/j.1748-1716.2009.02036.x

molecular genetic dissection of non-REM and REM sleep regulatory processes. *Neuron* **38**, 715–730.

Willie, J.T., Sinton, C.M., Maratos-Flier, E. & Yanagisawa, M. 2008. Abnormal response of melanin-concentrating hormone deficient mice to fasting: hyperactivity and rapid eye movement sleep suppression. *Neuroscience* **156**, 819–829.

Woolf, N.J. 1991. Cholinergic systems in mammalian brain and spinal cord. *Prog Neurobiol* **37**, 475–524.

Wu, M., Shanabrough, M., Leranth, C. & Alreja, M. 2000. Cholinergic excitation of septohippocampal GABA but not cholinergic neurons: implications for learning and memory. *J Neurosci* **20**, 3900–3908.

Wu, M., Zhang, Z., Leranth, C., Xu, C., van den Pol, A.N. & Alreja, M. 2002. Hypocretin increases impulse flow in the septohippocampal GABAergic pathway: implications for arousal via a mechanism of hippocampal disinhibition. *J Neurosci* **22**, 7754–7765.

Wu, M., Zaborszky, L., Hajszan, T., van den Pol, A.N. & Alreja, M. 2004. Hypocretin/orexin innervation and excitation of identified septohippocampal cholinergic neurons. *J Neurosci* **24**, 3527–3536.

Wu, M., Dumalska, I., Morozova, E., van den Pol, A.N. & Alreja, M. 2009. Gonadotropin inhibitory hormone inhibits basal forebrain vGluT2-gonadotropin-releasing hormone neurons via a direct postsynaptic mechanism. *J Physiol* **587**, 1401–1411.

Xi, M.C., Morales, F.R. & Chase, M.H. 2001. Effects on sleep and wakefulness of the injection of hypocretin-1 (orexin-A) into the laterodorsal tegmental nucleus of the cat. *Brain Res* **901**, 259–264.

Zaborszky, L. & Cullinan, W.E. 1989. Hypothalamic axons terminate on forebrain cholinergic neurons: an ultrastructural double-labeling study using PHA-L tracing and ChAT immunocytochemistry. *Brain Res* **479**, 177–184.

Zaborszky, L. & Duque, A. 2003. Sleep-wake mechanisms and basal forebrain circuitry. *Front Biosci* **8**, d1146–d1169.

Zaborszky, L., Gaykema, R.P., Swanson, D.J. & Cullinan, W.E. 1997. Cortical input to the basal forebrain. *Neuroscience* **79**, 1051–1078.

