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REVIEW
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# Basal Forebrain Cholinergic System and Orexin Neurons: Effects on Attention

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The basal forebrain (BF) cholinergic system has an important role in attentive functions. The cholinergic system can be activated by different inputs, and in particular, by orexin neurons, whose cell bodies are located within the postero-lateral hypothalamus. Recently the orexin-producing neurons have been proved to promote arousal and attention through their projections to the BF. The aim of this review article is to summarize the evidence showing that the orexin system contributes to attentional processing by an increase in cortical acetylcholine release and in cortical neurons activity.

**Keywords: attention, orexin, basal forebrain, lateral hypothalamus, acetylcholine**

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## INTRODUCTION

Attention may be defined as the behavioral and cognitive process that allows us to select the information present in our environment on the basis of their relevance along with the ability to ignore irrelevant stimuli (Sarter et al., 2001). It consists of several components such as sustained, selective and divided attention, which are responsible for the control of the flow of information in the cognitive system (Rieger et al., 2003). Attention involves both top-down processes (knowledge-driven mechanisms) and bottom-up processes (mechanisms driven mainly by the characteristics of the target stimulus and its sensory context; Sarter et al., 2001; Chieffi et al., 2004, 2012). These two processes drive the attentive focus control (Gazzaniga et al., 2002). Attentional processing comprises some generalized states of arousal which refers to the state of physiological reactivity ranging from sleep to excitement or panic (Coull, 1998; Fadel and Burk, 2010).

Changes in arousal typically are deduced from brain activity data (EEG), whereas the study of attention is based on behavioral studies. Moruzzi and Magoun (1949) first demonstrated that cerebral activation is related to changes in EEG waves and has a brainstem origin. The discovery and localization of the brainstem reticular arousal system (RAS) was subsequently made by Moruzzi and Magoun (1949). The more evident arousal effect on EEG activity is the "desynchronization" phenomenon. It refers to the rapid shift from high-amplitude low-frequency EEG activity, typical of sleep, to low-amplitude high-frequency electroencephalographic activity, typical of wakefulness. EEG was the earliest measure used to systematically examine human brain cortical activity. After a long period of decline in clinical interest, EEG is now

attracting increasing scientific and clinical interest. This resurgence is due to ongoing advances in signal processing and visualization that increase the spatial resolution of EEG imaging and exploit its ability to image quick transient cortical events and more precise regional changes in cortical activity. For the last few decades, scalp channel EEG data have been analyzed principally either in the time domain via ERP trial averaging, or in the frequency domain using FFT that estimate spectral power within a given frequency. Although phenomena and definitions may vary, EEG spectral power variations are typically dominated by distinct changes in power in few frequency bands. The standard terminology for these bands is: delta (<4 Hz), theta (4–7 Hz), alpha (8–12 Hz), beta (13–25 Hz; often split into beta-1/sensorimotor rhythm (SMR), 13–16 Hz, and beta-2, 17–25 Hz), and gamma (25–50 Hz or even higher frequency broadband activity extending to 200 Hz or greater). Cerebral activation is also detected during rapid eye movement (REM) sleep. Experimental evidence suggest that arousal systems work differently during the wake state and the REM sleep (Krueger et al., 2016). Arousal effects arise from the stimulation of the mesopontine cholinergic nuclei (Montplaisir, 1975; Jones and Webster, 1988) and the locus coeruleus (LC; Steriade and McCarley, 1990), which consist principally of noradrenergic neurons. Conversely, during REM the monoaminergic (noradrenergic and serotoninergic) neurons are silent (Hobson et al., 1975; McGinty and Harper, 1976). It is possible to distinguish brain mechanisms involved in attention from arousal, thanks to changes in task performance following manipulations known to affect attention (De Gangi and Porges, 1990; Schiff and Plum, 2000; Fadel and Burk, 2010). In this case, the interpretation of data resulting from these manipulations is primarily based on behavioral performance data (e.g., detection rates, false alarm rates, etc.) (Sarter and Bruno, 1999; Sarter et al., 2001). The relationship between arousal and attention is not simple. Attentional performance improves with a moderate increase of arousal but drops dramatically during high excitement state (Easterbrook, 1959). On the other hand, sustained attention reduces arousal and induces drowsiness (Babkoff et al., 1991).

Furthermore, physiological studies and data collected on patients suffering from injuries or neurological diseases provide a wealth of information on the neural mechanisms of attention processes. Thus, it is important to determine the brain networks mediating attention both to understand the neural mechanisms underlying these cognitive functions to expand knowledge on neurodevelopmental disorders characterized by impairments in attentional functions (Sarter et al., 2001; Esposito and Carotenuto, 2010, 2014; Carotenuto et al., 2016). Among these networks, the basal forebrain (BF) cholinergic system is considered as a major component of top-down processes in the mediation of attention, it is known to play a role in several aspects of attentional function (Fadel and Burk, 2010; Viggiano et al., 2014) and to be necessary for normal attentional performance (Sarter et al., 2001; Boschen et al., 2009). This system can be activated by different afferent inputs and can influence how attentional resources are allocated (Chieffi et al., 2009; Fadel and Burk, 2010). Among the various afferent inputs to the BF cholinergic projection system, the hypothalamus represents an important source of projections. The available data demonstrate that orexin neurons, whose cell bodies are present in the lateral hypothalamus, contribute substantially to these projections (Cullinan and Záborszky, 1991). Orexin neurons have widespread projections to a number of brain regions, including cholinergic BF structures. In the last decade, several studies have focused on specific neuronal pathways through which the orexin-producing neurons may promote not only arousal, but also attention. Their results suggest that the basal forebrain may be a key site through which these neurons act. In this article, we review the effects of orexin-producing neurons and their projection to the BF to support the hypothesis that orexin system may contribute to attentional processing through increased cortical-acetylcholine (Ach) release.

## THE CHOLINERGIC BASAL FOREBRAIN SYSTEM

In the BF, cholinergic neurons are codistributed with several other cell populations, including GABAergic and various neurons containing calcium binding protein for example calbindin, calretinin or parvalbumin (Fadel and Burk, 2010). These neurons project to all areas and layers of the cortex (Sarter and Bruno, 1997). The cholinergic projections modulate the response of pyramidal cells to other cortical-glutamatergic inputs (McCormick, 1993), facilitating the bottom-up sensory information processing within the cortex (**Figure 1**; Muir et al., 1994; Sarter et al., 2001). Furthermore, the long radiating dendrites of the cholinergic BF neurons receive inputs from all the brainstem and hypothalamic arousal systems, for example cholinergic ponto-mesencephalic neurons, noradrenergic LC neurons, dopaminergic ventral-mesencephalic neurons, histaminergic tubero-mammillary neurons and orexinergic perifornical neurons (Jones and Cuello, 1989; Panula et al., 1989; Zaborszky and Cullinan, 1996; Peyron et al., 1998; Semba et al., 1998).

The cholinergic basal forebrain neurons have been implicated in mechanisms of synaptic plasticity, learning, memory, arousal and attention (McCormick, 1993; Leanza et al., 1996); all these functions are related to cortical activation (Jones, 2003). For instance, pharmacological manipulations of cholinergic receptors in extra-striate occipital and superior-medial parietal cortices affect attentional performance (Bentley et al., 2004), and lesions of the BF in monkeys also interfere with attention (Voytko et al., 1994).

The cholinergic basal forebrain neurons are hyperpolarized by ACh released by brainstem or forebrain neurons; both muscarinic and nicotinic receptors are involved in this effect, and could modulate the cortical and forebrain activity during particular states across the sleep–waking cycle (McCormick, 1993; Khateb et al., 1997). In the cerebral cortex and in the hippocampus, Ach release is maximal during wakefulness and REM sleep (Jasper and Tessier, 1971; Marrosu et al., 1995), while it decreases during non-REM sleep (Arrigoni et al., 2010).

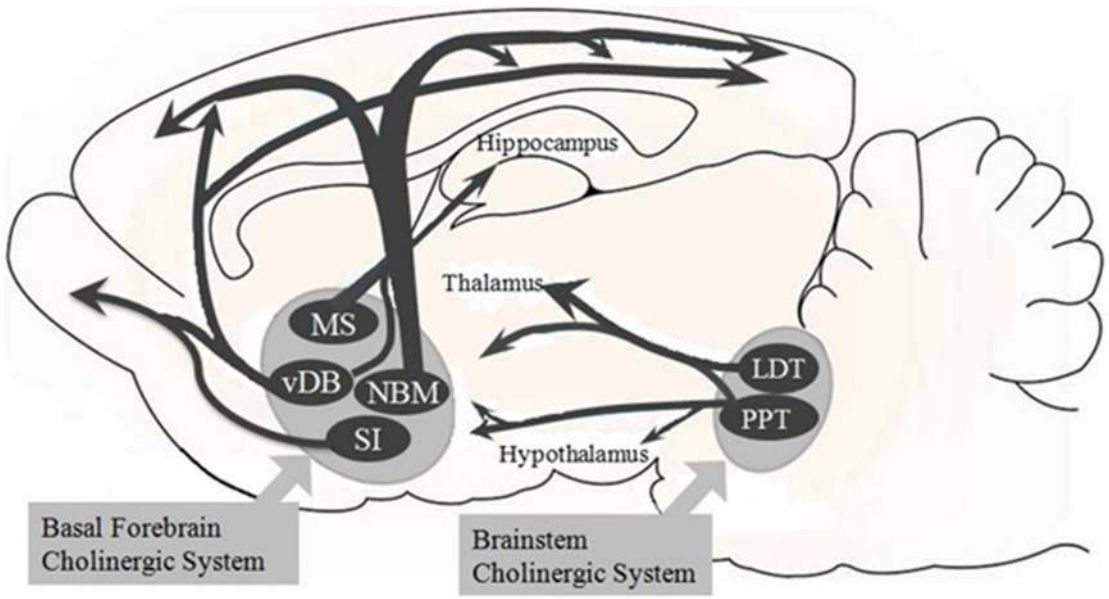


**FIGURE 1 | Overview of the basal forebrain (BF) cholinergic pathway.** The BF cholinergic system of the Sprague-Dawley rats includes the medial septum (MS), vertical limbs of the diagonal band of Broca (vDB), nucleus basalis of Meynert (NBM), and substantia innominate (SI). The vDB and NBM have diffuse projections to all parts of the neocortex and to basolateral amygdala and olfactory bulb (these latter two are not shown here). The MS and vDB project to hippocampus. Besides, the brainstem cholinergic system projects to the thalamus and hypothalamus but also to the BF region. This system includes the pedunculopontine tegmental nucleus (PPT) and laterodorsal pontine tegmentum (LDT).

Inglis et al. (1994) suggested that the BF neurons may be involved, together with dopaminergic neurons, in the regulation of attention and in rewarding activities including food intake because high amount of Ach is released during eating. Many other neurotransmitters can excite the BF neurons, for example glutamate (Khateb et al., 1995a), noradrenaline (NA; Fort et al., 1995), histamine (Khateb et al., 1995a,b), orexin (Eggermann et al., 2001), or can inhibit them for example serotonin (Khateb et al., 1993).

## OVERVIEW OF THE OREXIN NEURONS

The orexin/hypocretins are neuropeptides synthesized by a cluster of neurons within the postero-lateral hypothalamus that produce excitatory effects on target neurons. Two independent research groups discovered simultaneously these neuropeptides in the late 1990s. One group named these peptides orexins, from the Greek word "orexis", meaning "appetite", because they seemed to be involved in the control of feeding and metabolism (Sakurai et al., 1998; Sakurai, 2007). The other group named these peptides hypocretins, because these peptides share significant sequence homology with the members of the glucagon/vasoactive intestinal polypeptide/secretin (incretin) family (de Lecea et al., 1998). Therefore, as hypocretin, de Lecea et al. (1998), intended to indicate a hypothalamic member of the incretin family. However, the terms are interchangeable in the literature. Orexin-A (orexin-A/hypocretin-1, Orx-A) and orexin-B (orexin-B/hypocretin-2, Orx -B) are cleaved from a single gene product, prepro-orexin (Sakurai et al., 1998). Orexins act on two different G-protein coupled receptors: orexin 1 receptor (Orx1R), which binds selectively Orx A, and orexin 2 receptor (Orx2R), which binds both Orx-A and Orx-B with equal affinity (Sakurai et al., 1998; Sakurai, 2007). Orexin neurons also release other neurotransmitters, such as glutamate on histamine tubero-mammillary neurons (critical for the maintenance of arousal), the inhibitory neuropeptide dynorphin (which also modulate appetite) and pentraxin (regulator of AMPA receptors clustering; Chou et al., 2001; Reti et al., 2002; Schone et al., 2012). The orexin neurons may integrate a variety of interoceptive and homeostatic signals related to environmental, physiological and emotional stimuli to promote wakefulness and behavioral arousal in response to emotions, stress, hunger and circadian rhythms (Yoshida et al., 2006; Viggiano et al., 2009). Furthermore several brain regions involved in the central regulation of autonomic and endocrine processes or attention are targets of extensive orexin projections (Horvath et al., 1999; Chieffi et al., 2014a,b). Neurons containing the neuropeptide orexin send axons to numerous regions, throughout the central nervous system; their projections are widely distributed in the brain (Chemelli et al., 1999). These neurons innervate all brain regions known to promote wakefulness and arousal (Saper et al., 2005) including the cerebral cortex, BF, tubero-mammillary nucleus (TMN), LC, and dorsal raphe (DR; Peyron et al., 1998; Yoshida et al., 2006). Furthermore, they innervate brain nuclei that regulate motivation and emotions (Sakurai and Mieda, 2011; Thompson and Borgland, 2011; Di Bernardo et al., 2014), and brain regions that regulate motor

and autonomic functions (Nattie and Li, 2012). Thus, the orexin system is anatomically well positioned to coordinate many aspects of arousal and attention (Alexandre et al., 2013). Indeed, the orexin neurons are important in regulation of sleep/wakefulness states and lack of the peptide or the receptor caused narcolepsy in humans, dogs and mice (Chemelli et al., 1999; Lin et al., 1999; Thannickal et al., 2000).

## OREXIN AND ATTENTION

In attention regulation, orexins play a significant role likely via interactions with multiple ascending neuromodulatory systems, including dopamine neurons in the ventral midbrain (Vittoz and Berridge, 2006), noradrenergic neurons in the LC (Horvath et al., 1999; Espana et al., 2005) and the basal forebrain cholinergic system (Fadel and Burk, 2010). In the BF, orexin peptides increase cell activity and Ach release, thus they modulate attentional mechanisms (Fadel and Burk, 2010). Attentional deficits present in neurodegenerative conditions such as Alzheimer's disease, schizophrenia, drug addiction, and age-related cognitive decline may be related with alterations in the interactions between orexin neurons and cortical ACh neurons (Fadel and Burk, 2010). An imbalance in orexin regulation may also be involved in the pediatric Attention Deficit Hyperactivity Disorder syndrome (ADHD), comprising cognitive alterations, and in narcoleptic and/or obese and/or migrainous subjects as summarized in the Prader-Willi syndrome (Cortese et al., 2008; Carotenuto et al., 2009; Verrotti et al., 2013, 2015a,b; Morandi et al., 2015; Miano et al., 2016). Orexin neurons activity varies with the degree of arousal and is linked to heightened attentional states. Their activity promotes arousal, with maximal discharge during active wakefulness (Lee et al., 2005; Mileykovskiy et al., 2005; Viggiano et al., 2010), while their discharge decreases during quiet waking, in the absence of movement, and are silent in slow wave sleep and tonic periods of REM sleep, with occasional burst of activity during REM sleep (Lee et al., 2005; Mileykovskiy et al., 2005). In addition to arousal, orexins promote eating and are likely to have a role in physiological functions such as regulation of blood pressure, the neuroendocrine system, body temperature, and energy homeostasis (Peyron et al., 1998; Hara et al., 2001; Jones, 2003; Messina et al., 2014). Blouin et al. (2013) demonstrated that, in the human brain, Orx-A levels are maximal during positive emotion, social interaction, anger and increase at wake onset, suggesting that these levels are linked to specific emotions and state transitions.

## NETWORK REGULATION OF OREXIN NEURONS

Orexin neurons are controlled by positive and negative feedback mechanisms mediated by the lateral hypothalamus/perifornical area (LH/PFA; **Figure 2**). The Orx2R, Orx-A or Orx-B form a positive-feedback loop which opens nonselective cation channels and depolarizes orexin neurons and modulates presynaptic glutamate release (Yamanaka et al., 2010). Indirectly, glutamatergic transmissions stimulate orexin neurons through glutamate activation of astrocytes that release lactate and protons into the extracellular space through monocarboxylate transporters (MCTs; Pellerin et al., 1998; Burt et al., 2011). Furthermore, to sustain physical activity, orexin neurons metabolize astrocyte-derived lactate as an energy source; moreover, the release of protons due to MCT activity causes a local decrease in extracellular pH that can result in depolarization of orexin neurons (Williams et al., 2007). Even adenosine triphosphate (ATP), released by astrocytes and neurons, has an excitatory effect on orexin neurons through the ionotropic P2X receptors (Wollmann et al., 2005). ATP can be hydrolyzed by ectonucleotidases releasing adenosine in the extracellular space (Wall and Dale, 2008) which inhibits voltage-gated $Ca^{2+}$ currents in orexin neurons leading to their inhibition (Liu and Gao, 2007). Negative feedback pathways have also been identified, for example Dynorphin and Nociceptin/Orphanin FQ (N/OFQ), either co-expressed by orexin neurons (Chou et al., 2001; Maolood and Meister, 2010). Dynorphin attenuates glutamate release acting on presynaptic excitatory terminals, while N/OFQ inhibits both excitatory and inhibitory transmission (Li and van den Pol, 2006). The balance between the excitatory and inhibitory effects determines the activity levels of the postsynaptic cell (Burt et al., 2011). In addition, the glutamate released synaptically creates a negative feedback loop acting on presynaptic autoreceptors to inhibit glutamate and GABA released through group III metabotropic glutamate receptors (mGluRs; Acuna-Goycolea et al., 2004). Even other distinct neuronal populations in the LH/PFA create synaptic contacts with orexin neurons for example neurons expressing melanin concentrating hormone (MCH) and leptin receptor-expressing (LepRb+) neurons. The MCH neurons form reciprocal connections with orexin neurons and are directly depolarized by Orexin A and B which stimulate presynaptic glutamate release, whereas dynorphin and N/OFQ directly induce hyperpolarization of MCH neurons (Li and van den Pol, 2006). MCH can reduce the presynaptic glutamate release induced by orexin receptors to antagonize the excitatory effects on orexin neurons (Rao et al., 2008). Leptin receptor-expressing (LepRb+) neurons are excited by leptin and use GABA as a neurotransmitter (Leinninger et al., 2009). Leptin inhibits orexin neurons through hyperpolarization of these neurons (Yamanaka et al., 2003), because the activation of the LepRb+ neurons produces an inhibition of orexin neurons (Burt et al., 2011). Orexin neurons are also innervated by afferents of non-cholinergic terminals from the BF cholinergic cell area. BF glutamatergic neurons can excite orexin neurons involved in arousal, whereas GABAergic neurons can inhibit orexin neurons promoting behavioral quiescence and sleep (Henny and Jones, 2006). In summary, different peptides released by orexin neurons or distinct populations of LH/PFA neurons modulate orexin neurons and exert different excitatory and inhibitory influences during wake or sleep states. Others studies showed that LC neurons have an important role in waking and REM sleep (REMS; Mallick et al., 2012; Choudhary et al., 2014). Kumar et al. (2012) have constructed a mathematical model of waking, NREMS and REMS, showning the importance

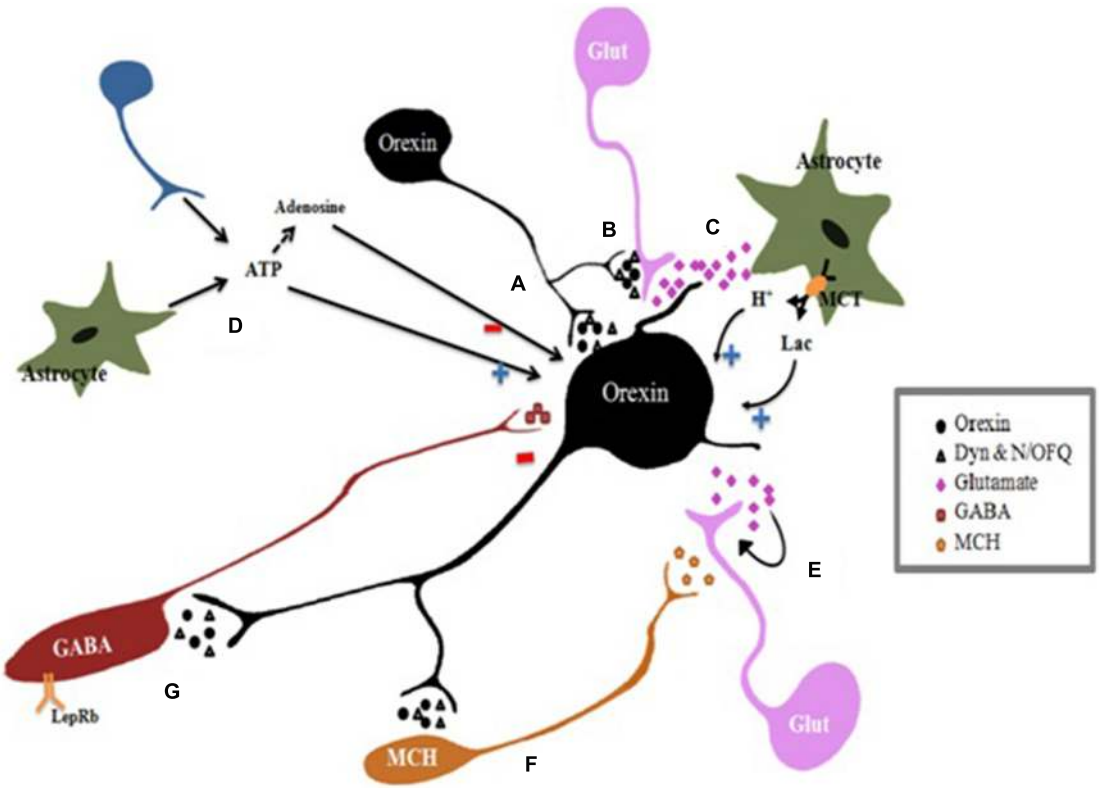


**FIGURE 2 | Regulation of orexin neurons.** Orexin neurons activity is controlled by positive and negative feedback mechanisms mediated by neurotransmitters released by lateral hypothalamus/perifornical area (LH/PFA) neurons. Orexin neurons corelease excitatory neurotransmitters orexin and inhibitory transmitters dynorphin (Dyn) and nociceptin/orphanin FQ (N/OFQ). **(A)** Direct effect: all of neurotransmitters coreleased by Orexin neurons form a feedback which directly affects postsynaptic orexin neurons. **(B)** Synaptic modulation: orexins modulate presynaptic glutamate release at excitatory synapses. Besides, Dyn attenuates glutamate release acting on presynaptic excitatory terminals, while N/OFQ inhibits both excitatory and inhibitory transmission. The balance between the excitatory and inhibitory effects determines the activity levels of the postsynaptic cell. **(C)** Indirect effects: Regulation of orexin neurons by astrocytes: Glutamate activates astrocytes which release lactate (Lac) and protons (H+) into the extracellular space through monocarboxylate transporters (MCTs). Orexin neurons metabolize astrocyte-derived lactate as an energy substrate to sustain activity. Furthermore, extracellular pH decreases due to MCT activity resulting in depolarization of orexin neurons. **(D)** Adenosine triphosphate (ATP) effects: ATP released by astrocytes and neurons, stimulates orexin neurons depolarizing them through the ionotropic P2X receptors. Ectonucleotidases hydrolyze ATP releasing into adenosine in the extracellular space which inhibits orexin neurons. **(E)** Autoinhibition: the glutamate released synaptically creates a negative feedback loop acting on presynaptic autoreceptors to inhibit glutamate release. **(F)** Melanin concentrating hormone (MCH) neurons are directly depolarized by Orexin A and B which stimulate presynaptic glutamate release, whereas dynorphin and N/OFQ induce direct hyperpolarization of MCH neurons. **(G)** Leptin receptor-expressing GABAergic neurons are excited by leptin and use GABA as a neurotransmitter. Leptin inhibits indirectly orexin neurons by activating these inhibitory LepRb+ neurons. In summary, the balance between the excitatory and inhibitory effects determines the activity levels of the orexin neurons. Glut, glutamate; (+), stimulation; (–) inhibition.

of orexinergic neurons in stabilizing the wake-sleep cycle and demonstrating that even small changes in inputs to or from those neurons can have a large impact on the ensuing dynamics. The results from this model help to understand the neural mechanisms of regulation and the patho-physiology of REMS.

## MODULATION OF THE BASAL FOREBRAIN CHOLINERGIC SYSTEM BY OREXIN NEURONS: EFFECTS ON ATTENTION

### Orexin Receptors in the Basal Forebrain

Orexin neurons have widespread projections to the basal forebrain that may promote arousal by activating the cortex. Orexin neurons also project onto BF cholinergic neurons and release orexins in the BF. Both Orx1R and Orx2R are expressed in the BF, and can activate cholinergic afferents (Marcus et al., 2001) and narcoleptic dogs lack OX2R (Lin et al., 1999). However, there are conflicting results from *in vitro* electrophysiological studies and BF orexin administration with regard to what type of orexin receptor subtypes are involved in the activation of cholinergic fibers. *in vitro* electrophysiological data indicate that both Orx-A and Orx-B can excite BF cholinergic cells, and that their effects are primarily Orx2R-mediated (Eggermann et al., 2001; Gotter et al., 2016). On the other hand, other studies suggested that the effects of orexin administration in the BF are primarily Orx1R-mediated (Espana et al., 2001). Using mice lacking orexin receptors, Alexandre et al. (2012) found that focal restoration of Orx1R and Orx2R in the substantia

innominate (SI) partially rescued their ability to produce long bouts of wakefulness. Furthermore, Boschen et al. (2009) have blocked in rats the Orx1Rs through the administration of the Orx1R antagonist SB-334867 prior to a two-lever sustained attention task performance. Their results showed that Orx1R blockade decreased the accuracy in attention-demanding tasks and that some of these effects on attention may be mediated by BF corticopetal neurons. In summary, the two receptors may play different and complementary roles in response to varying types of homeostatic challenges (Fadel and Frederick-Duus, 2008).

## Orexin Activation of the Basal Forebrain

Different *in vitro* studies have tried to understand how orexin neurons activate the BF focusing primarily on the effects of orexins on medial septum (MS) neurons that project to the hippocampus and to the cortically projecting neurons of the BF. In the MS, orexins directly excite septo-hippocampal cholinergic neurons through the activation of the sodium, calcium exchanger and inhibition of potassium channels, presumably an inward rectifier, increasing hippocampal acetylcholine release and promoting arousal (Wu et al., 2004). Orx-A excites BF cholinergic neurons inducing cortical release of acetylcholine and increasing attention (see **Figure 3**; Arrigoni et al., 2010). Cortical acetylcholine levels increase even more under demanding attention tasks (Hasselmo and McGaughy, 2004) and orexin neurons increase firing to promote arousal and during exploratory behaviors in response to salient external stimuli (Mileykovskiy et al., 2005). It is also important to consider that local application of orexins to the BF promotes wakefulness and improves cognitive performance. In fact, the administration of orexins into the BF excites cholinergic neurons that release acetylcholine in the cerebral cortex and thereby promotes wakefulness (Eggermann et al., 2001; Espana et al., 2001; Fadel et al., 2005). Within the prefrontal cortex, orexins can also directly improve attentional processes relevant to executive aspects of attention. Lambe et al. (2005) demonstrated that infusions of Orx-B into the prefrontal cortex improved accuracy under high attentional demand by exciting the same thalamo-cortical synapses that are activated by acetylcholine from the BF. Thus, through an increased cortical acetylcholine release and a direct action on thalamo-cortical projections, orexins may promote cortical activation and attention. Orexin A can also modulate cholinergic neuron activity indirectly, because it increases local glutamate release within the basal forebrain. Indeed, Fadel and Frederick-Duus (2008) demonstrated that the administration of Orx-A in the BF increases local glutamate efflux. Furthermore, via an excitatory autoreceptor mechanism, Orx-A might increase BF glutamate release. Even non-cholinergic neurons of the BF may be excited by orexins, for example most of the GABAergic neurons of the BF (Fadel and Frederick-Duus, 2008; Arrigoni et al., 2010). In fact, orexin excites GABAergic neurons of the MS that project to the hippocampus (Wu et al., 2004) and GABAergic neurons of the magnocellular preoptic nucleus and substantia innominata (MCPO/SI; Blanco-Centurion et al., 2006). Blanco-Centurion et al. (2006) studied the relative contribution of non cholinergic neurons to arousal; they found that after selective lesion of the basal forebrain-cholinergic neurons in the rats., the microinjection of Orx-A into the BF increased waking and still promoted arousal; this finding indicates that cholinergic neurons are not essential for the effects of Orx-A and, thus, suggests that Orx-A acts also on non-cholinergic neurons (Blanco-Centurion et al., 2006). These findings suggest that orexins may contribute to attentional processing in the BF, not excluding, however, that other neural circuits outside the BF may contribute to these effects (Boschen et al., 2009).

## Orexin, Circulating Factors and Basal Forebrain

Orexin neurons are responsive to circulating factors related to metabolic state, for example low plasma glucose, and are activated by food deprivation (Cai et al., 1999). It has been suggested that these neurons can provide a crucial regulation of arousal level in response to signals of energy balance, such as blood glucose, leptin, and food intake (Yamanaka et al., 2003; Esposito et al., 2014; Messina et al., 2014). Furthermore, Frederick-Duus et al. (2007) demonstrated that the administration of the toxin orexin B–saporin (which produces loss of orexin neurons in the LH/PFA) in food-restricted rats lead them to be insensible to the cholinergic response to presentation of palatable food. Moreover, animals pretreated with the Orx1R antagonist, SB-334867, show an increased feeding latency demonstrating that Orx1R activity is required for an appetitive food stimulus to increase cortical Ach released. Fadel and Frederick-Duus suggest that orexins are necessary for the activation of BF cholinergic neurons in response to a food-related stimulus and they are important for biasing the allocation of attentional resources toward cues related to the physiological status (Fadel and Frederick-Duus, 2008).

## Influence of Other Orexin Neuron Neurotransmitters on the Basal Forebrain

To better understand how orexin-producing neurons promote cortical activation, some studies have focused also on the neuropeptide dynorphin, which is synthesized in orexin neurons and have specific effects on different classes of BF neurons. Cholinergic neurons do not respond to dynorphin but are directly excited by Orx-A, but there are two more populations of non-cholinergic BF neurons. One of these populations is excited by Orx-A and do not respond to dynorphin; the other population of non-cholinergic sleep-promoting neurons, is inhibited by dynorphin but do not respond to Orx-A (Arrigoni et al., 2010). Therefore, the co-release of orexins and dynorphin can activate a synergistic mechanism that excites cholinergic and non-cholinergic wake-active neurons and inhibits non-cholinergic sleep-active neurons promoting attention and improving cognitive performance. In addition to dynorphin, orexin neurons also co-release glutamate that acts synergistically to excite BF via presynaptic glutamatergic

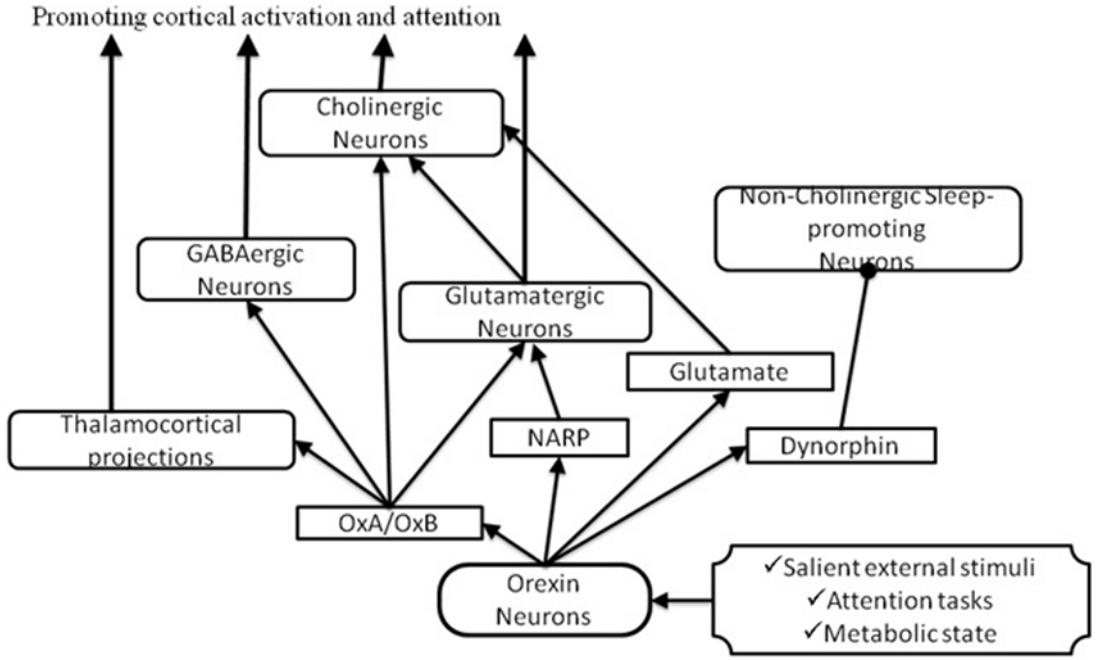


**FIGURE 3 | Pathways through which orexin could activate the BF to promote attention.** In response to salient stimuli, orexin neurons produce several neuropeptides which promote cortical activation and attention by acting on cholinergic and non-cholinergic neurons. Arrows indicate excitatory inputs; dots indicate inhibitory inputs.

mechanisms (Arrigoni et al., 2010; Fadel and Burk, 2010). Orexin neurons express also the neuronal activity-regulated pentraxin (Narp) that is involved in clustering of glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Reti et al., 2002) and may potentiate pre- or post-synaptic responses to glutamate (Arrigoni et al., 2010). Despite the need of more studies to understand the role of dynorphin, glutamate and NARP, targeted deletion of orexin seem to have different functional deficits than those induced by selective ablation of orexin neurons (Chemelli et al., 1999; Hara et al., 2001; Reti et al., 2002) suggesting that other secreted signaling molecules expressed in these neurons are involved in their effects.

## Involvement of the Orexin-Basal Forebrain Interactions in Narcolepsy

Narcolepsy is a disease characterized by excessive daytime sleepiness, sleep paralysis, instability of sleep onset and REM periods, and cataplexy (Weinhold et al., 2014). Reduced orexinergic function, due to a reduction of orexin peptides, or orexin neurons, or orexin receptors, is assumed to be a major cause of narcolepsy, clearly demonstrated by post mortem studies (Arrigoni et al., 2010). Neuropsychological impairments have been found in narcoleptic patients, for example a reduced performance in attention-demanding tasks (Fulda and Schulz, 2001). Some studies suggest that narcoleptic patients show deficits in attention even during normal wakefulness periods (Rieger et al., 2003). Furthermore, BF degeneration is associated with canine narcolepsy suggesting that a postsynaptic degeneration and, in turn, impaired ACh-dependent cognitive function may be caused by a deficit in orexin stimulation (Siegel et al., 1999; Fadel and Frederick-Duus, 2008; Monda et al., 2014). In human narcolepsy there are deficits in selective processing of relevant stimuli. It is possible that these deficits are due to a reduction in BF cholinergic signaling to the cortex (Fadel and Burk, 2010). Weinhold et al. (2014) have investigated the effect of intranasal administration of Orx-A in narcoleptic patients with cataplexy on sleep behavior and cognitive functions. In the test of divided attention these patients showed enhanced performances after orexin-A administration, as indicated by their mean reaction time and fewer false reactions. Their results confirmed the role of Orx-A as a REM sleep stabilizing factor and provided functional evidence for the Orx-A effects on attention in narcolepsy with cataplexy (Weinhold et al., 2014). Other studies demonstrated that the intranasal administration of Orx-A in sleep-deprived rhesus monkey is able to relieve the cognitive deficits produced by the loss of sleep (Deadwyler et al., 2007). Moreover, it has been suggested that nasal Orx-A administration may be an effective approach to the treatment of orexin deficiency in narcolepsy (Peyron et al., 2000; Thannickal et al., 2000).

## CONCLUSION

Collectively, the available data strongly support the hypothesis that orexin stimulation of the BF is able to promote

cortical activation and attention by acting on cholinergic and non-cholinergic neurons in response to salient stimuli. In fact, orexins excite cholinergic neurons, thus the increase in acetylcholine release within the cerebral cortex contributes to the cortical activation associated with attention. We have reviewed evidence suggesting that the BF may be a key target through which orexin neurons promote attention, even if many questions remain to be answered. Defining if orexin signaling in the BF is sufficient to maintain attention and the interaction between orexin and dynorphin within the BF should provide novel data to explain the role of orexin in several aspects of attention.

## AUTHOR CONTRIBUTIONS

IV, AM, MC and AVa carried out the study; AVi, FM, TE, VM, ME and FP participated in the design of the study; SC, GC, MM and GM participated in the design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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