

Role of Neurotransmitters in Regulating Sleep And Awake Cycle: A Review

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Abstract: During sleep, a person does not respond to the surrounding environment. Sleep disorders affect personal life of the individuals. In brain, various neurotransmitters are responsible for the sleep and wake cycle. Acetylcholine, serotonin, dopamine, histamine and orexin promote wakefulness while GABA neurotransmitters promote sleep. Two subtypes of histamine are involved in sleep/wake cycle. Histamine release is decreased by the activation of H₃ receptor which encourages sleep, while H₁ receptor promotes wakefulness. Dopamine D₂ receptors are responsible for controlling sleep/wake cycle. It is still not completely understood about which serotonin subtype is involved in controlling sleep/wake pattern. GABA neurons are present in preoptic area and basal forebrain and their release become increase during sleep. GABA neurotransmitters are divided into three subclasses e.g. GABA_A, GABA_B and GABA_C. GABA_A and GABA_C neurotransmitters produce their effects by inhibiting Cl⁻ ion channels. GABA_C neurons are mainly located in retina while GABA_A neurons are located throughout the brain. GABA_B neurons produce their effects by inhibiting Ca²⁺ in the presynaptic vesicles and these neurons are located in spines.

Keywords: GABA A; GABA B; Sleep awake cycle; Neurotransmitters; Brain receptors

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I. Introduction

Sleep: Sleep is a process in which body and mind are in the state rest, eyes are closed and body does not respond to surrounding. Sleep duration varies from individuals to individuals and age to age for example infant's duration of sleep is 12-15 hours. Child with the age between 3-4 years, duration of sleep is 11-14 hours. Teenager duration of sleep is 8-10 hours and adults duration of sleep is 7-8 hours. With the increase of age duration of sleep become decrease^[1]. Improper sleep also affects person social and personal life^[2]. There are two types of sleep non-rapid-eye-movement (NREM) and rapid-eye-movement (REM) sleep. NREM is further divided into four stages. NREM stage 1, this stage constitutes 2-5% of total sleep. Eyes are closed but individual is conscious with the surroundings and can awake easily. EEG shows slow range of 4-7 Hz spikes. NREM stage 2, this stage constitutes 45-55% of total sleep. Awareness to the surrounding is also occurring in this stage. K-complex and sleep spindles appeared on EEG. NREM stage 3&4 also known as deep sleep and EEG shows delta waves with the range of 1-3Hz^[3]. REM sleep constitutes 25% of the total sleep and about 70-90 minutes after sleeping this stage appeared which continue during whole night. Dreaming also occurs in this stage^[4]

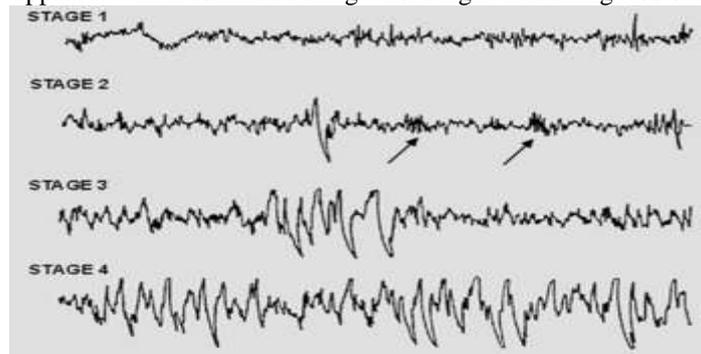


Fig 1: In the human, the gradual slowing of the EEG, and the increasing presence of delta activity, enables NREM sleep to be further classified into four stages. The presence of spindles is marked by the arrows during Stage 2^[5].

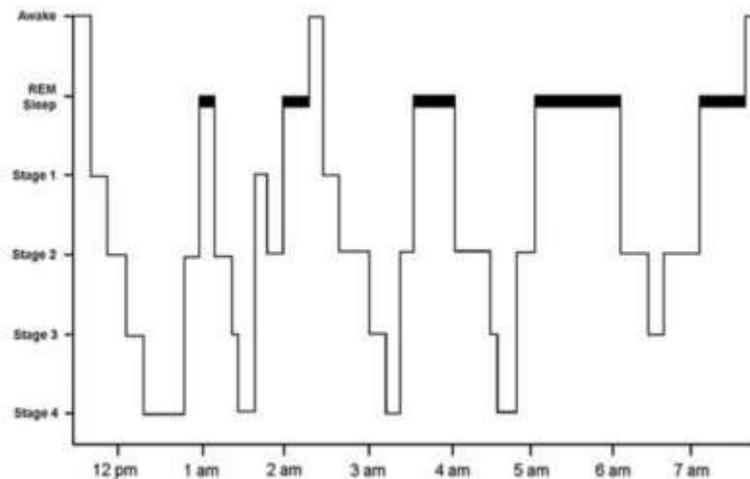


Fig 2: A typical night of sleep in a young human adult is displayed in this hypnogram^[5].
Neurotransmitter Role during Sleep-Wakefulness:

Norepinephrine:

This neuron is a center of flight-or-fight but also play important role during stressful sleep conditions. Main source of norepinephrine in the brain is locus coeruleus, Small amount of norepinephrine is also secreted by cerebral cortex, hippocampus and cerebellum. Locus coeruleus helps in regulation of sleep/wake cycle and LC activation become increase during wakefulness. Studies on rats, monkeys and cats showed that norepinephrine activity in locus coeruleus varied during sleep/wake cycle. Rapidly firing during wakefulness, slow during NREM and stop during REM^[6].

Histamine:

Histaminergic neurons are present in tuberomammillary nucleus (TMN) and also in posterior hypothalamus and from hypothalamus it is projected to main part of the central nervous system. Studied suggested that H₁/H₃ receptor are mainly involved in regulation of sleep/wake cycle. H₁ increased wakefulness whereas H₁ antagonize increased sleep. Release of histamine decreased by the activation of H₃ receptor which in turn induces sleep. By blocking H₃ receptor, wakefulness increased. Histamine mainly release from hypothalamus and also in small amount from other region of brain causes increased wakefulness. Activity of histaminergic neuron becomes increased during wakefulness but stop during NREM and REM sleep^[7].

Dopamine:

Dopamine is mainly responsible for movements and emotions. Dopamine activity becomes increase during wakefulness. Individual with neurological disorder like Parkinson's disease and schizophrenia, this neuron is involved to induce sleep. Previously, mechanism of dopamine during sleep/wake and which dopaminergic neuron is involved in it was unknown, but recent studies showed that REM sleep is due to activation of D₂ receptor not by D₁ receptor pathway. Changes occur in neurotransmitters level during sleep/wake cycle. As a result, firing rate of orexinergic, cholinergic, noradrenergic serotonergic and histaminergic neuron are also affected. These neurons send efferent impulses to cortical and subcortical, as a result of it brain generate a sleep/wake pattern and also increase or decrease muscle activity. During sleep, cortex shows low amplitude with fast oscillation in gamma range and also increases muscular activity. During SWS, cortex shows high amplitude, low oscillation and also low muscle activity. During REM sleep, cortex shows fast gamma oscillation, which are similar during the phase of sleep and muscle activity also decreased^[8].

Serotonin (5-HT):

Serotonin is produced by neuron located in dorsal raphe and raphe nuclei distributed along with the midline of the brainstem. Serotonin performed complex function in the brain like food intake, sexual function thermoregulation and also sleep/wake cycle. The firing rate of serotonin is high during wakefulness, decrease during NREM and stop in REM sleep. Serotonin involved in arousal, is still challenging because it binds with many receptors to produce it effects. Serotonin is divided into 7 classes (5-HT₁, 5-HT₂, 5-HT₃, 5-HT₄, 5-HT₅, 5-HT₆, 5-HT₇) and these 7 classes further subdivide into many subclasses.

Scientist suggested that 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2B}, 5-HT_{2C}, 5-HT₃, 5-HT₇ receptors play an important role in determining the 5-HT effect on sleep/wake cycle^[9].

Acetylcholine:

Cholinergic neurons are located in the brain stem and basal forebrain that stimulate wakefulness and REM sleep. Cholinergic neurons are also present in pons within laterodorsal and pedunculopontine (LD/PPT). LD/PPT neurons project to thalamus and hypothalamus, and are mostly found activated during wakefulness and REM sleep^[10].

Nicotinic and muscarinic receptors also play an important role. Nicotinic receptors are involved in presynaptic release of glutamate and causes depolarization of interneurons. Muscarinic receptors cause depolarization of neurons through blocking of leaked potassium conductance which in result activate mixed cation channel. Fluctuation in the beta and gamma range (20-20 Hz) and blockage of slow after hyperpolarization^[11].

Orexin:

It is also known as hypocretin and it has two types orexin A and orexin B. Orexin was identified as a ligand for orphan G protein-coupled receptor. Previously, orexin was identified for feeding behavior because of their production from the feeding center in lateral hypothalamic area. Recent studies had shown that deficiency of orexin causes narcolepsy in human which suggested that these neuropeptides play an important role in regulating sleep/ wake cycle because these neuropeptide activate wake active cholinergic and monoaminergic neurons in hypothalamus to maintain long awake period. Recent studies on mice suggested that efferent and afferent systems of orexin are in coordination with energy, homeostasis emotion and arousal. Orexin linked with limbic system which suggested that orexin increase alertness during emotional stimuli. These studies shown that orexin maintain proper wakefulness for the survival in animals^[12].

Gamma-aminobutyric acid (GABA):

GABA is mainly known for its inhibitory effect in brain. GABAergic neurons are located in basal forebrain and preoptic area. Many studies have suggested that the release rate of GABA is increase during sleep rather than during wakefulness. GABAergic neurons which are present in preoptic area shows an increased expression during sleep. GABAergic neurons from basal forebrain and preoptic area projected to posteriolateral hypothalamus where these neurons innervate with many other neurons including hypocretin neuron. In thalamus, GABAergic neurons inhibit the thalamocortical relay neuron, because these neurons are also present in thalamus. From thalamus, this neuron projects to cortex and in cortex they are responsible for producing the desynchronous EEG pattern related to REM sleep as well as wakefulness^[13].

GABA_A Receptor:

GABA_A receptors are widely spread throughout in the mammalian central nervous system. GABA receptor produces its inhibitory effect through GABA_AR receptor. GABA_A receptors are ligand gated chloride ion superfamily and the members of this family are heteropentamers. GABA_A receptors are composed of 5 protein subunits but these subunits belong to different subclasses. 16 subunits of GABA_AR receptors have been identified. They are divided into 7 subunit classes based on sequence (α 1-6, β 1-3, γ 1-3, δ , ϵ , θ , and π)^[14]. Studies have shown that α 1 β 1 β 2 β 3 and γ 2 subunits are present throughout the brain but they are also present at different sites and produce different effects. B and γ subunits form benzodiazepine sensitive receptor that facilitates phasic inhibition. α (4/6) β δ subunits are extra synaptic receptors that facilitates tonic inhibition^[15]. Synaptic transmission requires specialized structure called synapses, responsible for the communication between the cells. Number of receptors are located at the synapse determine the synaptic strength, GABA_AR insertion and internalization occur at the site of synapse^[16]. GABA_AR mediated inhibitory transmission which is located outside the synapse, activated by GABA present in the extracellular space. The inhibition produce in extracellular space is known as tonic inhibition, and conductance generated by GABA_AR is known as tonic conductance and such conductance has been found in variety of neurons and interneurons, also including those neurons which are present in cortex, cerebellum, thalamus, hypothalamus and spinal cord^[17].

To study the effect of GABA_A agonist on sleep/wake cycle, Nelson .et.al.monitored c-fos expression in the central nervous system after drug administration. By administering sub anesthetic dose of drugs like propofol, urethane, pentobarbital, muscimol, isoflurane and zolipidem, these drugs prompted a slow wave EEG pattern; increased delta range (0.5 HZ to 4 HZ) and also increased c-fos expression in ventrolateralpreoptic nucleus (VLPO). By administering of several drugs, the number of c-fos positive neurons varied in VLPO but were consistently less than half of the number observed during spontaneous sleep. GABA_A agonist drugs were tested, which confirmed that c-fos expression was inhibited in TMN. In cerebral cortex, the low level of c-fos expression was observed. In other ascending arousal system, c-fos expression was not always inhibited for example administration of alcohol induced c-fos expression in the ventral tegmental dopaminergic neuron while in supraoptic nucleus and hypothalamic periventricular nucleus induced c-fos expression after alcohol administration. This result suggested that GABA_A agonist drugs involve in the endogenous sleep/wake cycle at various site in wake promoting centers and also in sleep promoting VLPO, although lower activation of VLPO

is achieved during spontaneous sleep. In tuberomammillary nucleus (TMN), GABA_A antagonist blocked hypnotic effect produced by propofol, pentobarbital and muscimol because c-fos expression totally suppress in TMN. Experiments on mice showed that benzodiazepines produce sedative effect through α_1 subunit and anxiolytic effect through α_2 subunit [18].

GABA_B Receptor:

This type of receptor produces inhibitory effect in brain by acting through metabotropic and ionotropic receptors to control biochemical and electrical effect of neurons. GABA-Rs are metabotropic G-protein coupled receptors located in brain which release G $\beta\gamma$ subunits that obstruct Ca⁺² channels but activate K⁺ channels. Protein kinase A is also decrease by released Gai /Gao subunits. These subunits also inhibit adenylyl cyclase to decrease the level of cAMP. GABA_B is divided into two classes GABA_{B1} and GABA_{B2}. External agonist is required for the activation of GABA_{B1} whereas GABA_{B2} is liable for signaling and targeting membrane. GABA_{B1} is further subdivided into two classes GABA_{B1a} and GABA_{B1b}. N terminal of these two classes are different. GABA_{B1a} contains a pairs of sushi domains at the N terminal and sushi domains target GABA_{B1a} at the presynaptic terminals of excitatory synapses and control the release of glutamate. GABA_{B1a} and GABA_{B1b} both also found in the dendrites of postsynaptic side. GABA_{B1b} is only located in spines. GABA_{B1b} is responsible for the coupling of K⁺ channels and reduce postsynaptic K⁺ current by knocking out GABA_{B1b}. GABA_{B1b} neuron has direct effect voltage-sensitive Ca⁺² channels so it inhibits dendritic Ca⁺² spikes [19]. Sushi domains which are present on GABA_{B1a} also called complement control protein modules and also located on other G protein-coupled receptor [20].

Drugs which act on GABA_B receptor have several effects on body. Drugs which have agonist effect on GABA_B receptor produce analgesic and muscle relaxant properties and also useful for the treatment of asthma and gastrointestinal disorder. Baclofen is the only drug in the market that acts as a GABA_B agonist because of these side effects like asthenia confusion and sedation are the main obstructs for the development of GABA_B agonist. Drugs which have antagonist effect on GABA_B produce anticonvulsant and antidepressant properties. GABA_B receptor is a heterodimer which contains two seven transmembrane proteins so drugs target distinct receptor to reduce side effect. Gene deletion experiment studies conducted on mice to evaluate the influence of specific protein on phenotype. Studies also have shown that GABA_{B1} completely enhancing cognitive role depending on task. It is also found that deletion of the GABA_{B1} gene reduced immobility in the forced swim test corresponds with reports that GABA_B receptor antagonist shows antidepressant properties, hyperalgesia and hyper locomotion are also noted. GABA_B receptor agonist increases the pain threshold and reduces locomotor activity, Antidepressant drugs like desipramine, imipramine, mianserin and zimelidine does not act direct on GABA_B receptor but indirectly increases through enhancement in monoaminergic activity. Recent studies have shown administration of antidepressant increase GABA_B receptor on spinal cord and cerebral cortex and also shown that administration of baclofen reduce pain threshold when function of GABA_B receptor also reduced [21].

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GABA_C Receptor:

GABA_C receptors are a subfamily of GABA_A receptors and member of Cys-loop of ligand gated ion channel (LGICs). GABA_C are located in retinal neurons and their function is to control retinal signaling which is involved in the pathology of macromolecular degeneration. GABA_C are activated when drugs bind with GABA, but pharmacological properties of GABA_C receptor are different from GABA_A [22]. GABA_C receptor is a pentagonal i.e. composed of five ρ subunits and each subunit has extracellular N-terminal domains. The first response of GABA_C receptor were detected in spinal cord, later GABA_C receptor binding sites are also revealed in cerebellum but GABA_A and GABA_B receptors did not bind in this site [23]. Cis-4-aminocrotonic (CACA) is a GABA_C agonist which produces its inhibitory effect through neuronal firing in spinal cord and does not show response to its antagonist bicuculline. Further studies have revealed that GABA_A showed response against bicuculline whereas GABA_B receptors did not show response against bicuculline and were activated by baclofen. GABA_C receptors are homo oligomeric and pseudoheter-oligomeric receptor, composed of ρ subunits. GABA_A receptors are ligand gated ion channels composed of pentameric mixture of protein. GABA_B receptors are heterodimeric G protein coupled receptor.

GABA_A and GABA_C receptors are from the same subclass of nicotinoid superfamily of ligand gated ion channels. These two are different from each other on the availability of agonists, modulators and antagonists to produce their effects. Cis-4-aminocrotonic (CACA) is a GABA_C receptor agonist which inhibited neuronal

firing of GABA_A receptor antagonist. Methylphosphinic acid is a GABA_C receptor antagonist, isoguvacine ring of tetrahydropyridine make a difference between GABA_A and GABA_C receptors^[24].

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