REVIEW ARTICLE

REM sleep and its mechanism-an updated review

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Abstract

Normal human sleep comprises of two states rapid eye movement (REM) and Non-REM (NREM), which alternates cyclically across a sleep episode.REM sleep was identified by its most typical behavior rapid eye movements during sleep.Electroencephalogram (EEG) of neocortex of most mammals exhibit desynchronized,low-amplitude, and high frequency fast character previously seen in waking, but behavioral sleep persists.This state of sleep called as paradoxical sleep or activated sleep or activated brain in a paralyzed state.Hippocampus has regular high voltage theta waves throughout REM sleep. Multiple neuro-scientific and physiological studies were done to understand the mechanism and genesis of REM sleep.Results of different studies analyze that REM sleep is executed by widely distributed network, rather than single REM sleep center.A number of studies have been attempted to identify pedunculopontine tegmentum (PPT) cholinergic cells present in brainstem, which when activated causes generation and modulation of REM sleep when compared with either NREM or waking. REM sleep sign generator remains in Turned-off condition when aminergic and cholinergic neurotransmitter ratio is1:1 and generator is turned-on to express REM sleep signs when the ratio is 0:0.65. REM-on cells use the neurotransmitter GABA, Ach, glutamate, and glycine, whereas REM-off cells use nor-epinephrine,serotonin, and histamine. c-AMP-PKA intracellular signaling pathway is critically involved in cholinergic cell compartment of the PPT for regulation of cholinergic tone in REM sleep-sign-generators. This review reflects the recent updates on cellular and molecular mechanisms in regulation of REM sleep.

Keywords: REM sleep, PPT, Aminergic and cholinergic neurotransmitters, cAMP-PKA intracellular signaling pathway.

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Introduction

According to a behavioral definition, sleep is a reversible behavioral state of perceptual disengagement from unresponsiveness to the environment. It is a complex amalgam of physiologic and behavioral processes, usually accompanied by postural changes (recumbence,but not necessarily), behavioral

quiescence,closed eyes,increased sensory threshold, and typical electrographic signs[1].

Sleep comprises two states rapid eye movement (REM) and non-REM (NREM) sleep on the basis of physiological parameters. These sleep cycles alternate cyclically throughout each sleep episode. On the basis of pattern of EEG recording, NREM sleep is generally sub-divided into 3 stages. Sleep begins with NREM sleep and each period is approximately 90 minutes[2]. At the starting of sleep state the person is quiet, their respiratory rate, blood pressure, and pulse rate slows down, eyes drift slowly back and forthfrequently and changewith sleep positions[3]. Along with it, awareness to external world decreases,and it is associated with micro hallucinations and illusions. Supported by electroencephalogram (EEG) recording NREM sleep is associated with synchronous, low muscle atonia and minimum psychological activity, sleep spindles, k-complexes, and high voltage slow waves. Electrooculogram (EOG) shows, first slow rolling eye movements later replaced by rapid saccadic eye movements of waking and then subsides, finally eyes assume divergent upward gaze. Slow wave sleep predominates in the first third of the night. 3rd and 4th stages of NREMsleepare combinedly referred as SWS or delta sleep or deep sleep. An incrementally larger stimulus is required to produce an arousal from stage 3 and 4 of NREM sleep. Pulsatile release of GH, sex hormone from pituitary gland and increased production of antibody has been observed. Concurrent existence of all the above events suggests thatNREM sleep is an anabolic process benefiting the somatic tissues[4].

But in contrast, in REM sleep EEG of neocortex shows desynchronized, low-amplitude, high frequency fast character previously seen in waking, but behavioral sleep persists. Dreaming is typical. EOG shows episodic bursts of saccadic eye movements leading to this phase of sleep called as REM sleep. Specific physiological signs associated with REM sleep are atonic muscle, myoclonic twitches in facial, digital and major proximal skeletal muscles, marked variations in cardio-respiratory functions, body temperature, penile erection, and clitoral engorgement in females. It is also linked to circadian rhythm.The paradox of REM sleep is that, EEG shows the person is behaviorally sleeping but resembles that of waking state. That is why this state of sleep is also called paradoxical sleep or activated sleep[5] or activated brain in a paralyzed state.

In 1913, French scientist Henri Pieron authored a book titled "Le problem physiologique du sommeil," which examined sleep for the first time from a physiological perspective. This work is usually regarded as the beginning of the modern approach to sleep research. Dr. Nathaniel Kleitman, now known as the "Father of American sleep research," began work in Chicago in the 1920s questioning the regulation of sleep and wakefulness and of circadian rhythms. Kleitman's crucial work included studies of sleep characteristics in different populations and the effect of sleep deprivation. In 1953, he and one of his students Dr. Eugene Aserinsky postulated that the times infants awakened to nurse on a self-demand schedule would be integral multiples of a basic rest-activity cycle. He made the landmark discovery of REM during sleep. He described that, eye motility was a possible measure of depth of sleep[6].

Neuro-scientific and physiological basis of the nature of modern sleep research comprise brain structures, cell types, neurotransmitters and their receptors,intracellular molecular cascades, neuronal networks, and synaptic interactions. This review is a reflection on neural mechanisms of REM sleep.

Mechanisms of REM sleep

Over the past two decades, by the help of rapidly evolving technologies, progress has been made towards understanding mechanisms regulating sleep and its complexities. Brain transaction experiments like chronic decorticate and mesencephalic cat done by Jouvetand Michel[6] identified specific centers to well characterized states of NREM and REM sleep.Evidence from animal studies on both rat and cat done by Hobbson[7] and Dutta and Maclean[8] has suggested that each of the events of REM sleep is executed by distinct cell groups located in the brainstem. According toVertes[9] there are groups of discrete components of widely distributed network in brainstem, rather than a single REM sleep "center" particularly the pons and adjacent portion of midbrain. Muscle atonia is executed by the activation of neurons in the locus coeruleus alpha (LCa); rapid eye movements result from the activation of neurons in the peri-abducens reticular formation (PAb). As per the principle of sleep physiology, the key factor causing the atonia duringREM sleep is found due to augmented inhibitory (glycinergic and perhaps to a lesser extent GABAergic) synaptic inputs to motoneurons (MNs)[10]. Above study of Chase[10] was contradicted by Brooks[11] whoused local drug application

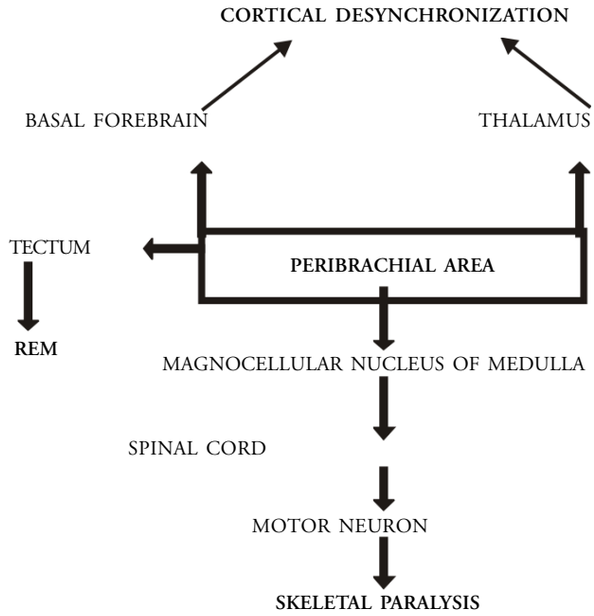
via micro dialysis into the trigeminal motor pool to demonstrate that glycine- and GABAA-receptor mediated inhibition is not the primary cause of REM atonia. As per the laboratory evidences, if glycinergic inhibition is indeed the dominating force driving motor inhibition during REM sleep, then why does blockade of glycine receptors at the trigeminal motor pool not prevent REM atonia in masseter muscles?[11] Currently several researches now provide evidence indicating that glycinergic inhibition plays only a minimal role in mediating REM atonia[12]. Concurrently, studies from multiple laboratories confirmed that the postsynaptic inhibition of motoneurons during REM sleep is initiated by neurons present in or near the vicinity of nucleus pontisoralis[13,14]. These cells also activate premotor inhibitory interneurons in the region of the nucleus gigantocellularis which, in turn, discharge selectively during REM sleep[15-19]. According to Chase et al[13]. in their experiment with hypoglossal postsynaptic inhibition of motoneurons produces atonia of the genioglossal muscle during REM sleep. During quiet wakefulness and NREM sleep, hypoglossal motoneurons exhibited spontaneous repetitive discharge. In the transition from NREM sleep to REM sleep repetitive discharge ceased and the membrane potential began to hyperpolarize; maximal hyperpolarization (10.5 mV) persisted throughout REM sleep. During REM sleep there was a significant increase in rheobase, which was accompanied by barrages of large-amplitude inhibitory postsynaptic potentials (IPSPs) which were reversed following the intracellular injection of chloride ions. The latter result indicates that they were mediated by glycine; IPSPs were not present during wakefulness or NREM sleep. So, it was concluded that the current theory of atonia of muscles during REM sleep is due to glycine receptor-mediated inhibition of post synaptic motorneurons. In contrast to above theory REM atonia persists even when glycine and GABAA receptors are blocked and potent glutamatergic agonists are simultaneously applied to the trigeminal motor pool.

It is interpreted as (1) glycinergic - and GABAA-mediated inhibition plays a minimal role in triggering REM atonia; (2) inhibition primarily functions to suppress muscle twitches during REM sleep; and, (3) a powerful, yet unidentified, inhibitory mechanism or mechanisms override excitation during REM sleep. So,a better possible alternative observation for solving the atonia in REM sleep should be attempted.

Neurotransmitters and receptors

In recent years, a number of studies have been attempted to identify pedunculopontine tegmentum (PPT) cholinergic cells present in brainstem, which when activated causes generation and modulation of REM sleep when compared with either NREM or waking[20]. PPT is situated in dorsolateral mesopontine tegmentum and contains a prominent group of cholinergic neurons projecting throughout brainstem and forebrain. Single cell recording from PPT of cats and rats done by Datta and Siwek[23] showed firing rates of different classes of cells which correlated with both wakefulness and REM sleep. Acetylcholine released from PPT activates medial pontine reticular formation (mPRF). Following activation ofmPRF cells, glutamate is released in the cholinergic cell compartment of the PPT leading to continuous release of acetylcholine into the mPRF and REM sleep sign generators.

Role of ACh-REM sleep initiation starts in the ACh neurons located in the pons, in peribrachial area. From peribrachial area fibers pass directly to thalamus and indirectly to basal forebrain through acetylcholine neurons,to control cortical desynchronization during REM sleep. ACh pathways pass from peribrachial area to tectum, part of midbrain to initiate and maintain rapid eye movement. Fibers from this area pass to magnocellular nucleus of medulla to motor neurons through spinal cord leading to skeletal paralysis.



Role of nor-epinephrine (NE)and 5-hydroxy tryptamine (5HT): Other neurotransmitters like aminergic neurotransmitter released from noradrenergic cells in nucleus locus coeruleus (LC) and serotonergic cells in raphe nuclei (RN) are available[21-26]. According to Hall[27] LC present in dorsal pons contains cell bodies with neurotransmitter nor-epinephrine. RN part of reticular formation located in pons and medulla contains the cell bodies with neurotransmitter serotonin. Fibers from these nuclei project throughout the brain. Experiments on animals showed that, stimulation of NE in LC and 5HT in RN causes cortical activation or arousal. During REM sleep both these NT are at their lowest level. Nor-epinephrine and serotonin complement with ACh. REM sleep is initiated and maintained by ACh. But, controlled and suppressed by NE and 5HT. Datta et al[25] identified cholinergic neurons in PPT that exhibit REM sleep associated immunoreactivity of cAMP response-element-binding protein (pCREB), indicating that the PPT intracellular transcription process is involved in the neurotransmitter receptor activation or inactivation-mediated regulation of wakefulness and REM sleep.

During wakefulness and onset of NREM sleep activity, both aminergic and cholinergic cells are equal. In contrast for generation and maintenance of REM sleep aminergic cell activities are less (<35% of wakefullness) or absent but cholinergic activities are high and important. If a hypothetical ratio made between aminergic and cholinergic neurotransmitter 1:1, REM sleep sign generator remains in turned - off condition. When the ratio is 0:0.65, generator is turned - on to express REM sleep signs[28,22]. It has been found that these cell groups are not critical for REM sleep generator, but likely they modulate expression of REM sleep. Activation of kainate receptors increases cytoplasmic free calcium concentration[29-33]. According to Gintty et al[34]. Ca^{2+} ions stimulate cAMP production and activates protein kinase A (PKA) via adenyl cyclase.Thus cAMP-PKA signal transduction pathway in PPT activates kainate to induce REM sleep[34-37]. According to Subimal Datta and Sarah L. Prutzman's hypothesis signaling pathway may be involved in the PPT to regulate spontaneous REM sleep[38]. Experimental evidence to prove the above hypothesis regarding brainstem PPT adenylyl cyclase in regulation of spontaneous REM sleep, specific adenyl cyclase inhibitor in different doses is microinjected bilaterally into PPT. Comparing alterations in sleep pattern following 4 different doses was evaluated and results analyzed. It demonstrated that reduction of REM sleep was due to increased latency and decreased frequency of REM sleep episodes. Effects on REM sleep were quantified in rats and results analyzed showed inhibition of AC within the cholinergic cell compartment of PPT reduced REM sleep. Recent experimental studies done by increasing catalytic subunit of PKA and PKA enzymatic activity in PPT cholinergic compartment increased REM sleep duration. The same study demonstrated REM sleep suppressionby inhibiting PPT intracellular cAMP-PKA activity.

Maintenance of normal REM sleep episodes

For regulation of cholinergic tone in REM sleep-sign-generators, c-AMP-PKA intracellular signalling pathway is critically involved in cholinergic cell compartment of the PPT. Kainate receptors (GluR6) are rapidly desensitized within seconds but PKA present in the cytoplasm gets activated and phosphorylates GluR6 subunits and modulates channel function for 3-25 minutes. This phosphorylation increases the number of active receptors and gating property of the channel. So for sustained activity of PPT cell and maintenance of REM sleep episodes increased PKA activity is responsible[39].

PPT-NMDA receptor activation-induced wakefulness involves Ca^{2+} or Calmodulin-dependent protein kinaseII (CaMKII)[39] and can conduct Ca^{2+}ions. Activation of NMDA receptors potentiates CaMKII activity, CaMKII transduces membrane-mediated $Ca2^{+}$current to affect transcriptional targets, like cAMP response element binding protein (CREB)[38]. As per recent development, in low wakefulness or high REM sleep, level of CaMKII and phosphorylated CaMKII expression in PPT decreases. In contrast, it increases with high wakefulness or low REM sleep periods. In normal sleep-wake cycle increased wakefulness at the expense of REM sleep which requires increased CaMKII activity in PPT[40].

Conclusion

REM sleep is characterized by cortical activation, muscle atonia and rapid eye movement. Alertness and REM sleep occurs in cyclical manner, regulated by hypothetical ratio made between aminergic and cholinergic neurotransmitter which is expressed as turn-on or turn-off signs. REM sleep is due to high cholinergic neurotransmitter level as compared with aminergic level. Cholinergic inputs from the PPTcholinergic cells and

aminergic inputs from the LC and RN. Cholinergic cells are of two types found in PPT i.e. REM-on and W-REM-on types. From transition of SWS to REM sleep REM-on cell are excited and firing occurs. During alertness and REM sleep W-REM-on cells activity increases causing firing. Glutamate receptors of low threshold kainate type present on PPT cholinergic cells, when activated generation of REM sleep increases. Research studies have also shown that, activated kainate receptors in PPT induce REM sleep via cAMP-PKA signalling pathway. So based on this present understanding, REM sleep is initiated by the kainate receptor activation-mediated excitation of PPT cholinergic cells, and maintained via a mutually excitatory positive feedback loop between the PPT cholinergic and the mPRF glutamatergic cells. As with all biomedical research, the ultimate goal of sleep research is to understand the basic mechanisms of sleep regulation and thereby find causes and cures for sleep disorders.

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