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Updates Regarding Neurocircuits and Neurotransmitters Involved in the Regulation of Wakefulness

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Abstract

Sleep is a universal phenomenon that is observed not only in humans but also in birds, fishes, and flies even in simpler organisms such as worms also we spend about 8 hours each day in sleep which represents nearly one-third of our life. All these observations indicate the physiological importance of sleep. study of sleep mechanisms requires the first study of the mechanisms, neurocircuits, and neurotransmitters involved in the promotion of wakefulness. Observation of Von Economo in 1930 of patients affected by "encephalitis lethargica", an epidemic that causes widespread and prolonged sleepiness most of the day, opened the door for the study of the brain regions responsible for wakefulness. Researchers recognized now many brain regions that are responsible for wakefulness and other brain regions responsible for the induction of different types of sleep. This review will try to discuss the most recent mechanisms and neural circuits involved in the promotion of wakefulness.

Keywords: wakefulness, orexin, neurocircuits

Introduction

Wakefulness in a physiological view can be defined as a condition of a person's alertness to the surrounding environment and his ability to communicate associated with low voltage fast electroencephalographic (EEG) activity[(1)]. Sleep is different in humans and mammals than sleep in other organisms so, finding a universal definition of sleep is difficult however Sleep in humans can be described as a transient natural, periodic, physiologic, reversible phenomenon in which there is impaired conscious perception of the external environment that may be accompanied by dreams[(2)].

Studying the sleep/wakefulness cycle is of increasing importance to find new strategies and drugs for the treatment of sleep disorders as sleep disorders, especially insomnia is of high prevalence all over the world, especially in old age. it is estimated that about 34% of people above 60 years in America have sleep disorders which leads to many health problems such as hypertension, diabetes, immune disorders, and even cancer[(3)].

Wakefulness-Generating Neural Circuits

The observations of Von Economo of brain autopsies obtained from patients who died after suffering from encephalitis lethargica displayed the most noticeable alterations in the upper part of the midbrain and substantia nigra, followed by the basal ganglia, thalam-

us, and other parts of the brainstem. [4] These observations lead to the discovery of what is known now as the ascending reticular activating system.

This system has two major pathways. The first pathway is the dorsal pathway which starts from the acetylcholine and glutamate-producing neurons within the brain stem. Then these fibers project to the thalamus at the intralaminar and midline nuclei and, accordingly, wide areas of the cerebral cortex are innervated. [5] although some fibers of this pathway send their discharge directly to the cerebral cortex. [6] Their discharge to the reticular nucleus is highly important, as it resides between the thalamic-relay nuclei and the cerebral cortex, which act as a gate that can inhibit communication between the thalamus and the cortex, which is important for wakefulness. [7]

The second pathway is the ventral pathway which starts from the same ponto-mesencephalic structures of the first pathway but bypasses the thalamus, reaching the lateral hypothalamic area (LHA) activating its neurons and activates also the tuberomammillary nucleus (TMN) in the posterior hypothalamus, basal forebrain (BF), and throughout the cerebral cortex. [5] So, Arousal systems can affect cortical activity either directly or indirectly through projections to the thalamus, LHA, or BF.[8]

Patients with lesions along the ventral pathway, especially in the LHA and rostral midbrain, are difficult to stay awake and enter into severe long-lasting hypersomnia or even coma. [7] on the other hand, The dorsal pathway allows the processing of information related to sensation, motor responses, and cognition at the thalamus. Patients with lesions limited to the thalamus are usually awake but in a vegetative state and unresponsive. [9]

Wakefulness promoting brain areas:

These include brain stem monoaminergic areas that secrete acetylcholine, noradrenaline, serotonin, dopamine, or glutamate. These areas discharge mainly during wakefulness and rapid eye movement (REM) sleep i.e. periods of increased brain electrical activity but their discharge diminishes during periods of non-REM sleep. [10] All these areas express orexinergic receptors. [11] These areas send projections to each other[12], and to the cortex either directly or indirectly through the thalamic nuclei as in the case of cholinergic neurons of the pedunculopontine nucleus (PPN) and laterodorsal tegmental nucleus (LDT) that their cortical direct connection is sparse. [9]

A. Acetylcholine producing cells:

Acetylcholine-producing neurons are present in two major collections (1) two pontine groups, which include the PPN and LDT, and (2) a BF group. [13]

The PPN and LDT are groups of neurons present at the junction between the pons and the midbrain.[9] In terms of cellular makeup and connectivity, they're very comparable and have no clear borders or landmarks separating them. [14]

PPN is found between the decussation of the superior cerebellar peduncle medially and the medial lemniscus laterally within the caudal mesencephalic tegmental area, superior to the locus coeruleus (LC) and inferior to the substantia nigra.[15]

The PPN is divided into pars compacta and pars dissipate. The pars compacta are located in the caudal half of the nucleus and primarily contain cholinergic and glutamatergic cells[12] with predominately cholinergic neurons. [15] while the pars dissipate is a small to medium-sized GABA producing neurons located throughout the rostrocaudal breadth of the PPN area. [12]

Thus, the rostral and caudal sections of the PPN have different neurochemical cell compositions, as both regions produce acetylcholine but the caudal portion produces also glutamate while the rostral portion produces GABA besides the acetylcholine. [14]

These neurons show a transitory discharge rate that is noticed to increase before the transition from sleep into an activated state i.e. either wakefulness or REM sleep. so it is believed that the primary role of these neurons is mostly the transitions from sleep to an activated state. [9]

The BF is defined by fields of acetylcholine-producing neurons that extend from the medial septum to the substantia innominata, it also contains GABAergic and glutaminergic neurons.[9] It is the primary cholinergic input to the cortex. [16]

Selective lesions of cholinergic neurons mainly increase the slow waves in EEG and lower non-REM sleep awakenings but not the amount of time spent awake. This suggests that BF cholinergic neurons are involved in the production of the fast cortical activity, but It's unclear if these neurons are required for wakefulness. While the GABAergic BF neurons are capable of promoting wakefulness by inhibiting the activity of inhibitory cortical interneurons. [9]

It contains also some GABAergic inhibitory neurons projecting to the cerebral cortex that mediate non-REM sleep. these neurons are inhibited by noradrenaline secreted from the LC. [6]

The glutamatergic BF neurons are less well understood, although they appear to enhance cortical activity as well. [9]

B. The Noradrenergic Wake-Promoting System Locus coeruleus (LC)

In the dorsolateral pons, there are seven noradrenergic neurons labeled A1–A7. The largest one (A6) is the LC[17] which sits in the heart of the sleep-arousal network, gathering arousal-related data from all wake and sleep-promoting nuclei. [6] It is necessary for maintaining high levels of alertness when responding to striking stimuli and stressors. [9]

In addition to promoting wakefulness, The LC inhibits REM sleep through inhibition of PPT & LDT that contain REM sleep-promoting cholinergic neurons. Activation of LC leads also to modulation of motor activity through excitatory projections to motor neurons in the spinal cord and the brainstem via α_1-adrenoceptors. When LC discharge declines during sleep, the muscle tone declines which when affects the muscles of the upper airways leading to the generation of sleep-related obstructive apnea. [6]

C. The serotoninergic Wake-Promoting System:

Dorsal Raphe nucleus (DRN):

The DRN is one of nine serotoninergic neurons labeled (B1-B9) that are located in the midline of the brainstem. The highest concentration of these serotoninergic neurons is present in the DRN (B7)[18] and it is involved in the regulation of sleep and wakefulness. [6]

It contains also GABAergic neurons that play a role in sleep promotion. [18] while the median raphe nucleus is integrated into the regulation of circadian rhythm. [16] The medullary caudal raphe group largely projects to the brainstem and spinal cord and supports wakefulness through facilitating autonomic and motor functions. [10]

Serotonin has a complex receptor system, so the effects of serotonin on wakefulness and sleep are still a debate, while some suggest its role in promoting wakefulness, others suggest its role in promoting sleep. This depends on the site of action of serotonin and the type of the receptor activated by serotonin. [6]

For example, the use of selective serotonin inhibitors in the cerebral cortex leads to wake promotion through activation of the 5-HT2A receptor while its use in the brainstem leads to sleep promotion through activation of 5-HT2C receptors which in turn activate GABAergic interneurons that inhibit the wake-promoting nuclei. [6] This explains insomnia happened after large lesions in raphe nuclei in rodents and cats. [9] Agonists for the 5-HT1A, 5-HT1B, or 5-HT2 receptors administered systemically increase wakefulness while decreasing non-REM and REM sleep. Many observations support that the main role of serotonin is the promotion of wakefulness. [19] Others suggest their role in the transition between stages as the acetylcholine system. [16]

However, because serotonin neurons are required for thermogenesis, it now appears that sleep disturbances caused by serotonin depletion were caused by hypothermia in a cool environment, as similar insomnia does not occur in a warm environment.[9]

D. Histaminergic wake-promoting system:

Histamine is a well-studied wakefulness-inducing molecule secreted from the TMN of the posterior hypothalamus. [20] The TMN is the only neuronal source of histamine in the central nervous system (CNS). [9] First-generation H_1-antihistamines that can cross the blood-brain barrier can cause sedation and sleep by blocking the action of histamine released from the TMN.[6]

There are four types of histamine receptors (H_1-H_4). The CNS contains H_1, H_2, and H_3.[6] while, H_4 receptors are only present in the periphery, where they mediate inflammatory, and immune responses by their action on mast cells, eosinophils, and T cells. [21]

H_1 and H_2 receptors are excitatory and located postsynaptically. While, H_3 receptors are inhibitory and located on the histaminergic neuron itself (autoreceptors) and also occur on the nerve terminals of other neurons where they inhibit the release of their neurotransmitters either monoamines, glutamate acetylcholine, GABA, or peptides. [6] H_3 receptors are strongly expressed in the hippocampus, basal ganglia, and cerebral cortex[21].

E. The Dopaminergic Wake-Promoting System:

Dopaminergic neurons are organized in several nuclei located in the midbrain, hypothalamus, and olfactory bulb. Only the midbrain nuclei are involved in the regulation of wakefulness. [6]

In the midbrain, there are three dopaminergic nuclei: the substantia nigra, VTA, and the ventral periaqueductal grey matter (VPAG). The pars compacta of the substantia nigra through its connection with the striatum via nigrostriatal fibers is concerned mainly with the regulation of motor activity. This pathway also contributes to wakefullness as it sends excitatory collaterals to the thalamic wake-promoting neurons. The damage of these dopaminergic fibers to the thalamus has been implicated in excessive sleepiness during daytime in patients with Parkinsonism. [6]

On the other hand, Dopamine at the level of the basal ganglia has complex effects on sleep and wakefulness, while the actions of dopamine on the nucleus accumbens lead to wakefulness, its actions on globus pallidus external lead to induction of sleep. [8]

Regarding The VTA, it is involved primarily in the regulation of motivation and reward through the mesolimbic pathway. Both VTA and VPAG influence wakefulness through their excitatory outputs to the LC and LHA / Perifornical area. [6] VTA activity is highest during wakefulness and REM sleep and the lowest activity is observed

during non-REM sleep. [10] Inhibition of VTA neurons reduces wake especially when significant motivation is required, such as when looking for tasty food or a possible mate. These observations show that dopaminergic neurons of the VTA promote wakefulness, especially in high-motivation situations. [9]

Nicotine increases dopamine release by activating nicotinic receptors on dopaminergic nerve terminals. This explains the wake-promoting effect of nicotine. [6]

Dopamine is inactivated mainly through active reuptake by the dopamine carriers. [8] Drugs that block dopamine transport lead to increase dopamine concentration as amphetamines and modafinil promote wakefulness strongly, whereas antipsychotics which are dopamine antagonists lead to sedation. [9]

F. The Glutamatergic Wake-Promoting System:

Glutamate is the most prevalent excitatory transmitter in the CNS. A wake-promoting glutamatergic system in the pons has been identified recently located in the parabrachial nucleus (PB) and precoeruleus area. [8] This is evidenced by injury to the PB produces sustained vegetation or even coma both in animals and humans. [9] They maintain wakefulness through excitation of the BF neurons. In rats, this glutaminergic system appears to be more important in maintaining wakefulness than the cortico-thalamic system. [6]

It is noticed that glutamate-producing neurons of the external lateral PB are also activated by elevated CO_2 levels in the blood. In addition to disruption of glutamate signaling in the lateral PB delays arousals to elevated CO_2 levels in a mouse model of obstructive sleep apnea. These neurons are also activeated by pain and cold. These observations suggest the involvement of this pathway in the promotion of wakefullness in response to several interoceptive stimuli. [9]

Infants with developmental anomalies in the integrated respiratory and arousal responses are more likely to have sudden infant death syndrome or other life-threatening conditions at night. Intermittent hypoxia can also lead to the damage of wakefulness-promoting neurons, increasing the likelihood of poor response to asphyxia during sleep. [10]

G. Orexin:

Orexins (A&B) are large molecular weight transmitters produced by neurons in the perifornical LHA. [8] They strongly stimulate all the wake-promoting neurons discussed above plus the midline thalamus and cortex[9] especially the LC which has the strongest projections. Orexinergic neuron activeity is highest during periods of wakefulness accompanied by apparent movements and motor engagement and lowest during periods of tonic REM sleep and non-REM sleep. [10] The orexin is especially important for sustaining long periods of wakefulness and maintenance of arousal when animals are subjected to stressful conditions, seeking reward, or exposed to conditions that can disturb homeostasis. [9]

Orexin neurons receive information from stress systems and the circadian system (indirectly via the dorsomedial nucleus of the hypothalamus). Then they integrate these data to adjust wakefulness levels to environmental needs. [22]

Orexin neurons projecting to the LC get input from many brain regions, including the amygdala and the nucleus accumbens, implying that this connection plays a role in emotions. a subpopulation of neurons in the LC that send to the lateral amygdala receives input from orexin neurons and inhibiting these connections leads to decreased conditioned fear responses and excitation of these connections may

lead to generalized fear responses. This suggests that this circuit mediates behavior related to fear. [22]

Loss of orexin resulted in decreased wakefulness and Narcolepsy,[13] a condition characterized by extreme daytime sleepiness, an early beginning of REM sleep, fragmented sleep during the night, and cataplexy, which is an emotionally-induced abrupt loss of muscle tone while awake. [22] It is also involved in the sleep disturbances of Alzheimer's disease, Parkinson's disease, and traumatized brain injuries. [8]

The orexins bind to specific receptors (OX1R and OX2R) exciting their targets. Their action lasts for several minutes. Glutamate and the inhibitory neuropeptide dynorphin are also produced by the orexin neurons. [9]

Orexin release is controlled by several feedback control mechanisms. The Wake-active noradrenergic and serotonergic neurons send inhibitory signals to orexin neurons. The histaminergic neurons' effect on orexin neurons is little. These negative feedback processes could be crucial for the fine-tuning of activity of the orexin neurons to maintain wakefulness. [23] In rats, a brief two-hour bout of total sleep loss was found to switch noradrenaline's effect on orexin neurons from excitation to inhibition. This mechanism could play a role in the increasing drowsiness that comes with sleep loss. [23]

Local feedback circuits may also play significant roles in orexin neuronal control. Orexin neurons activate themselves through local glutamatergic neurons both directly and indirectly, generating positive-feedback circuits that may maintain the activity of the orexin neuron discharge. On the other hand, negative feedback control is also activated via the activation of local GABAergic input to orexin neurons. [23]

H. Other neurotransmitters involved in the regulation of wakefulness:

Cocaine- and amphetamine-regulated transcript (CART) and Ghrelin: It supports wakefulness and inhibits REM and non-REM sleep by action on arousal-promoting nuclei. [22]

Corticotropin-releasing hormone (CRH) & Adrenocorticotropic hormone (ACTH): Short-term CRH enhances wakefulness and inhibits non-REM sleep, maybe through activation of LC and orexinergic neurons, while long-term CRH promotes REM sleep, potentially via LDT and PPT activation. ACTH promotes wakefulness but has no known role in REM or non-REM sleep. [22]

Endomorphin: It may promote wakefulness and inhibit non-REM sleep but has no known role in REM sleep. [22]

Neuropeptides Y &S: Neuropeptide S Promotes wakefulness[24] but injection of neuropeptide Y centrally enhances wakefulness in mice if injected at the onset of light when mice begin to sleep this occurs through suppression of the sleep center in the hypothalamus. [22]

Neurotensin: It Promotes wakefullness, Inhibits non-REM sleep, and may promote REM sleep. [22]

The contribution of the previously mentioned wake-promoting nuclei is evidenced by the electroencephalographic pattern of wakefulness upon their stimulation. During wakefulness, EEG shows low voltage (5-10 μV), and high-frequency waves (20-30 Hz). These waves are called the beta waves[25]. The acetylcholine-producing neurons in the PPN/LDT for example, transform the slow waves of EEG characteristic of sleep (delta waves) into fast waves characteristic of wakefulness (beta waves). Also, lesions of the BF cholinergic neurons prolong the slow waves[9].

Many factors affect the activity of these wake-promoting nuclei such as the body temperature and feeding state. Falling body temperature and satiety suppress the activity of the wake-pr-

omoting nuclei[26]. all of the above-mentioned wake-promoting regions can be suppressed by the GABA released by the ventrolateral preoptic nucleus in the anterior hypothalamus which is considered a sleep center that is responsible for the onset of sleep[27]. Other neurotransmitters are involved in the regulation of sleep as galanin, prostaglandin D_2, adenosine[27], melatonin, and melanocyte-stimulating hormone[28] but a detailed discussion of their roles is beyond the scope of this review.

Conclusion:

Wakefulness is not a function of a sole area in the brain. It is a matter of contribution of many brain centers in the brain stem, thalamus, hypothalamus, and basal ganglia. Some of these areas are responsible for wakefulness in certain conditions that need alertness. Orexin from the lateral hypothalamic area act as a key regulator of wakefulness.

List of abbreviations

ACTH	Adrenocorticotropic hormone	LHA	Lateral hypothalamic area
BF	Basal forebrain	PB	Parabrachial nucleus
CRH	Corticotropin-releasing hormone	PPN	Pedunculopontine nucleus
CNS	Central Nervous System	REM	Rapid eye movement
DRN	Dorsal Raphe nucleus	TMN	Tuberomamillary nucleus
EEG	electroencephalography	VPAG	Ventral periaqueductal grey matter
LC	Locus coeruleus	VTA	Ventral tegmental area
LDT	Laterodorsal tegmental nucleus		

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