# *Modulation of Cortical Activation and Behavioral Arousal by Cholinergic and Orexinergic Systems*

**BARBARA E. JONES**

*Department of Neurology and Neurosurgery, McGill University, Montreal Neurological Institute, Montreal, Quebec, Canada*

**Multiple neuronal systems contribute to the promotion and maintenance of the wake state, which is characterized by cortical activation and behavioral arousal. Using predominantly glutamate as a neurotransmitter, neurons within the reticular formation of the brainstem give rise to either ascending projections into the forebrain or descending projections into the spinal cord to promote through relays fast cortical activity or motor activity with postural muscle tone. Using acetylcholine, cholinergic neurons in the brainstem project to forebrain relays and others in the basal forebrain to the cortex, by which they stimulate fast gamma activity during waking and during rapid eye movement or paradoxical sleep (PS). Other neuromodulatory systems, such as noradrenergic locus coeruleus neurons, give rise to highly diffuse projections through brain and spinal cord and simultaneously stimulate cortical activation and behavioral arousal. Although such neuromodulatory systems were thought to be redundant, a recently discovered peptide called orexin (Orx) or hypocretin, contained in diffusely projecting neurons of the hypothalamus, was found to be essential for the maintenance of waking with muscle tone, since in its absence narcolepsy with cataplexy occurred. Orx neurons discharge during active waking and cease firing during sleep. Since cholinergic neurons discharge during waking and PS, they would stimulate cortical activation in association with muscle tone when orexinergic neurons are also active but would stimulate cortical activation with muscle atonia when orexinergic neurons are silent, as in natural PS, or absent, as in pathological narcolepsy with cataplexy.**

*Key words:* **acetylcholine; orexin; hypocretin; noradrenaline; sleep–wake states**

## Introduction

An alert, active, and responsive awake state depends upon influences ascending from the brainstem to the cerebral cortex to stimulate cortical activation and descending from the brainstem to the spinal cord to stimulate behavioral arousal with muscle tone (FIG. 1). These ascending and descending influences arise predominantly from neurons of the reticular formation, which is known to be critical for the maintenance of a waking state.[1,2] Comprising some 100,000 neurons, the reticular formation contains a minor proportion of GABAergic cells, which are primarily local projection neurons,[3–5] together with presumed glutamatergic neurons that likely comprise most of the long-projection neurons.[6,7] Originating predominantly from neurons in the rostral pons and midbrain, the projections into the forebrain go mainly to subcortical relay stations, first along a dorsal pathway to the nuclei of the nonspecific thalamocortical projection system and second along a ventral pathway to the lateral hypothalamus and basal forebrain where other neurons in turn project to the cerebral cortex.[8] Originating predominantly from neurons of the caudal pons and medulla, the projections to the spinal cord go mainly to the intermediate zone where other neurons in turn project to sensory or motor neurons. The presumed glutamatergic projection neurons provide tonic or phasic inputs to the forebrain and spinal cord, which serve to facilitate cortical activation or motor activity.[9,10] Many GABAergic locally projecting and spinally projecting neurons appear to be active during sleep, particularly paradoxical sleep (PS), and could be responsible for the motor disfacilitation and inhibition underlying muscle atonia (FIG. 1).[11,12]

In addition to the glutamatergic and GABAergic neurons of the reticular formation, other neurons containing modulatory neurotransmitters are distributed

Address for correspondence: Barbara E. Jones, Montreal Neurological Institute, 3801 University Street, Montreal, Quebec, Canada, H3A 2B4. Voice: +1-514-398-1913; fax: +1-514-398-5871.
barbara.jones@mcgill.ca

Ann. N.Y. Acad. Sci. 1129: 26–34 (2008). 
doi: 10.1196/annals.1417.026

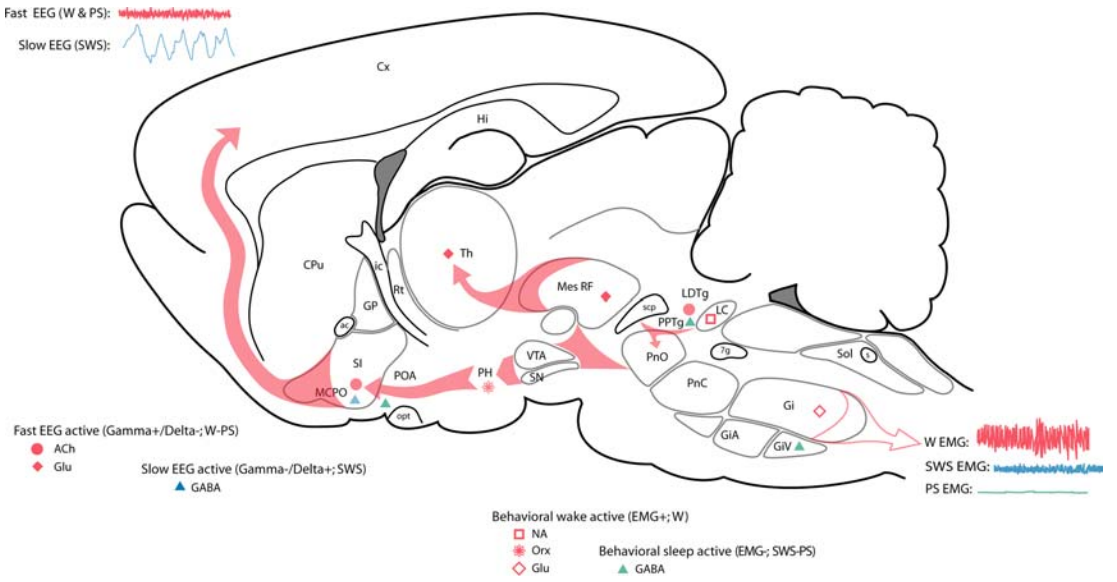


**FIGURE 1.** Cholinergic, orexinergic, and other neurons involved in sleep–wake state control. Sagittal schematic view of the rat brain depicting neurons with their chemical neurotransmitters and pathways by which they influence cortical activity or behavior across the sleep–wake cycle. Wake (W) is characterized by fast gamma activity on the cortical electroencephalogram (EEG) (upper left) and high postural muscle tone on the neck electromyogram (EMG) (lower right); slow wave sleep (SWS) by slow delta EEG and low tone on the EMG; and paradoxical sleep (PS) by fast gamma EEG and atonia on the EMG. Neurons that are active during waking (*red symbols*) include cells with ascending projections toward the cortex, which stimulate fast cortical activity, and cells with descending projections toward the spinal cord, which stimulate postural muscle tone and behavioral arousal. The cholinergic (ACh) neurons and other glutamatergic (Glu) neurons, which have predominantly ascending projections (*red filled arrows*), discharge in association with fast EEG activity (gamma+) and cease firing with delta activity (delta−) to be active during W and PS (W-PS, *filled red symbols*). The orexinergic (Orx), like noradrenergic (NA) locus coeruleus (LC) neurons, give rise to diffuse projections and, together with some putative glutamatergic (Glu) neurons, which have descending projections (*red open arrows*), discharge in association with behavioral arousal and EMG activity (EMG+) and cease firing with muscle atonia to be active during W and silent during PS (W, *empty red symbols*). When orexinergic, along with noradrenergic and other neurons, are silent, cholinergic neurons can promote PS with muscle atonia. Neurons that discharge with slow wave activity (delta+) and during SWS include GABAergic neurons (*blue triangle*) in the basal forebrain. Other GABAergic neurons in the forebrain and brainstem are active during behavioral sleep, including SWS and PS (SWS-PS) and in a manner negatively correlated with EMG (EMG-) such that they could diminish behavioral arousal and muscle tone. Abbreviations: 7g, genu 7th nerve; ac, anterior commissure; ACh, acetylcholine; CPu, caudate putamen; Cx, cortex; EEG, electroencephalogram; EMG, electromyogram; Gi, gigantocellular RF; GiA, gigantocellular, alpha part RF; GiV, gigantocellular, ventral part RF; Glu, glutamate; GP, globus pallidus; Hi, hippocampus; ic, internal capsule; LC, locus coeruleus nucleus; LDTg, laterodorsal tegmental nucleus; Mes RF, mesencephalic RF; MCPO, magnocellular preoptic nucleus; NA, noradrenaline; opt, optic tract; Orx, orexin; PH, posterior hypothalamus; PnC, pontine, caudal part RF; PnO, pontine, oral part RF; POA, preoptic area; PPTg, pedunculopontine tegmental nucleus; PS, paradoxical sleep (also called REM sleep); RF, reticular formation; Rt, reticularis nucleus of the thalamus; s, solitary tract; scp, superior cerebellar peduncle; SI, substantia innominata; SN, substantia nigra; Sol, solitary tract nucleus; SWS, slow wave sleep; Th, thalamus; VTA, ventral tegmental area; W, wake. (Modified with permission from Jones, 2005.[28])

in clusters within the brainstem and project in parallel with the reticular neurons to influence forebrain and/or spinal processes.

## Cholinergic Systems Stimulate Cortical Activation

Acetylcholine (ACh) has been known to play a critical role in stimulating cortical activation since very early pharmacological studies.[13] Yet, ACh plays this role in waking, during attentive or active states, and also in rapid eye movement (REM) sleep or PS, during immobility, and during complete muscle atonia. Cholinergic neurons can thus stimulate cortical activation irrespective of behavioral arousal or motor activity and muscle tone. Indeed ACh in the brainstem likely even promotes muscle atonia along with other processes of PS, as evident from the elicitation of these by

injections of the cholinergic agonist carbachol into the pontine tegmentum.[14–17] In the brainstem, cholinergic neurons are distributed within the medullary reticular formation from where brainstem and descending projections emerge and within the pontomesencephalic tegmentum in the laterodorsal and pedunculopontine tegmental nuclei (LDTg and PPTg) from where brainstem and ascending projections arise (FIG. 1).[18–21] The LDTg and PPTg cholinergic neurons project in parallel with reticular neurons particularly along the dorsal pathway to the thalamus y*et al*so to a lesser extent along the ventral pathway into the lateral hypothalamus and basal forebrain. The basal forebrain relay to the cerebral cortex is comprised importantly of cholinergic neurons located in the magnocellular preoptic (MCPO) nucleus (in the rat) and substantia innominata, as well as the medial septum and diagonal band of Broca more rostrally and medially.[22] Although this relay is composed by other, including GABAergic, projections,[23,24] the cholinergic component plays a key role in stimulating fast activity, particularly gamma activity (30–60 Hz), in the cerebral cortex.[13,25] In single-unit recording studies, identified cholinergic basal forebrain neurons were found to discharge at their highest rates in association with gamma activity during attentive or active Wake (W) and during PS (FIG. 2).[26] They virtually cease discharge during slow wave sleep (SWS), such that their discharge is positively correlated with gamma activity and negatively correlated with delta activity across the sleep–waking cycle (gamma+/delta−, W-PS; FIGS. 1 and 2). Their pattern of discharge is also unique, manifesting rhythmic bursting during cortical activation that is cross-correlated with, and thus potentially involved in, modulating rhythmic theta activity in the cerebral cortex, including medial prefrontal and retrosplenial (or posterior cingulate) cortex (FIG. 2). The cholinergic basal forebrain neurons can thus stimulate gamma with theta activity and associated heightened attention or sensory-motor processing. Such processes can occur in absence of behavioral arousal or movement and do occur during PS with muscle atonia, as what we know as dreams.

Cholinergic neurons lie intermingled with a larger population of GABAergic neurons in the basal forebrain. Some of these have a reciprocal profile of discharge to the cholinergic neurons, discharging maximally in association with slow waves and minimally in association with fast cortical activity (gamma−/delta+, SWS; FIG. 1).[27–29] Moreover, such slow wave- and slow wave sleep-active cells were found to bear α2-adrenergic receptors (ARs),[30,31] indicating that they would be hyperpolarized and inhibited by noradrenaline (NA) in contrast to the cholinergic cells, which are depolarized and excited by NA through α1-ARs.[32,33] Thus, W-PS-active cholinergic neurons would be recruited by other activating systems, including the noradrenergic arising from locus coeruleus (LC) neurons, whereas the SWS-active, GABAergic, basal forebrain neurons would be inhibited by these.

## Noradrenergic and Other Diffuse Neuromodulatory Systems Stimulate Arousal

The LC noradrenergic neurons differ from neurons of the reticular formation and from the cholinergic neurons of the brainstem and basal forebrain in that they give rise to highly diffuse projections ascending into the forebrain where they go to the subcortical relay stations and continue directly up to the cerebral cortex and descending into the spinal cord where they go to the intermediate zone but also more directly onto sensory relay, somatic, and visceral motor neurons (FIG. 1).[8] NA has either excitatory or inhibitory actions upon postsynaptic neurons, depending upon the receptors (above), and has the capacity to directly excite the thalamo–cortical relay neurons and the cholinergic basalo–cortical neurons, as well as cortical pyramidal neurons through α1-ARs,[33,34] while inhibiting sleep-promoting neurons through α2-ARs.[31] NA also excites motor neurons.[35] Thus, when LC neurons discharge, they can simultaneously stimulate cortical activation and behavioral arousal with muscle tone. In contrast to the cholinergic neurons, LC noradrenergic neurons discharge selectively during waking and cease firing during sleep to be completely off during PS with muscle atonia.[36,37] They are likely inhibited by local GABAergic neurons during sleep (FIG. 1).[38–40] According to their projections and activity, the noradrenergic LC neurons were accordingly once thought to represent an ideal substrate for an arousal system that would control the waking state. Yet, massive destruction of the noradrenergic neurons of the LC in the cat[41] or more recently rat[42] did not result in a comatose state, as large lesions of the reticular formation did, or even a reduction in amounts of waking measured by cortical activation or behavioral arousal. It thus appeared that arousal systems, which also include histaminergic and possibly serotonergic neurons, are highly redundant and that no one system is necessary for the maintenance of a waking state.[43]

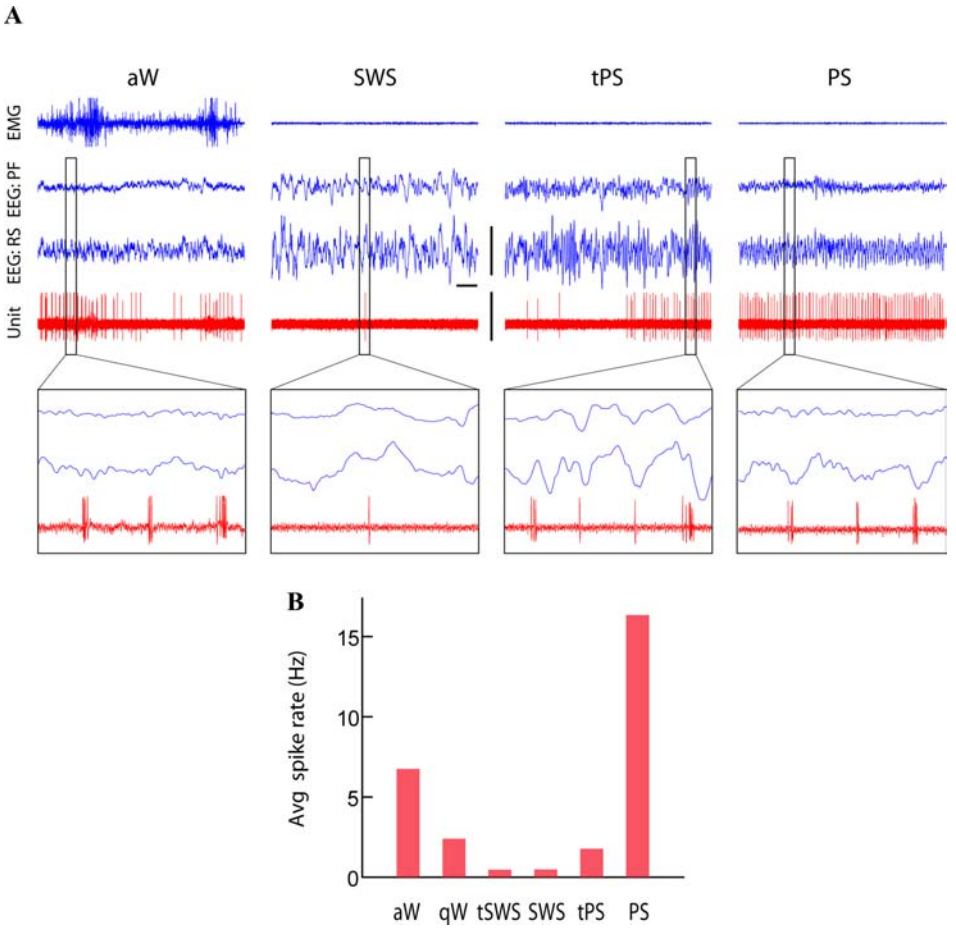


**FIGURE 2.** Cholinergic basal forebrain unit. Discharge of a cholinergic basal forebrain neuron across sleep–wake states. (**A**). Record of a neuron labeled by juxtacellular technique with Neurobiotin (Nb) and identified by immunohistochemistry for choline acetyltransferase (ChAT) as cholinergic in the magnocellular preoptic nucleus (MCPO) of the rat. As evident in 10-s traces (above), the unit fired during active W (aW), virtually ceased firing during SWS, resumed firing during tPS, and discharged maximally during PS. As evident in expanded 0.5-s traces (below), the unit discharged in rhythmic bursts of spikes with theta EEG activity that was present intermittently during periods of aW, toward the end of tPS, and continuously during PS. (**B**). Average discharge rate (Hz) across the sleep–wake states and transitions (t) of the same cell. Abbreviations: Avg, average; aW, active wake; EEG, electroencephalogram; EMG, electromyogram; PF, prefrontal cortex; PS, paradoxical sleep; RS, retrosplenial cortex; SWS, slow wave sleep; tPS, transition to PS; tSWS transition to SWS. Bar for horizontal scale: 1 s. Bar for vertical scales: 1 mV for EEG/EMG and 1.5 mV for Unit. (Reprinted with permission from Lee, Hassani, and Jones, 2005.[26])

## Orexinergic System Stimulates and Sustains Behavioral Arousal

Surprisingly, a newly identified peptide called orexin (Orx) by one group[44] and hypocretin (Hcrt) by another[45] was subsequently discovered to be necessary for the maintenance of waking and behavioral arousal. Indeed, the absence of this peptide, the receptors to the peptide, or the neurons that contain the peptide in the hypothalamus results in the condition of narcolepsy with cataplexy.[46–49] In humans, this disease is characterized by excessive daytime sleepiness, sleep-onset REM sleep, and motor paralysis or loss of muscle tone that can occur with sleep and dreaming or with continued conscious awareness of the environment. Like the noradrenergic LC neurons, orexinergic neurons give rise to highly diffuse projections through the forebrain, including the subcortical relays and entire cerebral cortex, the brainstem, and the spinal cord (FIG. 1).[50] Through these regions, Orx exerts excitatory effects

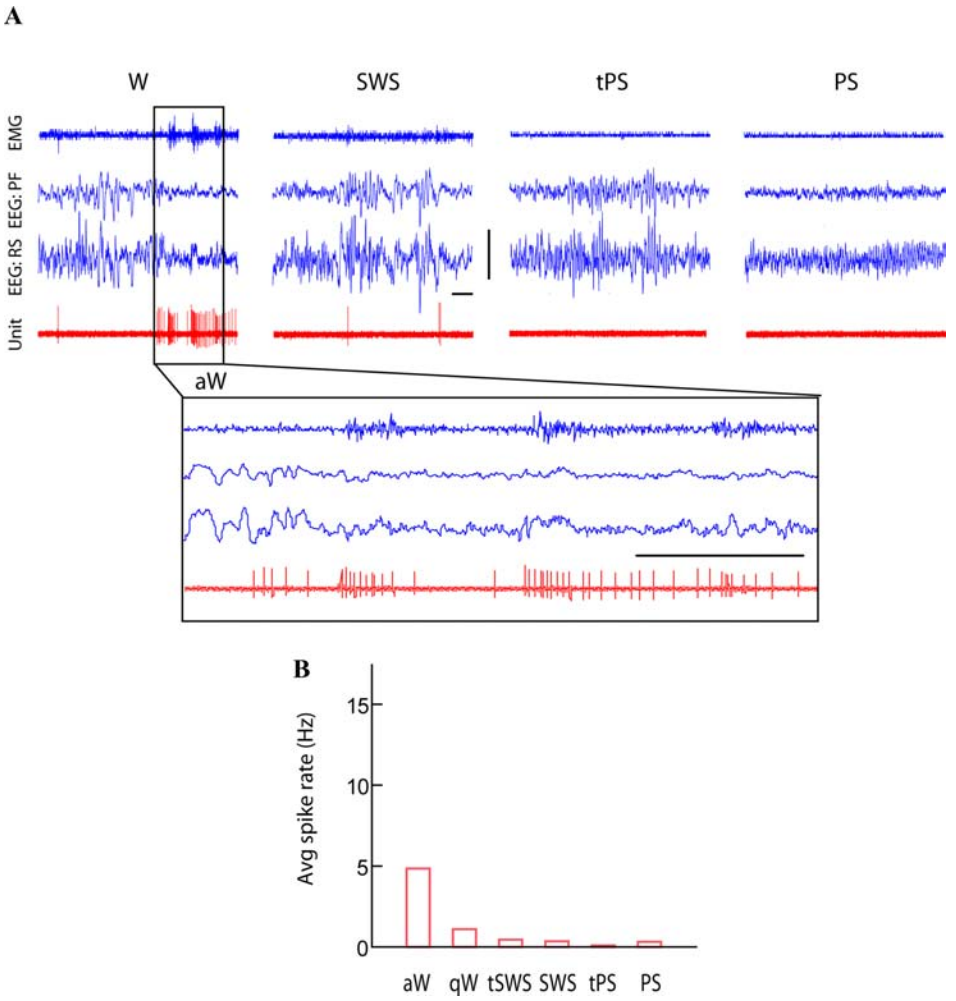


**FIGURE 3.** Orexinergic lateral hypothalamic unit. Discharge of an Orx neuron across sleep–wake states. Record of a neuron labeled by juxtacellular technique with Neurobiotin (Nb) and identified by immunohistochemistry for Orx in the rat. (**A**). As evident in 10-s traces (above), the unit fired during W and was virtually silent during SWS, tPS, and PS. As evident in an expanded trace (of approximately 4 s, below), the unit discharged during aW and increased firing phasically in association with increases in muscle tone seen on the EMG. (**B**). Average (Avg) discharge rate (Hz) across the sleep–wake states and transitions (t) of the same cell. Abbreviations as in Fig. 2. Horizontal scale bars: 1 s. Vertical scale bar: 1 mV for EEG, 0.5 mV for EMG and 2 mV for Unit. (Reprinted with permission from Lee, Hassani, and Jones, 2005.[63])

upon select neurons through Orx1 or Orx2 receptors (also called HrctA or HcrtB).[51–56] It does not appear to exert any direct inhibitory effects. In contrast to NA, Orx thus does not inhibit the GABAergic presumed sleep-promoting neurons but instead very selectively excites the cholinergic neurons of the basal forebrain.[51] It similarly selectively excites neurons of the nonspecific thalamo–cortical projection system and particular neurons in the cerebral cortex.[53,57] In addition, Orx recruits all the other arousal systems, including the noradrenergic LC neurons, the histaminergic tuberomammillary neurons, and the serotonergic raphe neurons.[52,54,56,58] It also directly excites somatic motor neurons[59] and the sympathetic nervous system.[60–62] By recruiting central and peripheral systems, Orx can thus stimulate and maintain a waking state with cortical activation and behavioral arousal. Perhaps not surprisingly, by single-unit recording of identified Orx neurons using the juxtacellular technique, they were found to discharge selectively during W and to turn off during sleep, including SWS and PS (FIG. 3).[63,64] Their discharge is positively correlated with amplitude

of the electromyogram (EMG+, W, FIG. 1). Even during W, Orx neurons fire in association with muscle tone and movement during active periods (FIG. 3). Yet, they increase firing prior to the return of muscle tone with awakening from PS and thus appear to stimulate awakening by recruiting other arousal systems.

From *in vitro* studies, it was learned that Orx neurons have the unique capacity for sustained excitation and spiking in absence of synaptic input through their intrinsic membrane properties.[65] They would accordingly need to be inhibited during sleep when they cease firing. This inhibition can occur through GABAergic inputs which arise, in part, from basal forebrain neurons with descending projections.[66] Such neurons would likely include those that are active during both SWS and PS (SWS-PS, FIG. 1) and are inhibited by NA.[29,31]

## Opponent Processes of Cholinergic and Orexinergic Systems Can Determine Normal Behavioral States or Pathologies

Although cholinergic neuronal activity and ACh release reach maximal levels during PS,[26,67–69] they are also high during wakefulness,[70] particularly in association with attentive behavior.[71] From very early pharmacological studies, it has been known that enhancement of ACh, by inhibition of its catabolic enzyme acetyl cholinesterase with physostigmine, evokes waking with cortical activation.[72] On the other hand, following depletion of the monoamines (with reserpine in the same study), physostigmine evokes PS with cortical activation and muscle atonia. With local injections of physostigmine or the cholinergic agonist carbachol into the pons, PS with muscle atonia can also be evoked.[14,16,17,73,74] The agonists act in the pons where cholinergic neurons of LDTg and PPTg project and normally release ACh during PS with muscle atonia (FIG. 1).[19,68,75] Here in the same region, monoaminergic afferents arrive and can oppose the action of ACh in eliciting PS with muscle atonia.[76]

Orx fibers also project into the pontine tegmentum and could oppose the action of ACh there.[50,77,78] Indeed, block in the synthesis of Orx receptors in the pons by local injection of specific antisense was found to result in PS enhancement and the occurrence of cataplectic-like attacks during the active period in rats.[79] In this region, many reticular neurons bear the Orx2 receptor (Orx2R),[77,78] which is lacking in narcoleptic dogs.[49] The Orx2R-bearing neurons would be excited by Orx (above). Some large Orx2R-bearing neurons also bear muscarinic 2 receptors (M2Rs),[77,78] upon which the cholinergic elicitation of PS depends.[80–83] The M2R is associated with hyperpolarizing responses to ACh through opening of $K^+$ channels.[84] Accordingly, the same neurons that would be excited by Orx would be inhibited by ACh. Through putative large reticulo–spinal neurons, Orx could thus facilitate, whereas ACh would disfacilitate, muscle tone and activity. By such opponent processes, Orx could override any inhibitory effects of ACh upon reticulo–spinal neurons when present. Thus, concurrent release of ACh in forebrain and brainstem would be associated with cortical activation in the presence of muscle tone and activity during waking when Orx is also present, but it would be associated with cortical activation in the absence of muscle tone and activity during PS when Orx is naturally absent or in cases of narcolepsy with cataplexy when Orx is pathologically absent. Evoked by strong emotions in humans and dogs,[85] cataplexy might represent the pathological unmasking of an otherwise adaptive response to danger or predation known as "tonic immobility" or "feigning death", a loss of muscle tone with maintenance of cortical activation and awareness.[86] Like PS, tonic immobility and cataplexy are both increased by enhanced cholinergic transmission[73,87–89] and could thus result from greater cholinergic activity relative to orexinergic activity.

## Summary

Cortical activation is stimulated by cholinergic neurons of the basal forebrain and brainstem during waking with movement but also during attentive waking without movement. It is also stimulated by cholinergic neurons during PS in association with postural muscle atonia and could be so during tonic immobility in certain mammals. Behavioral arousal along with high postural muscle tone and movement is stimulated by orexinergic neurons of the hypothalamus. With the natural cessation of orexinergic activity during sleep, PS or REM sleep with muscle atonia is stimulated by cholinergic transmission. With the pathological loss or decrease of Orx in narcoleptics, cataplexy or muscle atonia can be stimulated by cholinergic transmission to intrude into wakefulness, sometimes in association with REM sleep and dreams or with continued waking and awareness as in tonic immobility.

### Acknowledgments

The recent research of the author reviewed in this article was funded by grants from the Canadian

Institutes of Health Research and U.S. National Institutes of Health and was performed at the Montreal Neurological Institute by Frederic Brischoux, Maan Gee Lee, Oum Hassani, Ian Manns, Mandana Modirrousta, Pablo Henny, and Lynda Mainville to whom I am most grateful.

### Conflicts of Interest

The author declares no conflicts of interest.

## References

1. Moruzzi, G. & H.W. Magoun. 1949. Brain stem reticular formation and activation of the EEG. Electroencephalogr. Clin. Neurophysiol. **1:** 455–473.
2. Jones, B.E. 2005. Basic mechanisms of sleep-wake states. *In* Principles and Practice of Sleep Medicine. M.H. Kryger, T. Roth & W.C. Dement, Eds.: 136–153. Elsevier Saunders. Philadelphia.
3. Ford, B. *et al*. 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.
4. Holmes, C.J., L.S. Mainville & B.E. Jones. 1994. Distribution of cholinergic, GABAergic and serotonergic neurons in the medullary reticular formation and their projections studied by cytotoxic lesions in the cat. Neuroscience **62:** 1155–1178.
5. Jones, B.E. *et al*. 1991. GABA-synthesizing neurons in the medulla: their relationship to serotonin-containing and spinally projecting neurons in the rat. J. Comp. Neurol. **312:** 1–19.
6. Kaneko, T., F. Fujiyama & H. Hioki. 2002. Immunohistochemical localization of candidates for vesicular glutamate transporters in the rat brain. J. Comp. Neurol. **444:** 39–62.
7. Jones, B.E. 1995. Reticular formation. Cytoarchitecture, transmitters and projections. *In* The Rat Nervous System. G. Paxinos, Ed.: 155–171. Academic Press Australia. Sydney.
8. Jones, B.E. & T.-Z. Yang. 1985. The efferent projections from the reticular formation and the locus coeruleus studied by anterograde and retrograde axonal transport in the rat. J. Comp. Neurol. **242:** 56–92.
9. Steriade, M. 1981. Mechanisms underlying cortical activation: neuronal organization and properties of the midbrain reticular core and intralaminar thalamic nuclei. *In* Brain Mechanisms and Perceptual Awareness. O. Pompeiano & C. Ajmone Marsan, Eds.: 327–377. Raven Press. New York.
10. Siegel, J.M. 1979. Behavioral functions of the reticular formation. Brain Res. **180:** 69–105.
11. Maloney, K.J., L. Mainville & B.E. Jones. 2000. c-Fos expression in GABAergic, serotonergic and other neurons of the pontomedullary reticular formation and raphe after paradoxical sleep deprivation and recovery. J. Neurosci. **20:** 4669–4679.
12. Holmes, C.J. & B.E. Jones. 1994. Importance of cholinergic, GABAergic, serotonergic and other neurons in the medullary reticular formation for sleep-wake states studied by cytotoxic lesions in the cat. Neuroscience **62:** 1179–1200.
13. Jones, B.E. 2004. Activity, modulation and role of basal forebrain cholinergic neurons innervating the cerebral cortex. Progr. Brain Res. **145:** 157–169.
14. George, R., W. Haslett & D. Jenden. 1964. A cholinergic mechanism in the brainstem reticular formation: induction of paradoxical sleep. Int. J. Neuropharmacol. **3:** 541–552.
15. Baghdoyan, H.A. *et al*. 1984. Site-specific enhancement and suppression of desynchronized sleep signs following cholinergic stimulation of three brainstem regions. Brain Res. **306:** 39–52.
16. Vanni-Mercier, G. *et al*. 1989. Mapping of cholinoceptive brainstem structures responsible for the generation of paradoxical sleep in the cat. Arch. Ital. Biol. **127:** 133–164.
17. Gnadt, J.W. & G.V. Pegram. 1986. Cholinergic brainstem mechanisms of REM sleep in the rat. Brain Res. **384:** 29–41.
18. Jones, B.E., M. Pare & A. Beaudet. 1986. Retrograde labeling of neurons in the brain stem following injections of [3H]choline into the rat spinal cord. Neuroscience **18:** 901–916.
19. Jones, B.E. 1990. Immunohistochemical study of choline acetyl transferase-immunoreactive processes and cells innervating the pontomedullary reticular formation. J. Comp. Neurol. **295:** 485–514.
20. Mesulam, M.-M. *et al*. 1983. Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). Neuroscience **10:** 1185–1201.
21. Hallanger, A.E. *et al*. 1987. The origins of cholinergic and other subcortical afferents to the thalamus in the rat. J. Comp. Neurol. **262:** 105–124.
22. Rye, D.B. *et al*. 1984. Cortical projections arising from the basal forebrain: a study of cholinergic and noncholinergic components employing combined retrograde tracing and immunohistochemical localization of choline acetyltransferase. Neuroscience **13:** 627–643.
23. Gritti, I. *et al*. 1997. GABAergic and other non-cholinergic basal forebrain neurons project together with cholinergic neurons to meso- and iso-cortex in the rat. J. Comp. Neurol. **383:** 163–177.
24. Henny, P. & B.E. Jones. 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.
25. Metherate, R., C.L. Cox & J.H. Ashe. 1992. Cellular bases of neocortical activation: modulation of neural oscillations by the nucleus basalis and endogenous acetylcholine. J. Neurosci. **12:** 4701–4711.
26. Lee, M.G. *et al*. 2005. Cholinergic basal forebrain neurons burst with theta during waking and paradoxical sleep. J. Neurosci. **25:** 4365–4369.

27. MANNS, I.D., A. ALONSO & B.E. JONES. 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.
28. JONES, B.E. 2005. From waking to sleeping: neuronal and chemical substrates. Trends Pharmacol Sci. **26:** 578–586.
29. HASSANI, O.K., M.G. LEE & B.E. JONES. 2006. Discharge properties of identified GABAergic basal forebrain neurons across the sleep-waking cycle in head-fixed rats. Neuroscience Meeting Planner. Atlanta, GA: Society for Neuroscience, 458.417.
30. MANNS, I.D. *et al*. 2003. Alpha 2 adrenergic receptors on GABAergic, putative sleep-promoting basal forebrain neurons. Eur. J. Neurosci. **18:** 723–727.
31. MODIRROUSTA, M., L. MAINVILLE & B.E. JONES. 2004. GABAergic neurons with alpha2-adrenergic receptors in basal forebrain and preoptic area express c-Fos during sleep. Neuroscience **129:** 803–810.
32. FORT, P. *et al*. 1998. Pharmacological characterization and differentiation of non-cholinergic nucleus basalis neurons *in vitro*. Neuro Report. **9:** 1–5.
33. FORT, P. *et al*. 1995. Noradrenergic modulation of cholinergic nucleus basalis neurons demonstrated by *in vitro* pharmacological and immunohistochemical evidence in the guinea pig brain. Eur. J. Neurosci. **7:** 1502–1511.
34. MCCORMICK, D.A. 1992. Neurotransmitter actions in the thalamus and cerebral cortex and their role in neuromodulation of thalamocortical activity. Prog. Neurobiol. **39:** 337–388.
35. FUNG, S.J. *et al*. 1991. Locus coeruleus control of spinal motor output. Prog. Brain Res. **88:** 395–409.
36. ASTON-JONES, G. & F.E. BLOOM. 1981. Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. J. Neurosci. **1:** 876–886.
37. HOBSON, J.A., R.W. MCCARLEY & P.W. WYZINSKI. 1975. Sleep cycle oscillation: reciprocal discharge by two brainstem neuronal groups. Science **189:** 55–58.
38. MALONEY, K.J., L. MAINVILLE & B.E. JONES. 1999. Differential c-Fos expression in cholinergic, monoaminergic and GABAergic cell groups of the pontomesencephalic tegmentum after paradoxical sleep deprivation and recovery. J. Neurosci. **19:** 3057–3072.
39. NITZ, D. & J.M. SIEGEL. 1997. GABA release in the locus coeruleus as a function of sleep/wake state. Neuroscience **78:** 795–801.
40. GERVASONI, D. *et al*. 1998. Electrophysiological evidence that noradrenergic neurons of the rat locus coeruleus are tonically inhibited by GABA during sleep. Eur. J. Neurosci. **10:** 964–970.
41. JONES, B.E., S.T. HARPER & A.E. HALARIS. 1977. Effects of locus coeruleus lesions upon cerebral monoamine content, sleep-wakefulness states and the response to amphetamine. Brain Res. **124:** 473–496.
42. BLANCO-CENTURION, C. *et al*. 2004. Effects of hypocretin2-saporin and antidopamine-beta-hydroxylase-saporin neurotoxic lesions of the dorsolateral pons on sleep and muscle tone. Eur. J. Neurosci. **19:** 2741–2752.
43. JONES, B.E. 2003. Arousal systems. Front Biosci. **8:** S438–451.
44. SAKURAI, T. *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.
45. DE LECEA, L. *et al*. 1998. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc. Natl. Acad. Sci. U. S. A. **95:** 322–327.
46. CHEMELLI, R.M. *et al*. 1999. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell. **98:** 437–451.
47. PEYRON, C. *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.
48. THANNICKAL, T.C. *et al*. 2000. Reduced number of hypocretin neurons in human narcolepsy. Neuron. **27:** 469–474.
49. LIN, L. *et al*. 1999. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell. **98:** 365–376.
50. PEYRON, C. *et al*. 1998. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J. Neurosci. **18:** 9996–10015.
51. EGGERMANN, E. *et al*. 2001. Orexin/hypocretins excite basal forebrain cholinergic neurones. Neuroscience **108:** 177–181.
52. ERIKSSON, K.S. *et al*. 2001. Orexin/hypocretin excites the histaminergic neurons of the tuberomammillary nucleus. J. Neurosci. **21:** 9273–9279.
53. BAYER, L. *et al*. 2002. Selective action of orexin (hypocretin) on nonspecific thalamocortical projection neurons. J. Neurosci. **22:** 7835–7839.
54. LIU, R.J., A.N. VAN DEN POL & G.K. AGHAJANIAN. 2002. Hypocretins (orexins) regulate serotonin neurons in the dorsal raphe nucleus by excitatory direct and inhibitory indirect actions. J. Neurosci. **22:** 9453–9464.
55. BURLET, S., C.J. TYLER & C.S. LEONARD. 2002. Direct and indirect excitation of laterodorsal tegmental neurons by Hypocretin/Orexin peptides: implications for wakefulness and narcolepsy. J. Neurosci. **22:** 2862–2872.
56. HORVATH, T.L. *et al*. 1999. Hypocretin (orexin) activation and synaptic innervation of the locus coeruleus noradrenergic system. J. Comp. Neurol. **415:** 145–159.
57. BAYER, L. *et al*. 2004. Exclusive postsynaptic action of hypocretin-orexin on sublayer 6b cortical neurons. J. Neurosci. **24:** 6760–6764.
58. BAYER, L. *et al*. 2001. Orexins (hypocretins) directly excite tuberomammillary neurones. Eur. J. Neurosci. **14:** 1571–1575.
59. YAMUY, J. *et al*. 2004. Hypocretinergic control of spinal cord motoneurons. J. Neurosci. **24:** 5336–5345.
60. VAN DEN POL, A.N. 1999. Hypothalamic hypocretin (orexin): robust innervation of the spinal cord. J. Neurosci. **19:** 3171–3182.
61. KROUT, K.E., T.C. METTENLEITER & A.D. LOEWY. 2003. Single CNS neurons link both central motor and cardiosympathetic systems: a double-virus tracing study. Neuroscience **118:** 853–866.
62. SHIRASAKA, T. *et al*. 1999. Sympathetic and cardiovascular actions of orexins in conscious rats. Am. J. Physiol. **277:** R1780–R1785.

63. Lee, M.G., O. Hassani & B.E. Jones. 2005. Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. J. Neurosci. **25:** 6716–6720.
64. Mileykovskiy, B.Y., L.I. Kiyashchenko & J.M. Siegel. 2005. Behavioral correlates of activity in identified hypocretin/orexin neurons. Neuron. **46:** 787–798.
65. Eggermann, E. *et al*. 2003. The wake-promoting hypocretin-orexin neurons are in an intrinsic state of membrane depolarization. J. Neurosci. **23:** 1557–1562.
66. Henny, P. & B.E. Jones. 2006. 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.
67. Marrosu, F. *et al*. 1995. Microdialysis measurement of cortical and hippocampal acetylcholine release during sleep-wake cycle in freely moving cats. Brain Res. **671:** 329–332.
68. Kodama, T., Y. Takahashi & Y. Honda. 1990. Enhancement of acetylcholine release during paradoxical sleep in the dorsal tegmental field of the cat brain stem. Neurosci. Lett. **114:** 277–282.
69. Vazquez, J. & H.A. Baghdoyan. 2001. Basal forebrain acetylcholine release during REM sleep is significantly greater than during waking. Am. J. Physiol. Regul. Integr. Comp. Physiol. **280:** R598–R601.
70. Williams, J.A. *et al*. 1994. State-dependent release of acetylcholine in rat thalamus measured by *in vivo* microdialysis. J. Neurosci. **14:** 5236–5242.
71. Himmelheber, A.M., M. Sarter & J.P. Bruno. 2000. Increases in cortical acetylcholine release during sustained attention performance in rats. Brain Res. Cogn. Brain Res. **9:** 313–325.
72. Karczmar, A.G., V.G. Longo & A. Scotti deCarolis. 1970. A pharmacological model of paradoxical sleep: the role of cholinergic and monoamine systems. Physiol. Behav. **5:** 175–182.
73. Baghdoyan, H.A. *et al*. 1984. Microinjection of neostigmine into the pontine reticular formation of cats enhances desynchronized sleep signs. J. Pharmacol. Exp. Ther. **231:** 173–180.
74. Baghdoyan, H.A. *et al*. 1987. A neuroanatomical gradient in the pontine tegmentum for the cholinoceptive induction of desynchronized sleep signs. Brain Res. **414:** 245–261.
75. Jones, B.E. 2004. Paradoxical REM sleep promoting and permitting neuronal networks. Arch. Ital. Biol. **142:** 379–396.
76. Semba, K. 1993. Aminergic and cholinergic afferents to REM sleep induction regions of the pontine reticular formation in the rat. J. Comp. Neurol. **330:** 543–556.
77. Brischoux, F., L. Mainville & B.E. Jones. 2005. Muscarinic-2 and orexin-2 receptors on GABAergic and other neurons of the mesopontine reticular formation. Sleep **26:** A30.
78. Brischoux, F., L. Mainville & B.E. Jones. Submitted. Muscarinic-2 and orexin-2 receptors on GABAergic and other neurons in the rat mesopontine tegmentum and their potential role in sleep-wake state control.
79. Thakkar, M.M. *et al*. 1999. REM sleep enhancement and behavioral cataplexy following orexin (hypocretin)-II receptor antisense perfusion in the pontine reticular formation. Sleep Res. Online **2:** 112–120.
80. Velazquez-Moctezuma, J., J.C. Gillin & P.J. Shiromani. 1989. Effect of specific M1, M2 muscarinic receptor agonists on REM sleep generation. Brain Res. **503:** 128–131.
81. Velazquez-Moctezuma, J. *et al*. 1991. Cholinergic antagonists and REM sleep generation. Brain Res. **543:** 175–179.
82. Imeri, L. *et al*. 1994. Selective blockade of different brain stem muscarinic receptor subtypes: effects on the sleep-wake cycle. Brain Res. **636:** 68–72.
83. Baghdoyan, H.A. & R. Lydic. 1999. M2 muscarinic receptor subtype in the feline medial pontine reticular formation modulates the amount of rapid eye movement sleep. Sleep **22:** 835–847.
84. Egan, T.M. & R.A. North. 1986. Acetylcholine hyperpolarizes central neurones by acting on an M2-muscarinic receptor. Nature **319:** 405–407.
85. Nishino, S. *et al*. 2000. Is narcolepsy a REM sleep disorder? Analysis of sleep abnormalities in narcoleptic Dobermans. Neurosci. Res. **38:** 437–446.
86. Overeem, S., G.J. Lammers & J.G. van Dijk. 2002. Cataplexy: 'tonic immobility' rather than 'REM-sleep atonia'? Sleep Med. **3:** 471–477.
87. Monassi, C.R., A. Hoffmann & L. Menescal-de-Oliveira. 1997. Involvement of the cholinergic system and periaqueductal gray matter in the modulation of tonic immobility in the guinea pig. Physiol. Behav. **62:** 53–59.
88. Nishino, S. *et al*. 1995. Muscle atonia is triggered by cholinergic stimulation of the basal forebrain: implication for the pathophysiology of canine narcolepsy. J. Neurosci. **15:** 4806–4814.
89. Reid, M.S. *et al*. 1994. Cholinergic mechanisms in canine narcolepsy–I. Modulation of cataplexy via local drug administration into the pontine reticular formation. Neuroscience **59:** 511–522.