Activation of the Basal Forebrain by the Orexin/Hypocretin Neurons: Orexin International Symposium

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Abstract

The orexin neurons 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 neurons promote arousal. Here we review anatomical, pharmacological and electrophysiological studies on how the orexin neurons may promote arousal by exciting cortically-projecting neurons of the BF. Orexin fibers synapse on BF cholinergic neurons and orexin-A is released in the BF during waking. Local application of orexins excites BF cholinergic neurons, induces cortical release of acetylcholine, and promotes wakefulness. The orexin neurons 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 neurons that project to the cortex. Cholinergic neurons were directly excited by orexin-A, but did not respond to dynorphin. Non-cholinergic BF neurons that project to the cortex seem to comprise at least two populations with some directly excited by orexin that may represent wake-active, GABAergic neurons, whereas others did not respond to orexin but were inhibited by dynorphin and may be sleep-active, GABAergic neurons.

This evidence suggests that the BF is a key site through which orexins activate the cortex and promotes behavioral arousal. In addition, orexins and dynorphin may act synergistically in the BF to promote arousal and improve cognitive performance.

Keywords

orexin/hypocretin; dynorphin; basal forebrain

Orexin-A and -B (also known as hypocretin-1 and -2) are two neuropeptides produced by a cluster of wake-active neurons in the lateral hypothalamus (de Lecea *et al.* 1998, Sakurai *et al.* 1998, Lee *et al.* 2005b, Mileykovskiy *et al.* 2005). The orexin neurons heavily innervate brain regions involved in arousal and excite postsynaptic neurons 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 neurons (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

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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 neurons are necessary for normal arousal, but the specific brain regions through which orexins promote arousal remain unknown.

A growing body of evidence suggests that the basal forebrain (BF) is a key site through which the orexin neurons promote arousal. This paper comprehensively reviews the anatomical, pharmacological and electrophysiological studies, including data from our own *in vitro* recordings on how the orexin neurons can promote arousal by exciting BF neurons 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 behavioral 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 neurons (Szymusiak 1995, Semba 2000, Jones 2004). In conjunction with monoaminergic and cholinergic projections from more caudal regions, the BF is considered a key extra-thalamic relay to the cerebral cortex from the brainstem reticular activating system initially proposed by Moruzzi and Magoun (Moruzzi and Magoun 1949) (Fig. 1). BF neurons project to the cortical mantle 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 neurons 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).

The BF is the major source of cholinergic input to the cortex (Woolf 1991). During wakefulness and rapid eye movement (REM) sleep, cholinergic neurons of the MCPO and SI fire most rapidly and acetylcholine release in the cortex is maximal (Jasper and Tessier 1971, Marrosu *et al.* 1995). During non-REM sleep, the cholinergic neurons 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 neurons produce GABA and a smaller number produce glutamate (Freund and Gulyas 1991, Gritti *et al.* 1997, Hur and Zaborszky 2005, Henny and Jones 2008). GABAergic neurons account for about one-third of the MCPO/SI cortically-projecting neurons, 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 neurons 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 neurons with glutamate agonists increases wake (Manfridi *et al.* 1999, Cape and 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 neurons increases 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 neurons with an adenosine A1 receptor agonist promotes sleep, even after lesioning 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 neurons across much of the BF act synergistically to promote wake (Szymusiak *et al.* 2000, Jones 2005).

Anatomical studies

Although the orexin peptides are produced by a relatively small number of neurons in the perifornical region of the lateral hypothalamus, these neurons 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 and Cullinan 1989, Cullinan and Zaborszky 1991). More recently, studies have shown that projections from orexin neurons 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 and 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 fibers closely appose and synapse on cholinergic neurons of the BF (Wu *et al.* 2004, Espana *et al.* 2005, Fadel *et al.* 2005, Fadel and Frederick-Duus 2008). An ultrastructural study reveals that 70% of the cholinergic neurons 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 neurons by the orexin neurons (Fadel *et al.* 2005, Fadel and Frederick-Duus 2008).

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

BF neurons 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). Neurons 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 neurons are more responsive to orexin-B suggesting an Ox2R effect, whereas others conclude that orexin-A signaling 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 pharmacologic 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 neurons 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 neurons 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 neurons can trigger awakenings from sleep (Adamantidis *et al.* 2007), and it is possible that in addition to promoting wakefulness, the orexin neurons 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 behavioral response and the rise in acetylcholine is blunted in rats with lesions of the orexin neurons and adjacent cells in lateral hypothalamus (Frederick-Duus *et al.* 2007). This observation suggests that orexins are necessary for the activation of BF cholinergic neurons, though it should be interpreted cautiously as this type of lesion kills much more than just the orexin neurons (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 neurons of the BF. Orexin-B excites GABAergic neurons 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 neurons of the MCPO/SI region (see below). In fact, microinjection of orexin-A into the BF still promotes arousal after selective lesioning the BF cholinergic neurons (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 behavioral arousal by acting on cholinergic and non-cholinergic neurons.

Electrophysiologic responses to orexins

Several studies using *in vitro* slice recordings have shed light on how the orexin neurons activate the BF (Eggermann *et al.* 2001, Wu *et al.* 2002, Wu *et al.* 2004). Most of these studies focused on the effects of orexins on medial septum neurons that project to the hippocampus (Wu *et al.* 2002, Wu *et al.* 2004), and so far, the cortically-projecting neurons of the caudal BF have received less attention. Mühlethaler and collaborators reported early on that orexins directly excites MCPO cholinergic neurons (Eggermann *et al.* 2001). 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 neurons.

Much more is know about the responses of neurons in the medial septum. Wu and colleagues found that orexins directly excite septohippocampal cholinergic neurons 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 (Wu *et al.* 2004). Similar effects of orexin-A on a constitutively active, inwardly rectifying potassium current were also reported in cultured BF neurons of the nucleus basalis (Hoang *et al.* 2004). In about 80% of septohippocampal cholinergic neurons, these two effects co-exist, whereas orexins only reduce a K^+ current in the locus coeruleus, central amygdala and thalamic neurons (Ivanov and Aston-Jones 2000, Bayer *et al.* 2002, Bayer *et al.* 2004, Bisetti *et al.* 2006) and only activate a Na^+/Ca^{2+} exchanger in neurons 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 neurons 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 neurons 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 neurons, and this effect was spike-dependent suggesting that it was mediated by the activation of local GABAergic neurons 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 neurons to orexins and dynorphin, another neuropeptide synthesized in the orexin neurons (Chou *et al.* 2001, Crocker *et al.* 2005). We identified cortically-projecting MCPO/SI neurons 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 neurons in the BF as nearly all express the p75 receptor (Hartig *et al.* 1998, Wu *et al.* 2000, Arrigoni *et al.* 2006). Thus, cholinergic neurons projecting to mPFC were recognized by the presence of both green beads and red Cy3-p75-IgG (Fig. 2). Non-cholinergic, cortically-projecting neurons contained green beads but lacked red Cy3-p75-IgG (Fig. 3).

We found that SI cholinergic neurons were directly excited by orexin-A but did not respond to dynorphin-A. In addition, orexin-A increased the amplitude of evoked glutamatergic excitatory postsynaptic currents (EPSCs) in cholinergic MCPO/SI neurons (Fig. 2). We found two populations of non-cholinergic MCPO/SI neurons 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 neurons showed the same electrophysiological properties previously reported in GABAergic neurons of the medial septum that project to the hippocampus (Wu *et al.* 2000). These may be GABAergic, cortically-projecting neurons (Fig. 3). An additional class of non-cholinergic cortically-projecting neurons that display different firing properties, including the 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 neurons (Duque *et al.* 2000, Manns *et al.* 2000, Modirrousta *et al.* 2004). These results show that orexin-A and dynorphin have specific effects on different classes of BF neurons. These responses may provide a synergistic mechanism by which the co-release of orexins and dynorphin can activate cholinergic and non-cholinergic wake-active neurons and can inhibit non-cholinergic sleep-active neurons to promote wakefulness and improve cognitive performance.

Dynorphin and glutamate may act synergistically to excite BF neurons

In addition to the orexin peptides, the orexin-producing neurons contain other neurotransmitters. In rats, mice and humans, essentially all orexin-producing neurons 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 presynaptic 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 neurons 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 neurons, but dynorphin has inhibitory effects.

Possibly, orexin and κ receptors reside on different target neurons or are located on different part of the target neurons. For example while orexins directly excite TMN neurons and NPY neurons of the arcuate nucleus (Eriksson *et al.* 2001, van den Top *et al.* 2004, Acuna-Goycolea and van den Pol 2005) dynorphin has no post-synaptic effects but reduces GABAergic synaptic input to these neurons (Eriksson *et al.* 2004, Li and 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) neurons 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 and van den Pol 2006). Perhaps this same phenomenon occurs in neurons 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 and Aston-Jones 2000, Brown *et al.* 2001, Brown *et al.* 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 neurons remain active, the dynorphin signaling would desensitize and the excitatory effects of orexins would then help sustain wakefulness.

In addition to dynorphin, the orexin neurons 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 are 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 neurons. Since the release of neuropeptides may require a higher firing frequency than the release of glutamate (De Camilli and Jahn 1990), it is conceivable that low frequency firing of the orexin neurons 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 signaling protein that stimulates clustering of glutamatergic AMPA receptors (Tsui *et al.* 1996, Fong and Craig 1999, O'Brien *et al.* 1999). The orexin neurons of mice and humans express NARP (Reti *et al.* 2002, Blouin *et al.* 2005, Crocker *et al.* 2005), and it is possible that the 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 neurons. However, mice lacking the orexin neurons seem to have a slightly different narcolepsy phenotype and a greater tendency toward obesity than mice simply lacking orexins (Chemelli *et al.* 1999, Hara *et al.* 2001, Hara *et al.* 2005, Kantor *et al.* 2009), perhaps due to loss of the other signaling molecules.

Role of the melanin-concentrating hormone (MCH) neurons

In addition to the orexin neurons the lateral hypothalamus also contains neurons that produce the inhibitory peptide MCH. Their firing pattern is roughly opposite to the orexin neurons;

MCH neurons 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 and de Lecea 2008, Willie *et al.* 2008). MCH neurons contain GABA, they project to the BF, and MCH-R1 are expressed in the BF (Bittencourt and 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 neurons, but this has not yet been tested directly.

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

Considerable evidence suggests that the BF is a key site through which the orexin neurons 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 neurons 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 neurons 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 neurons. Most likely these cells produce GABA (Duque *et al.* 2000, Manns *et al.* 2000) and reduce the activity of inhibitory cortical interneurons (Freund and Gulyas 1991, Semba 2000). We found that non-cholinergic cortically-projecting MCPO/SI neurons that display the electrophysiologic characteristics of GABAergic neurons 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 neurons. In support of this mechanism, dialysis of orexin-A into the BF increases local release of glutamate. Furthermore, we have found that orexin-A increases evoked excitatory postsynaptic currents in cholinergic and non-cholinergic (putative GABAergic) cortically-projecting neurons. In BF, the source of this glutamate is currently unknown; it may be released from the terminals of BF neurons (Manns *et al.* 2001, Hur and Zaborszky 2005, Henny and Jones 2008, Wu *et al.* 2009), orexin neurons, or inputs from the cortex, midline thalamus, or pedunculopontine 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 neurons in the BF and GABAergic neurons that inhibit the wake-promoting neurons. These sleep-active neurons 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, Manns *et al.* 2003a, Manns *et al.* 2003b, Zaborszky and 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 neurons produce sustained periods of wakefulness? The orexin neurons 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 neurons (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) neurons (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 signaling in neurons of the BF through a presynaptic mechanism or by up-regulation of postsynaptic glutamatergic receptors. This would make wake-promoting BF neurons 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 neurons helps sustain long periods of wakefulness.

Alternative mechanisms

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

Another hypothesis is that orexins directly excite cortical neurons. However, only neurons 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. Orexin also has been hypothesized to indirectly excite the cortex by acting on neurons of the midline and intralaminar thalamic nuclei (Bayer *et al.* 2002, Ishibashi *et al.* 2005, Govindaiah and Cox 2006, Huang *et al.* 2006, Kolaj *et al.* 2007) and on their cortical inputs (Lambe and Aghajanian 2003, Lambe *et al.* 2005). These "nonspecific" 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 behavioral arousal.

Future directions

We have reviewed evidence suggesting that the BF is a key target through which the orexin neurons promote wake, yet many fundamental questions remain unanswered. Is orexin signaling in the BF necessary or sufficient to maintain normal wakefulness? Which BF neurons 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 neurons sustain arousal, improve alertness, and regulate other key functions of the basal forebrain.

Acknowledgments

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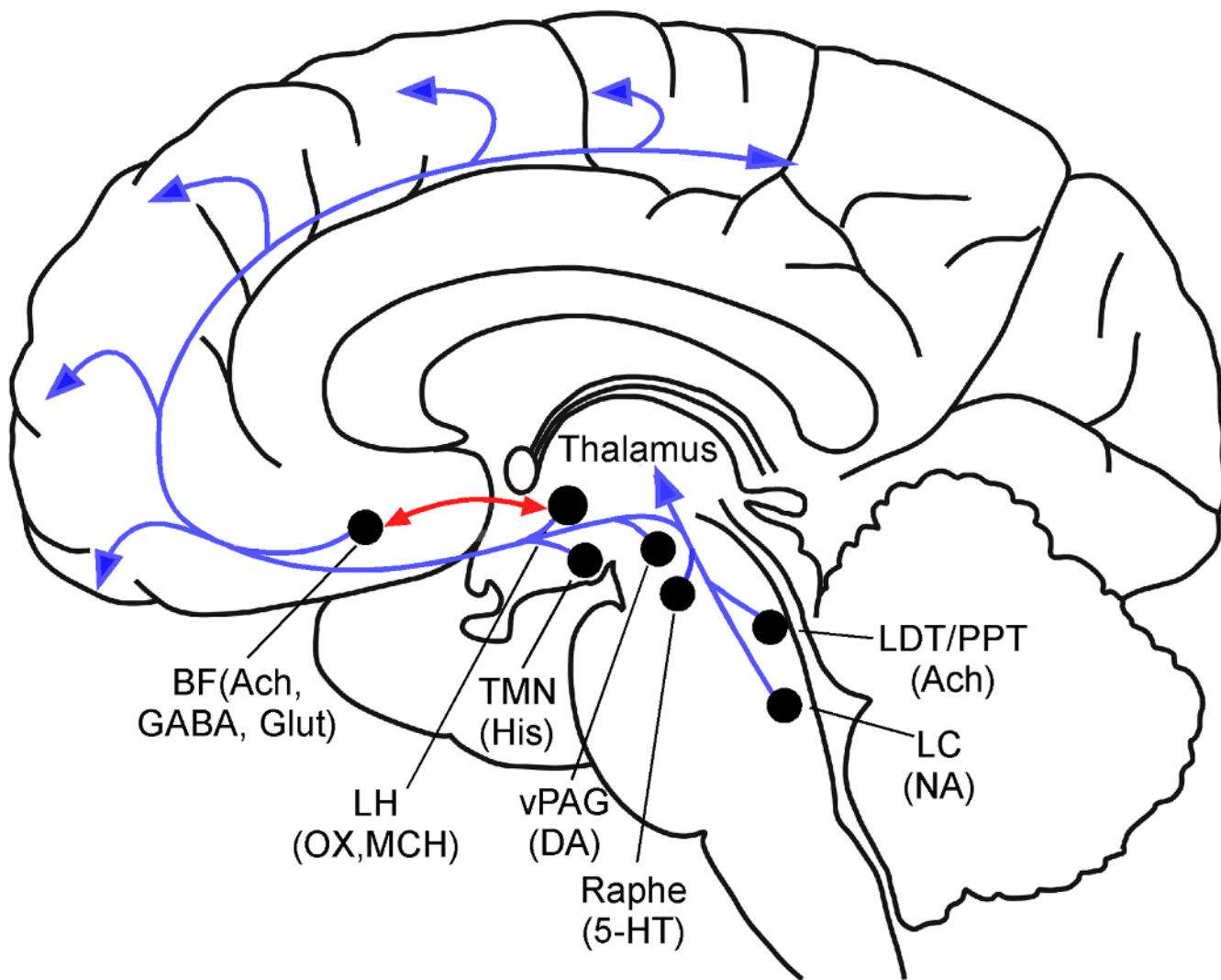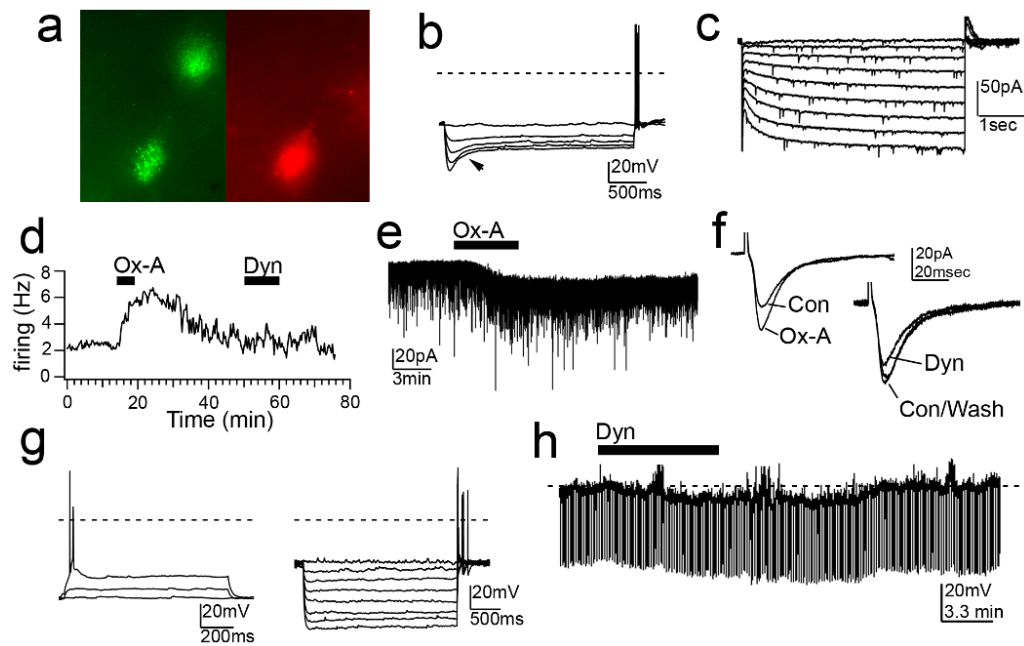
Figure 1.
The ascending arousal systems are diffusely projecting neurons (blue) that use acetylcholine, monoamines, or neuropeptides to produce broad changes in neuronal activity. The pedunculopontine (PPT) and laterodorsal tegmental (LDT) nuclei are the major cholinergic inputs to the thalamus. The 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 gray matter (vPAG) which produces dopamine (DA), and the tuberomammillary nucleus (TMN) which produces histamine. In addition, peptidergic neurons in the lateral hypothalamus (LH) produce orexins and melanin-concentrating hormone (MCH). All these regions innervate the BF, and BF neurons send descending projections back to the lateral hypothalamus (red), thalamus, and brainstem.
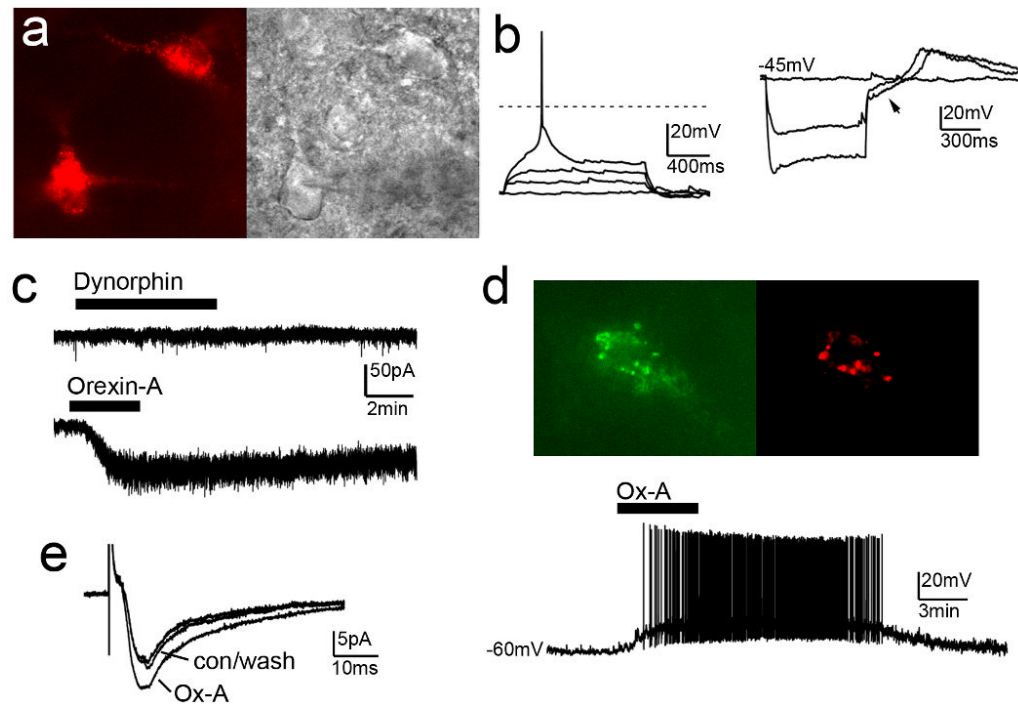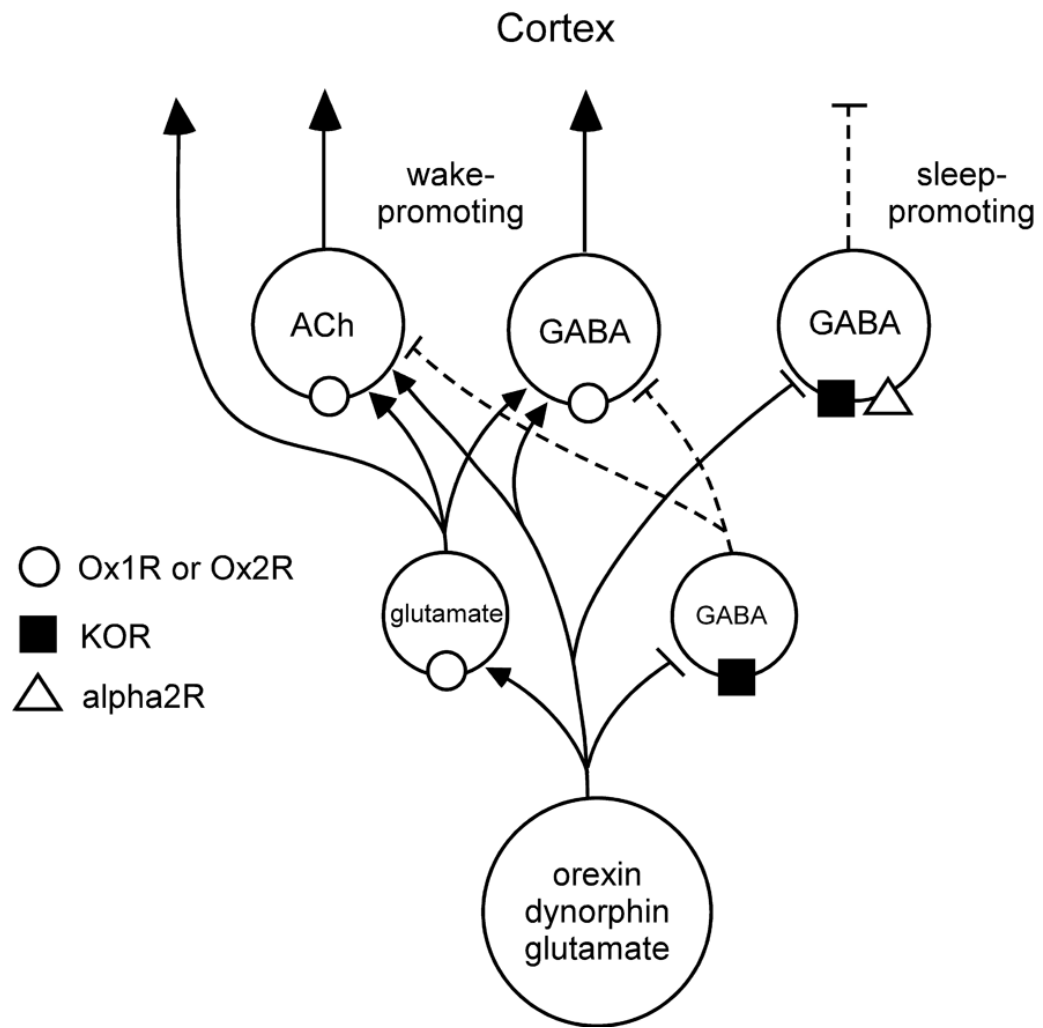
Figure 2.
Cholinergic neurons of the MCPO and SI are excited by orexin-A but do not respond to dynorphin. (**a**) Two SI cholinergic neurons labeled with Cy3-p75-IgG (left) and the same neurons under infrared differential interference contrast (IR-DIC) visualization. (**b**) Firing properties of MCPO/SI neurons during depolarizing (left) and hyperpolarizing current pulses (in 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 neurons do not respond to dynorphin (10 μM), but orexin-A (300 nM) activates an inward current (Vh = -60 mV). (**d**) A SI cholinergic neuron that projects to the mPFC is double labeled 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 EPSCs evoked by local electrical stimulation (Vh =-60 mV)

Figure 3.
Non-cholinergic, cortically-projecting neurons in the MCPO and SI have two types of responses to orexin-A and dynorphin. (**a**) Two SI neurons retrogradely labeled with green fluorescent beads from mPFC. The lower cell is also labeled with red Cy3-p75-IgG, a marker for the BF cholinergic neurons; the upper cell is a non-cholinergic. (**b**) A subset of these neurons have pronounced depolarizing sags during negative current pulses (arrowhead) due to the activation of Ih. (**c**) Ih recorded in voltage clamp mode (Vh = -50mV; -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 (Vh = -60 mV). (**f**) Evoked EPSCs are potentiated by orexin-A and inhibited by dynorphin (Vh = -60 mV). (**g**) A second subset of non-cholinergic cortically-projecting neurons in the MCPO/SI have burst discharges, no Ih, and no IK$_A$. (**h**) These type of neuron is inhibited by dynorphin (dotted line = -60 mV).

Figure 4.
Pathways through which the orexin neurons may activate the BF to promote wakefulness. Orexins excite wake-promoting cholinergic and non-cholinergic neurons (most of which probably contain GABA). Orexins also enhance release of glutamate in the BF. In contrast, dynorphin released from the orexin neurons acts through κ opiate receptors (KOR) to inhibit sleep-active cells, including GABAergic interneurons. 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.