ABSTRACT Sleep is a complex process that has an essential role in physiological function. However, the mechanism of how exactly the human wake-sleep cycle is running has not been identified. The classical theory has been a cornerstone of the explanation since the early 20th century. According to the theory, various neurotransmitters link several structures in the brainstem, diencephalon, and cortex to maintain the cycle. However, several aspects of sleep cannot be explained by the theory, such as how the process of transition from slow-wave sleep to REM sleep, how the regulation of muscle atony and eye movement during REM sleep, and why damage to one nucleus of the awareness system has little effect on daily sleep quantity.

A new paradigm in the wake-sleep cycle by adding the role of the rapid neurotransmitters, the balance of glutamate and GABA, has become the focus of sleep research in the last ten years. Recent evidence points to the important role of glutamate to fill in gaps in the classical theory of the wake-sleep cycle, sleep homeostasis and its effects on neuroplasticity, and the development of various neurodegenerative diseases.

KEYWORDS Wake-sleep cycle, glutamate, neurotransmitters

Introduction

Sleep, one of the basic biological needs for humans, involves a complex neurobiology process and has an essential role in regulating the physiological functions of various systems in the human body. Sleep is essential for maintaining the balance of the immune system, removal of metabolic waste products, learning and memory processes, and synaptic homeostasis and neural plasticity. Various studies have shown that sleep deprivation and poor sleep quality are associated with impaired brain development, physical and mental health problems, and cognitive dysfunction, which ultimately affects humans’ quality of life and function as social beings.[2]

Baron Constantine von Economo first introduced the basic understanding of brain activity in regulating the wake-sleep cycle in the early 20th century. Post mortem observations in lethargic encephalitis patients with excessive sleep led to the occurrence that the hypothalamus, brainstem and basal forebrain (BF) has an important role in the wake-sleep cycle.[3,4]

As technology advances to support medical research, a theory has been developed that the waking state is mainly determined by the monoaminergic and cholinergic systems of the nuclei in the brain stem, which transmits to the thalamus, which is then projected onto the cerebral cortex. On the other hand, sleep is induced by the GABAergic system of the hypothalamic preoptic nuclei.[3,4]

In the last decade, various studies on sleep in experimental animals have found that the classical theory of sleep has many weaknesses. The damage to one or more nuclei that play a role in the classical theory of the monoamine-cholinergic system does not have a consistent effect on changes in the wake-sleep cycle.[4] A new paradigm in the wake-sleep cycle by adding the role of the rapid neurotransmitters, the balance of glutamate and GABA, has become the focus of sleep research. Recent evidence points to the important role of glutamate to fill in gaps in the classical theory of the wake-sleep cycle, sleep homeostasis and its effects on neuroplasticity, and the development of various neurodegenerative diseases.[5]

A. Classical Theory of Monoaminergic and Cholinergic Systems

The theory of the wake-sleep cycle continued to develop after Von Economo first declared the theory of the structures in the brain that contributed to awareness in the early 20th century. Since then, discussion of the arousal circuit (awareness) and sleep in the brain has centred on the monoaminergic and cholinergic systems.[3,4] Based on this classical theory, the arousal...
system of the awareness system that maintains the waking condition consists of two main systems, namely the cholinergic system or dorsal pathway and the monoaminergic system or ventral pathway. According to the theory, sleep occurs due to inhibition of the entire alert system by GABAergic innervation from the nuclear population in the preoptic area.[3] In addition to the monoaminergic and cholinergic systems, the alert system is also maintained by the orexin hypocretin system produced by the lateral hypothalamus area. Furthermore, several non-neurotransmitter substances, the named sleep-regulatory substances (SRSs), were also identified to influence the wake-sleep cycle, including adenosine, cytokines (IL-1β and TNF-α), and prostaglandins. The SRSs have been shown to act on brain circuits responsible for waking and sleeping, influencing the wake-sleep cycle because levels vary with the cycle, and changes or inhibition of SRSs cause significant changes in the wake-sleep cycle.[3] The classical theory argues that many neurotransmitters are involved in maintaining alertness to ensure that the dysfunction of a system does not necessarily lead to overall disruption. In addition, previous studies have concluded that each of these neurotransmitters contributes to different aspects of the alert system.

1. Dorsal Cholinergic Awareness System

The cholinergic system consists of acetylcholine-producing neurons in the periaqueductal grey matter, namely the segmental laterodorsal nucleus (LDT) and the segmental pedunculopontine (PPT) nucleus, as well as the nuclei in the basal forebrain, namely the medial septum nucleus, the preoptic magnocellular nucleus, and substantia innominata. Cholinergic signals from LDT and PPT form a dorsal awareness pathway to the thalamus, projected to the cortex. These nuclei are active in the wake-up phase and the Rapid Eye Movement (REM) in the sleep phase. During the NREM phase, the tone of this cholinergic system is at its lowest.[3] This dorsal cholinergic pathway plays a role in transmitting sensory input from the thalamus to the cortex. Acetylcholine is a regulator of thalamic depolarization, thereby allowing the transmission of sensory signals. The connection between the subcortical structures, thalamus and cortex via acetylcholine is a source of rapid electroencephalographic wave activity (EEG) during the wake and REM sleep phases. In the NREM sleep phase, the neurons in the thalamus hyperpolarize so that their reciprocal relationship with the cortex is disturbed. The predominance of GABAergic during this phase in the thalamus and cortex gives slow-wave EEG images and sleep spindles.[3]

2. Ventral Monoaminergic Awareness System

The monoaminergic system involves four types of neurotransmitters, namely norepinephrine, histamine, dopamine and serotonin, produced from the nuclei in the rostral pons and the hypothalamus. Norepinephrine is produced by the ceruleus locus (LC), histamine is produced by the tuberomammillary nucleus (TMN), and serotonin is released by the dorsalis raphe nucleus (DRN). There are four sources of dopamine in the human brain, namely mesolimbic, mesocortical, nigrostriatal and ventral periaqueductal grey areas. In the wake-sleep cycle, only dopaminergic neurons in the ventral periaqueductal grey are involved.[3] Norepinephrine and histamine play a role in maintaining attention, especially to new stimuli (novel stimuli) and/or stressors. Dopamine is associated with reward-motivated behaviour because of its association with the limbic system.[3] The projections of the nuclei in the rostral pons in this ventral pathway are projected directly to the area of the lateral hypothalamus, BF and cortex without passing through the thalamus. The activity of this ventral pathway is highest on awakening, decreases in the NREM phase and completely disappears in the REM phase.[3]

3. Hypocretin/Orexin System

Hypocretin/Orexin is produced by a group of neurons in the lateral area of the hypothalamus. This system is responsible for maintaining alertness. Hypocretin/orexin was projected onto TMN, LC and DRN to maintain the waking state. These neurons are maximally active when awake, especially when someone is doing voluntary activities. Hypocretin/orexin is also hypothesized to be involved in the process of addiction, food intake, learning and memory.[3,6]

4. Sleep as a Result of Inhibition of the Awareness System

The brain area responsible for initiating sleep is a cluster of neurons that produce GABA and galanin in the preoptic area. The ventrolateral preoptic (VLPO) is the specific nucleus that acts as a wake-sleep switch. Activated VLPO releases GABA and galanin, which inhibit the awareness system.[2] VLPO activation is influenced by two mechanisms, namely homeostatic control and circadian control.[7]

Adenosine is a significant substance in the control of sleep homeostasis. Adenosine works through the main purine receptors (P1), which consists of A1 receptors widely distributed in the brain and A2a located in the meninges around VLPO. Adenosine has an excitation effect at A2a receptors so that its increased levels will contribute to the activation of VLPO.[3,7]

Circadian control is carried out by the suprachiasmatic nucleus (SCN), a brain’s biological clock. Light is the primary stimulus for adjusting circadian rhythms to the external environment. This circadian rhythm works in a 24-hour cycle. The neuronal signalling from the SCN induces the synthesis and release of melatonin from the pineal gland when light intensity is already low. The highest sleep drive occurs 2 hours after ventricular and circulatory melatonin 3 levels start to increase. Conversely, melatonin itself also serves as a timestamp (a marker of darkness) for the SCN. In blind people, where the light stimulus does not act on the SCN, melatonin acts as a neuroendocrine regulator for the wake-sleep cycle rhythm in 24 hours. Efferent projection from the SCN to the dorsomedial nucleus (DMH) of the hypothalamus, which activates VLPO, is a determinant of sleep initiation.[8]

5. Transition to REM Sleep

The brain transitions in EEG images from slow-wave to fast, low voltage wave, the REM sleep phase during sleep. This phase is characterized by autonomic instability, skeletal muscle atony, and desynchronization of cortical EEG images that cause sleep, also known as paradoxical sleep. At the beginning of identifying this paradoxical sleep, a theory developed that this condition arises due to the interaction between the cholinergic neurons in PPT and LDT that are active during the REM phase with monoaminergic neurons that are inactive during that phase.[9] Various studies later concluded that two types of cholinergic neuron populations are active during the REM phase in this area of the pons, namely those that project rostral to the thalamus, hypothalamus and BF and those that project caudally to the
ventrolateral area of the reticular formation in the medulla oblongata. The first population is responsible for the EEG images that reflect cortical activation, while the second population plays a role in muscle atony during this sleep phase.[9]

B. Gap in Classical Theory
The development of research on the wake-sleep cycle in the last 10 years identified the shortcomings of the classical theory. Damage to one nucleus of the awareness system has little effect on daily sleep quantity. Animal studies have found that damage to some components such as LC and BF still does not provide a substantial change in the wake-sleep cycle. Lesions in the thalamus (such as in patients with the persistent vegetative state) and LDT / PPT still show an EEG image such as awake, NREM and REM. These findings suggest that the reticulo-thalamocortical pathway acts as a modulator in the alert system and is not a major determinant.[10] As already mentioned above, sleep EEG images have long been known to originate in the lower brainstem. Cholinergic activity in PPT and LDT is the source of EEG desynchronization in the REM phase because these areas actively activate BF during paradoxical sleep. However, classical theory cannot explain how the process of transition from slow wave sleep to REM sleep and how the regulation of atony and REM eye movement occurs.[4]

C. Glutamate in the Wake-Sleep Orchestration
The gap from the classical theory of the wake-sleep cycle has led to the study of the role of the fast neurotransmitters, namely the glutamatergic and GABAergic systems. The hypothesis about the important role of glutamate in the wake-sleep cycle originates from the finding that damage to the glutamatergic neuron population in the parabrachial nucleus (PB) and the pedunculopontine nucleus led to a 40% increase in total sleep time.[3]

1. Glutamate in the Structure of the Brainstem and Thalamus
In addition to the neurotransmitters that act as modulators of the wake-sleep cycle, it turns out that the nuclei in the brainstem, hypothalamus, and BF involved in the wake-sleep rhythm also have a population of neurons that produce glutamate and GABA. The studies of the effect of these two neurotransmitters on the wake-sleep cycle were carried out by assessing their effect if there were lesions in these structures and the inhibition and activation processes with optogenetic and chemogenetic stimuli.[4]

These studies identified that glutamate input from PB and PPT nuclei to BF was a major determinant of the awareness system.[11] In addition, glutamate-producing neurons were identified in the supramamillary nucleus (SUM). Damage to glutamatergic neurons in PB and PPT causes impaired consciousness, while lesions in SUM only cause a 20% reduction in waking time.[4]

All thalamocortical projections also use glutamate as the main neurotransmitter. The release of neuronal signals from these glutamatergic neurons in the thalamus is influenced by GABAergic neurons located in the reticular nucleus of the thalamus (TRN). During the wake and REM sleep phase, the GABAergic neurons in these TRNs release signals tonically, whereas they only release bursting mode signals during the NREM phase.[11]

2. Projection of Ascenden and Descenden Glutamate from BF
The classical theory concluded that cholinergic neurons are responsible for the projections from BF to the cortex. However, recent studies have identified that in addition to these cholinergic neurons, in BF, there are also glutamatergic and GABAergic neurons that also project into the cortex.[4,11] A part of BF’s glutamatergic and GABAergic neurons were also identified to transmit descending projections toward the posterior hypothalamus and pontomedullary tegmentum.[11]

Studies using juxtacellular recording have identified that all cholinergic neurons in BF are active in the wake and REM sleep phase. Meanwhile, glutamatergic and GABAergic neurons are heterogeneous, consisting of several functional groups, namely groups active during the SWS sleep phase, groups active in the wake phase, and groups active during REM sleep. Glutamatergic neurons that are active in the wake and REM phase and the active groups during the SWS phase are neurons that send descending signals to the cortex. Neurons that are active in the wake and REM phases send descending signals to influence muscle tone and behavioural elements during the wake and REM sleep phases.[11]

3. Glutamatergic Neurons in Paraventricular Nucleus (PVN)
For a long time, it has been known that PVN is the regulatory center for the sympathetic nervous system. This structure also plays a role in maintaining homeostasis under stressful conditions by increasing the physiological response through the hypothalamus-pituitary-adrenal (HPA axis) axis. Various studies have shown that the HPA axis also helps to maintain alertness and modulate sleep and that PVN, which is the main neurotransmitter is glutamate, is a determining factor of this system.[12] A study conducted by Liu et al. in 2017 identified an association between PVN and sleep-regulating centres. Liu et al. identified two types of glutamatergic neuron populations in PVN: neurons that are active in the wake phase and active during REM sleep. Neurons active in the wake-up phase are further divided into two groups, namely alert neurons and action neurons. The first group plays a role in the formation of the EEG image in the wake phase, but this group’s neuronal activity was not sufficient to activate the motor as reflected in the EMG image. The second group, the action neuron, causes autonomic, somatomotor activation and plays a role in desynchronizing EEG images. The awareness neuron group is active in the wake-up phase and REM sleep, while action neurons are only active during the wake-up phase. However, this study found that these neurons only create alertness and does not trigger REM.[12]

4. Glutamatergic in Sleep Control
Sleep is triggered when the activity of the glutamatergic centre is inhibited. In 2014, Anaclet et al. identified that the parafacial zone, an area of the reticular formation in the ventrolateral of the facial nerve genu, is the source of GABAergic input, which is responsible for the EEG image during sleep. GABAergic neurons from this area inhibit glutamatergic neurons in the PB nucleus.[13] The role of the glutamatergic system in the sleep phase is largely related to the REM sleep phase. Recent studies have reported that the glutamatergic neurons involved in the awareness process are also active during the REM phase. This explains the activation of the cortex during this paradoxical
phase of sleep.[4] The glutamatergic neuron population in the ventral of LDT, sublaterodorsal neurons (SLD), is thought to trigger the REM sleep phase. A recent theory hypothesizes that glutamatergic activation of this area is responsible for the desynchronized EEG image during the paradoxical sleep phase by activating PPT and LDT, which pass REM neuronal circuits to the BF and cortex.[4]

The state of atony in the REM phase occurs because glutamatergic neurons from SLD activate inhibit interneurons in the medulla oblongata and spinal cord. The activation of extraocular muscle movements is thought to be due to the projection of the SLD to the reticular formation at Pons.[3,4]

D. Fast and Modulator Neurotransmitters, How It Connected?

Various studies have identified the role of fast neurotransmitters glutamate and GABA to fill the classical wake-sleep cycle theory gap. However, how certain the system interacts with the modulator neurotransmitters has not been identified. The involvement of structures in the brain and a neurotransmitter in the wake-sleep cycle have been identified by performed studies investigating the effect of lesions on a structure on the wake-sleep cycle. Another technique used is to evaluate the levels of neurotransmitter transporter molecules concerning wake-sleep activity, sleep deprivation or sleep induction, or by stimulation or inhibition by optogenetics and chemogenetics. Thus, the results obtained were in the form of information on whether a structure and its neurotransmitters are activated or not during a sleep phase.

Further studies are needed to map the neuronal circuits that link the determinants of neurotransmitters and modulators in the wake-sleep cycle and how exactly the effects of the interactions between these structures and neurotransmitters on alertness, sleep, behaviour and autonomic responses.

Conclusion

Studies in the last decade have further strengthened the role of fast neurotransmitters, glutamate and GABA, as determinants of the wake-sleep cycle. Identifying several structures in the brainstem, hypothalamus and BF with fluctuating populations of glutamate-producing neurons during the wake-sleep cycle completes a gap that classical theory cannot explain. However, how the various neuronal circuits that influence the wake-sleep cycle have been identified by performed studies investigating the effect of lesions on a structure on the wake-sleep cycle. Another technique used is to evaluate the levels of neurotransmitter transporter molecules concerning wake-sleep activity, sleep deprivation or sleep induction, or by stimulation or inhibition by optogenetics and chemogenetics. Thus, the results obtained were in the form of information on whether a structure and its neurotransmitters are activated or not during a sleep phase.

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Conflict of interest

There are no conflicts of interest to declare by any of the authors of this study.

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