Local network regulation of orexin neurons in the lateral hypothalamic

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Burt J, Alberto CO, Parsons MP, Hirasawa M. Local network regulation of orexin neurons in the lateral hypothalamus. Am J Physiol Regul Integr Comp Physiol 301: R572–R580, 2011. First published June 22, 2011; doi:10.1152/ajpregu.00674.2010.— Obesity and inadequate sleep are among the most common causes of health problems in modern society. Thus, the discovery that orexin (hypocretin) neurons play a pivotal role in sleep/wake regulation, energy balance, and consummatory behaviors has sparked immense interest in understanding the regulatory mechanisms of these neurons. The local network consisting of neurons and astrocytes within the lateral hypothalamic area and perifornical area (LH/PFA), where orexin neurons reside, shapes the output of orexin neurons and the LH/PFA. Orexin neurons not only send projections to remote brain areas but also contribute to the local network where they release multiple neurotransmitters to modulate its activity. These neurotransmitters have opposing actions, whose balance is determined by the amount released and postsynaptic receptor desensitization. Modulation and negative feedback regulation of excitatory glutamatergic inputs as well as release of astrocyte-derived factors, such as lactate and ATP, can also affect the excitability of orexin neurons. Furthermore, distinct populations of LH/PFA neurons express neurotransmitters with known electrophysiological actions on orexin neurons, such as melanin-concentrating hormone, corticotropin-releasing factor, thyrotropin-releasing hormone, neurtensin, and GABA. These LH/PFA-specific mechanisms may be important for fine tuning the firing activity of orexin neurons to maintain optimal levels of prolonged output to sustain wakefulness and stimulate consummatory behaviors. Building on these exciting findings should shed further light onto the cellular mechanisms of energy balance and sleep/wake regulation.

food intake; MCH neuron; sleep

THE LATERAL HYPOTHALAMUS (LH) is the most extensively interconnected area of the hypothalamus, allowing it to control and convey a variety of essential autonomic and somatomotor functions. Neuroanatomical studies have demonstrated direct projections from the LH to other hypothalamic areas, cortical/limbic areas, and the autonomic and motor system of the brainstem (101, 102, 104). Such extensive connectivity is thought to represent the anatomical underpinning that supports sleep-wake regulation (10, 90, 106), energy homeostasis, as well as cognitive, reward-related, and emotion-related functions (8, 24, 103).

There are a number of neuronal populations that have been identified within this hypothalamic region. To a significant degree, the function of the LH can be attributed to orexin neurons that synthesize orexin A and B (also called hypocretin-1 and -2), 33 and 28 amino acid peptides, respectively, cleaved from the precursor protein prepro-orexin (22, 96). Orexin effects are mediated by two subtypes of orexin receptors, OX1R and OX2R, which have extensive, yet distinct, expression patterns in the brain (118). Orexin neurons are almost exclusively localized in the LH and adjacent perifornical area (PFA) in animal and human brains and have wide-ranging projections (14, 30, 90, 121), including the LH/PFA itself, where these neurons make synaptic contacts onto one another (41). Physiological functions of the orexin peptides include stabilization of wakefulness (97, 105), energy homeostasis (83, 96, 112), behavioral responses to food reward and addictive drugs (7, 38), neuroendocrine and autonomic outflow (99), and analgesia (17).

Many of orexin’s known behavioral effects, such as stimulation of food intake, wheel running, and spontaneous physical activity can be induced by local injection of orexins into the LH/PFA (27, 60, 61, 79, 111, 113, 116, 125). An orexin injection into the LH/PFA also results in Fos expression, a marker for neuronal activation (79), suggesting that endogenous orexin release within this area has an excitatory effect, which may lead to an amplification of excitation by further activating other orexin neurons. The idea of orexin neurons working in concert explains how a relatively small population of neurons (90) can coordinate diverse physiological functions. Furthermore, many previous studies, mainly using in vitro electrophysiological and histological approaches, collectively suggest complex interactions that take place among orexin neurons and other cell types within the LH/PFA. These interactions can ultimately determine the sensitivity to and integration of incoming signals and the levels of outgoing signals.
Excitation of Orexin Neurons

Orexin neurons have intrinsic features and the local environment that can promote a long-lasting firing activity. This may be because neuropeptide release from dense core vesicles needs prolonged depolarization (21) and/or because of its primary role in the maintenance of wakefulness and homeostasis where sustained output may be more relevant than precise timing.

Orexin neurons are intrinsically in a depolarized state (28), largely due to a constitutively active nonselective cation current mediated by transient receptor potential C channels (20). Furthermore, the local network of orexin neurons allows them to sustain an active state and/or to recruit a larger number of orexin neurons, utilizing excitatory neurotransmitters, such as orexins and glutamate (1, 94, 117). Orexin A or B activate OX2Rs, which in turn open nonselective cation channels to depolarize orexin neurons (137) (Fig. 1A and B). The balance between the excitatory and inhibitory effects determines the activity levels of the postsynaptic cell. C: at excitatory synapses, glutamate acts on presynaptic autoreceptors to inhibit glutamate release, completing a short, negative feedback loop. In addition, depolarization of orexin neurons induces release of endocannabinoids (eCB), which in turn act as retrograde messengers to inhibit excitatory inputs (D). Reciprocal communications between orexin neurons and MCH/GABA neurons (E) as well as GABAergic interneurons (F) also represent local feedback mechanisms.

Glutamatergic transmission may also stimulate orexin neurons indirectly in an unconventional manner (Fig. 2). Glutamate is known to stimulate lactate production in astrocytes, which in turn release lactate into the extracellular space through monocarboxylate transporters (MCTs) (45, 89). Since MCTs cotransport protons along with lactate, lactate release accompanies a local decline in extracellular pH (45). A decrease in pH would result in depolarization of orexin neurons (129). Moreover, orexin neurons utilize astrocyte-derived lactate, but not glucose, as an energy substrate to maintain spontaneous firing activity (86). In conditions where lactate supply is insufficient or ATP production is impaired, ATP-sensitive K+ channels become active and hyperpolarize these neurons (86). Therefore, the MCT-mediated astrocyte-neuron-lactate shuttle can mediate the excitatory action of glutamatergic transmission.

Activation of astrocytes or neurons can also induce release of ATP, which can, in turn, act as a transmitter molecule (26, 42) (Fig. 2). Extracellular ATP directly depolarizes orexin neurons via ionotropic P2X receptors expressed on the plasma membranes as shown by ultrastructural and electrophysiological studies (56), which provide the structural basis for fast glutamatergic transmission that supplies a major excitatory drive mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-d-aspartate (NMDA), but not kainate receptors (4, 66, 92).

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membrane, while having little effect on synaptic inputs (131). Adenosine, an inhibitory purine, is released endogenously onto orexin neurons by stimulation of afferent fibers at a frequency as low as 10 Hz in vitro (133), and an A1-receptor antagonist has been shown to increase wakefulness when injected locally into the LH/PFA in vivo (114). Whether or not neurons are the source of adenosine in this case remains unresolved, as adenosine is thought to be primarily produced by hydrolysis of astrocyte-derived ATP in the extracellular space by ectonucleotidases (124, 142) and affect neuronal activity (88). In orexin neurons, adenosine inhibits voltage-gated Ca\(^{2+}\) currents (69) and suppresses firing activity by A1-receptor-mediated reduction of presynaptic glutamate release (69, 133). The ratio of the two purines would depend on the rate of ATP release, conversion of ATP to adenosine, and uptake by nucleoside transporters (124) and would determine the polarity of the effect (excitatory or inhibitory) on orexin neurons. This may have important functional implications in sleep homeostasis (44, 114) and survival of LH neurons (141).

In summary, there are a number of local mechanisms that drive orexin neurons to be in a sustained active state. These include intrinsic ion channels (transient receptor potential C channels), paracrine and/or autocrine actions of excitatory neurotransmitters released by orexin neurons, and neighboring astrocytes that can amplify excitatory inputs and further stimulate orexin neurons by releasing lactate, protons, and ATP. Astrocytes may also mediate the propagation of excitatory signals among neurons that are not physically coupled (85, 140). These mechanisms may be important for orexin neurons in exerting their physiological functions that require prolonged output, such as maintaining wakefulness and stimulating consummatory behaviors.

**Self-Regulatory Mechanisms**

Excitatory positive-feedback mechanisms can conceivably continue until exhaustion without regulatory mechanisms that keep them in check. Indeed, many negative feedback pathways exist that regulate orexin neurons upon their excitation, including inhibitory neurotransmitters released by the same neurons. Dynorphin, a neuropeptide coexpressed by orexin neurons (18), directly hyperpolarizes these neurons by a \(\kappa\)-opioid receptor-mediated activation of G protein-dependent inwardly rectifying potassium channels and suppression of Ca\(^{2+}\) currents (67) (Fig. 1A). Interestingly, the dynorphin effect desensitizes faster than that of orexins, which results in a time-dependent shift in the balance between inhibitory (dynorphin) and excitatory (orexin) influence over the neurons (67). Another neuropeptide recently identified to be coexpressed by orexin neurons is nociceptin/orphanin FQ (N/OFQ) (73). N/OFQ inhibits orexin neurons through activation of K\(^+\) currents and inhibition of Ca\(^{2+}\) currents via N/OFQ peptide receptors (135). This, unlike dynorphin, is a long-lasting effect that can last for over an hour (M. P. Parsons, unpublished observation).

Glutamate, a classical neurotransmitter, can be expected to be released with less intense activity compared with peptide transmitters (21). Thus, it is tempting to speculate that the intensity and duration of firing activity dictate the release and postsynaptic response to the four conotoxins of orexin neurons. At low-to-moderate activity levels, orexin neurons will preferentially release glutamate and increase the excitability of postsynaptic orexin neurons. With higher firing frequencies, glutamate release becomes depressed (133), while peptide release would be favored. This will initially induce an inhibitory postsynaptic response due to the inhibitory actions of dynorphin and N/OFQ, masking the excitatory orexin effect. However, during a prolonged firing activity, \(\kappa\)-receptors will be desensitized and the excitatory orexin effect will eventually prevail. Nonetheless, the excitability will be kept in check due to the nondesensitizing N/OFQ effect. It has been postulated that the dynamic balance of orexin neuron’s excitatory and inhibitory neurotransmitters underlie the apparent lag between the electrophysiological activity and functional outcome (128). Specifically, on one hand, the timing of orexin neuron’s spiking activity precedes wakefulness (64, 75), which suggests that orexins should affect every wake bout. On the other hand, orexins are only effective at maintaining sustained wake bouts and not brief wake bouts of < 1 min (25). Perhaps the initial glutamate release is not sufficient to fully recruit and maintain the activity of a large number of orexin neurons, and the orexin effect needs to be unmasked from the inhibitory dynorphin for the maintenance of wakefulness.

Excitatory synaptic inputs are also subject to negative regulation (Fig. 1B). Dynorphin acts on presynaptic excitatory terminals to attenuate glutamate release (67, 135), while N/OFQ inhibits both excitatory and inhibitory transmission (135). Furthermore, synthetically released glutamate negatively regulates the presynaptic release of glutamate and GABA through group III metabotropic glutamate receptors (mGluRs) (2) (Fig. 1C). This would primarily be an autoinhibitory mechanism that limits glutamatergic transmission, since the inhibitory mGluRs on excitatory terminals (autoreceptors) are tonically activated by endogenous glutamate, whereas those expressed on GABAergic terminals seem to require more intense synaptic activation and glutamate spillover to be activated (2). Endocannabinoids, on the other hand, are typically released by the postsynaptic neuron upon a strong depolarization and act as a retrograde messenger (5). In orexin neurons, postsynaptic depolarization results in an inhibition of glutamatergic transmission (depolarization-induced suppression of excitation) but not GABAergic transmission, subsequently hyperpolarizing the postsynaptic cell (58) (Fig. 1D). These processes are designed not to turn on when the excitatory synapse or the postsynaptic cell is quiescent, thus providing negative feedback mechanisms to prevent overexcitation.

**Intra-LH/PFA Neuronal Network**

Several neuronal populations have been identified within the LH/PFA that are distinct from the orexin-expressing population. There is no doubt that these neurons also play important roles in the LH/PFA functions, including shaping the output and/or mediating the functions of orexin neurons (Fig. 3).  

**Melanin-concentrating hormone neurons.** Melanin-concentrating hormone (MCH) neurons are concentrated in the LH/PFA as well as in the zona incerta, where they are densely intermingled with orexin neurons but constitute a distinct cell group (14, 30, 90, 121). These neurons have also been the subject of intense research because of the crucial roles of MCH in promoting positive energy balance (91, 95, 108), sleep (3, 51, 123), and anxiety (11, 37). MCH neurons, like orexin
neurons, project widely within the central nervous system (10, 14, 16, 90) and form reciprocal connections with orexin neurons within the LH/PFA (41). MCH neurons are also known to express GABA (31, 50) as well as anorexic peptides nesfatin-1 (34, 35) and cocaine- and amphetamine-regulated transcript (13, 29, 49). It remains to be seen whether and how these peptides, which are functionally opposite to MCH (in terms of feeding effect), exert electrophysiological effects on LH/PFA neurons.

Communication between orexin and MCH neurons is bidirectional (Fig. 1E). Orexin A and B directly induce depolarization of MCH neurons (67, 122) and stimulate presynaptic glutamate release (122), whereas dynorphin and N/OFQ have direct hyperpolarizing effects, each through activation of GIRK channels (67, 87). Unlike orexin neurons, not only dynorphin (67) but also N/OFQ, induce an effect that desensitizes with repeated applications (87), while the orexin effect does not desensitize (67). Together, the effect of coreleased excitatory and inhibitory peptides from orexin neurons onto MCH neurons can be expected to shift towards excitation with prolonged activity, because the sustained excitatory orexin effect outlasts the desensitizing dynorphin and N/OFQ effects. MCH neurons also receive glutamatergic inputs, some of which may be from orexin neurons. Fast EPSCs are mediated by NMDA and non-NMDA receptors (122), whereas postsynaptic group I mGluRs provide another excitatory pathway to MCH neurons that induces a slow depolarization mediated by Na⁺/Ca²⁺ exchanger and potentiation of NMDA currents (57). Thus, somewhat akin to orexin neurons, MCH neurons are regulated by a balance between excitatory (orexins and glutamate) and inhibitory (dynorphin and N/OFQ) neurotransmitters originating from orexin neurons, a balance influenced by the quantity and time course of each transmitter release.

On the other hand, MCH released onto orexin neurons can act as a gatekeeper to prevent excess excitatory inputs. MCH does not have any apparent presynaptic effect on its own, but can attenuate presynaptic glutamate release induced by activation of orexin and D1-like receptors (93). At the postsynaptic side, MCH downregulates AMPA receptors in orexin neurons (93). Therefore, in response to the excitatory input from orexin neurons, MCH neurons send a negative feedback signal via inhibitory neurotransmitters MCH and GABA. Such mechanisms may be underlying the reciprocal firing activities of MCH neurons and orexin neurons observed in vivo (51).

MCH neurons can also self-regulate their activity levels. The MCH peptide does not affect the resting membrane potential of MCH neurons, but inhibits voltage-gated Ca²⁺ channels (36). Thus, it is possible that MCH can negatively regulate its own release via modulation of Ca²⁺ channels expressed at the axon terminals. GABA also conveys important information to MCH neurons, because inhibitory inputs are dominant over excitatory inputs, evident from spontaneous EPSCs being relatively scarce compared with inhibitory postsynaptic currents (58, 68) in stark contrast to orexin neurons that receive dominant excitatory inputs (56). In neonate rodents, GABA_A currents are depolarizing until about postnatal days 8–9, providing the main excitatory inputs to MCH neurons (68). In mature MCH neurons, the GABA_A current becomes inhibitory and mediates a tonic inhibitory tone by endogenous GABA (58, 68). In addition, the GABA_B receptor agonist baclofen has been observed to hyperpolarize a subpopulation of MCH neurons (n = 2 out of 6 cells examined; C. O. Alberto, unpublished data).

Endocannabinoids are also released by depolarization of MCH neurons, which inhibit presynaptic GABA release (depolari- zation-induced suppression of inhibition). This works as a positive feedback, since the postsynaptic cell is consequently disinhibited from a tonic GABA_A tone (58, 59). Although endocannabinoids also inhibit glutamate release to MCH neurons, depolarization-induced suppression of inhibition seems to have an overwhelming influence (58). Since depolarization-induced endocannabinoid release is dependent on Ca²⁺ currents (59), a concurrent MCH release (although likely from MCH neurons that induces a slow depolarization mediated by Na⁺/Ca²⁺ exchanger and potentiation of NMDA currents (57). Thus, somewhat akin to orexin neurons, MCH neurons are regulated by a balance between excitatory (orexins and glutamate) and inhibitory (dynorphin and N/OFQ) neurotransmitters originating from orexin neurons, a balance influenced by the quantity and time course of each transmitter release.

Leptin receptor-expressing GABAergic neurons. Another distinct neuronal population that exists in the LH/PFA is the leptin receptor-expressing (LepRb +) neurons (65). These neurons are excited by leptin, use GABA as a neurotransmitter (65), and make synaptic contacts with orexin neurons but not MCH neurons (70). This suggests that leptin not only inhibits

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**Fig. 3.** Local network within the lateral hypothalamus/perifornical area (LH/PFA). A number of neuronal populations have been identified within the LH/PFA in addition to orexin (Orx) neurons, including those expressing melanin-concentrating hormone (MCH), thyrotropin-releasing hormone (TRH), corticotropin-releasing factor (CRF), neuropeptide Y (NPY), galanin (Gal) and leptin receptor (LepRb +)-expressing GABAergic neurons. With the exception of galanin, whose electrophysiological effect remains unknown, these neurotransmitters have been demonstrated to modulate the excitability of orexin neurons. Therefore, these neurons, along with astrocytes, constitute a local network that can fine-tune the activity levels of this brain area through complex interactions. Further investigation is necessary to fully elucidate the intricate mechanisms that control the functional output of the LH/PFA in physiological and pathological conditions.
orexin neurons directly, as demonstrated as a reversible hyperpolarization in isolated orexin neurons (136), but also indirectly by activating these inhibitory LepRb+ neurons. Leptin also increases the transcription factor STAT3 immunoreactivity in orexin neurons (43), and local action of leptin within the LH results in a robust orexin mRNA expression after 26 h (71). Thus, leptin induces an acute reversible inhibition as well as a slow, long-term stimulation of the orexin system.

At inhibitory synapses to orexin neurons, GABA<sub>A</sub> receptors mediate fast inhibitory postsynaptic currents (66), whereas GABA<sub>B</sub> receptors mediate a slow hyperpolarization (134). GABA<sub>B</sub> agonists also inhibit excitatory and inhibitory transmission presynaptically (134). Genetic knockout of GABA<sub>B</sub> receptors on orexin neurons indirectly activates inhibitory interneurons, which in turn increase the membrane conductance of orexin neurons and shunt other synaptic inputs (74). Therefore, it seems that there is reciprocal communication between orexin and GABAergic interneurons, where orexin neurons activate interneurons, which in turn send negative feedback to orexin neurons (Fig. 1F). Whether or not LepRb+ GABAergic neurons play a part in this reciprocal network remains to be elucidated.

Obviously, local interneurons are not the sole source of glutamatergic and GABAergic synaptic inputs to orexin neurons; major projections from other brain areas are also meditated by these important neurotransmitters. Sources of glutamatergic afferents, in addition to orexin neurons, include those originating from energy balance-related arcuate proopiomelanocortin neurons (53), arousal-related basal forebrain (52), and lateral parabrachial neurons (82), as well as the dorsomedial hypothalamus that relays circadian rhythms from the suprachiasmatic nucleus to the LH/PFA (19, 23). MCH and LepRb+ neurons are one source of GABAergic inputs to LH/PFA neurons, but other GABAergic sources include the sleep-wake-related preoptic area (98) and basal forebrain (52), energy balance-related neuropeptide Y, proopiomelanocortin neurons of the arcuate nucleus (14, 30, 53–55), and emotion-related central amygdaloid nucleus (80). Therefore, it seems that there is reciprocal communication between orexin and GABAergic interneurons, where orexin neurons activate interneurons, which in turn send negative feedback to orexin neurons (Fig. 1F). Whether or not LepRb+ GABAergic neurons play a part in this reciprocal network remains to be elucidated.

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Other peptidergic neurons. Other neuropeptides have also been detected in cells within the LH/PFA (Fig. 3). Galanin-positive neurons have relatively small cell bodies and are separate from the orexin-expressing population (43, 132), although it has been shown that some orexin neurons may coexpress galanin (43). Galanin has no effect on Ca<sup>2+</sup> signaling in orexin neurons (119); however, no study has examined the electrophysiological effect.

Corticotrophin-releasing factor (CRF) neurons located in the LH/PFA are also distinct from orexin and MCH neurons but coexpress neuropeptide Y (127). CRF depolarizes a subpopulation of orexin neurons (25%) via CRF1 receptors. The effect is reversible but nondesensitizing as long as the ligand is present (130). Neuropeptide Y has been shown to activate Ca<sup>2+</sup> signaling (119).

A significant number of neurons expressing thyrotropin-releasing hormone (TRH) are also found in the LH/PFA according to the Allen Institute for Brain Science Atlas (http://mouse.brain-map.org.brain/gene/71016631.html). At the cellular level, TRH has been found to directly depolarize orexin neurons by activating nonselective cation channels (39, 47). However, TRH also increases the firing activity of presynaptic GABAergic interneurons, increasing the inhibitory synaptic influence (47). Therefore, the location of synaptic release of TRH would likely determine whether TRH excites or inhibits orexin neurons.

It is interesting that these neuropeptides that excite orexin neurons are known to promote energy expenditure and inhibit food intake [CRF (107), TRH (63), and neuropeptide Y (72)]. Some of these effects may indeed be mediated by orexin neurons; for example the TRH effect on locomotor activity is reduced in orexin neuron-ablated mice (47). Unlike other orexigenic neuropeptides that inhibit energy expenditure to promote positive energy balance, orxins not only induce feeding but also spontaneous physical activity (61, 81, 110, 115), sympathetic outflow (32, 33, 99, 100, 109), thermogenesis (84, 138, 139), and oxygen consumption (107). In fact, the role of orexins in energy expenditure may be more substantial than in food intake, because, in the absence of orexin signaling, animals become obese despite accompanying hypophagia (46, 48). Thus, it seems plausible that orexin neurons mediate the effects of catabolic neuropeptides expressed in the LH/PFA. Likewise, orexins (9, 76, 126, 135), CRF (62), and TRH (12) have been shown to have antinociceptive properties. Therefore, orexin neurons could possibly mediate the effects of CRF and TRH. On the other hand, N/OFQ is known to block opioid-mediated stress-induced antinociception (78), which involves suppression of orexin neurons and the downstream analgesic effect (135).

**Perspectives and Significance**

Previous studies have revealed intricate interactions within the network of neurons and astrocytes that may fine tune the output of the LH/PFA. A given neuron can release multiple neurotransmitters to other LH/PFA neurons, some with opposing actions on the postsynaptic cell, and the impact of each neurotransmitter can change in a time-dependent manner. What remains largely unknown is whether or not these cotransmitters are regulated in parallel, with respect to synthesis, storage (are they packaged in the same vesicle?), release (amount and site of release), and receptor expression (number and subcellular localization).

Many of the neurotransmitters expressed in the LH/PFA are not exclusive to this brain area, with the exception of orexins and MCH that are largely confined. Future studies should investigate the roles of LH/PFA-specific expression of neurotransmitters to fully elucidate the characteristics of local neurochemical interactions as well as specific physiological functions of the LH/PFA. Site-specific knockdown of individual neurotransmitters may prove to be useful in this regard.

Not all LH/PFA neurons make synaptic contacts onto one another simply because of their proximity, as seen with MCH neurons not receiving synaptic inputs from the nearby LepRb+ neurons (70). Nonetheless, unlike glutamate or GABA, whose diffusion is restricted, neuropeptides can act as volume transmitters even in the absence of synaptic specialization between the pre- and postsynaptic cell (143) and can be expected to play an important role within the local circuitry.
These local interactions may also affect the sensitivity of LH/PFA neurons to incoming projections from other brain areas (15). The functioning of the LH/PFA circuitry is further complicated by functional plasticity and dynamic synaptic remodeling of orexin neurons that occurs in response to changing physiological needs, as seen following sleep (40, 77, 92, 120) and food deprivation (56) and in relation to circadian rhythm (6). It is highly likely that the intra-LH/PFA network activity discussed here would be altered as a result. Further research on neurotransmitter effects and synaptic integration in various physiological and pathological contexts will help provide a clearer picture of the regulatory mechanisms for orexin neurons and the LH/PFA.

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REFERENCES


NEUROTRANSMITTER INTERACTIONS IN THE LATERAL HYPOTHALAMUS


124. Wall M, Dale N.

125. Watanabe S, Kuwaki T, Yanagisawa M, Fukuda Y, Shimoyama M.


133. Wollmann G, Acuna-Goycolea C, van Den Pol AN.

134. Wollmann G, Acuna-Goycolea C, van Den Pol AN.


