4 Neurobiology of the REM–Non-REM Sleep Cycle

Jerome M. Siegel

• Generation of Cortical Electroencephalogram

The electroencephalogram (EEG) (brain waves) recorded from the cerebral cortex result from the synchronized occurrence of excitatory and inhibitory postsynaptic potentials in cortical neurons. The generation of "sleep spindles" and "slow waves" has been shown to result from the activity of neurons that are able to discharge rhythmically because of their membrane properties. Interconnections between such neurons synchronize their discharges and transmit the rhythmicity to cortical structures. McCormick and Pape[1]; McCormick[2]; Steriade, Gloor, Llinas, et al[3]; and Steriade, McCormick, and Sejenowski[4] have demonstrated that the spindle waves that characterize the sleep EEG are generated by interactions between the nucleus reticularis, which forms a shell surrounding the thalamus, and the thalamic nuclei. The nucleus reticularis is composed of gamma-aminobutyric acid secreting (GABAergic) cells. These cells fire in a 7- to 14-Hz rhythm because of the membrane time course of low-threshold calcium spikes, so named because calcium rather than sodium is admitted through voltage-sensitive channels that open only when the cell is relatively hyperpolarized. After the calcium spikes, membrane currents return the cell to the hyperpolarized state, restarting the process. Through GABA release by their projections into the thalamus, reticularis neurons synchronize rhythmically recurring hyperpolarizations in thalamocortical neurons. Reticularis-induced inhibitory postsynaptic potentials (IPSPs) result in rebound depolarizations in the thalamocortical cells because the hyperpolarization also "turns on" a low-threshold calcium current in these cells. These depolarizations of thalamocortical cells produce action potentials and cortical excitatory and inhibitory postsynaptic potentials (excitatory postsynaptic potential [EPSPs] and IPSPs), which cause the waves recorded as sleep spindles. Delta waves are produced by a similar process occurring at higher levels of membrane hyperpolarization, which produce a slower membrane oscillation.

Ponto-Geniculo-Occipital (PGO) Spikes

PGO spikes are high-voltage potentials that occur singly or in bursts primarily, but not exclusively, during rapid eye movement (REM) sleep. They are so named for the structures in which they appear most prominently, that is, the pons, lateral geniculate body, and occipital cortex. These spike potentials originate in neurons in the laterodorsal tegmental (LDT) nucleus and Pedunculopontine nucleus (PPN) (LDT/PPN), which are largely cholinergic cell groups in the dorsal pons.[5,6] PGO spikes are correlated with but do not necessarily precede the other phasic events of REM sleep.[7,8] Morrison and Bowker[9] found that a wave like the PGO may be evoked in alert waking by abrupt stimuli, similar to those that elicit the startle response. It has been hypothesized that the "spontaneous" PGO spikes of REM sleep may be generated by an internal activation of startle response circuits.

Neuronal Activity across the Sleep Cycle

For most neurons, discharge rates and patterns during REM sleep resemble those during active

waking. Neuronal activity reaches its lowest level during non-rapid eye movement (NREM) sleep. The general pattern of reduced neuronal activity during NREM sleep relative to active waking, and high rates and irregular discharge patterns in REM sleep, is reflected in metabolic rate and brain temperature. In NREM sleep, brain metabolic rate and brain temperature are at their lowest levels. In REM sleep, brain temperature and metabolic rate are maximal, typically equal to or greater than their peak waking values.

• Hypothalamic and Brainstem Neurons Actively Generate Sleep Phenomena

Unlike other behavioral drives, such as feeding, drinking, respiration, or sex, people do not know what the adaptive function of sleep is. However, although the function of sleep remains one of the greatest biologic mysteries, great progress has been made in understanding the mechanisms gen erating sleep states. The further analysis of mech anism is a sure route to a better understanding of the function(s) of sleep. The question of how sleep is generated can be subdivided into the questions of how REM and NREM sleep are generated.

NREM Sleep Generation

Although transection (i.e., making a complete cut across the brainstem) of the neuraxis at the spinomedullary junction does not greatly affect sleep in the forebrain, transection further forward in the brain does. "Midpontine-pretrigeminal" transection in front of the entry point of the trigeminal nerve prevents trigeminal, as well as spinal, sensory inputs from reaching the forebrain. Contrary to the concept that sensory input is responsible for maintaining waking, these animals have considerably reduced sleep (assessed by EEG and eye movement measures) in the deafferented forebrain.[10] The region where stimulation is most potent in producing waking, the midbrain reticular formation, is just in front of this transection.[11] Therefore, the increased waking of the midpontine-pretrigeminal animal can be explained as a release of the midbrain arousal system from more caudal inhibitory influences.

An important component of the midbrain arousal system arises from cholinergic neurons in the LDT/PPN.[12] These nuclei are located in the midbrain and adjacent dorsal pons. Acetylcholine released by axons of these cells terminating in the diencephalon blocks the oscillatory discharge of the thalamic mechanisms that are responsible for EEG spindles and slow waves. Recent in vitro studies have shown that this is accomplished, by reducing K^+ conductances and thereby producing depolarization of thalamic cells and hyperpolarization of nucleus reticularis cells.[1,2] This prevents oscillatory discharge in both populations. Most cholinergic cells in the pons are maximally active in both waking and REM sleep, consistent with the EEG desynchrony seen in both of these states.

Major changes in NREM sleep can be induced by manipulation of the posterior hypothalamus. The posterior hypothalamus contains cells that release histamine into brainstem and forebrain regions mediating arousal.[13-15] The inactivation of these neurons by ingestion of antihistamines or by lesions inactivating histaminergic and adjacent neurons produces sleep and EEG synchrony. Stimulation produces arousal resembling that produced by midbrain stimulation.

The posterior hypothalamus and regions around the fornix contain groups of neurons that synthesize hypocretin (also called orexin).[16,17] This neurotransmitter has an important role in maintaining arousal and also in facilitating muscle tone. Malfunction of this system either through mutations in the gene responsible for synthesizing the hypocretin or its receptors or damage to the hypocretin neurons causes symptoms of narcolepsy, that is, an inability to maintain wakefulness and muscle tone during wakefulness.[18-21]

Going further forward in the brain, the anterior hypothalamus and adjacent basal forebrain region have the most potent sleep-promoting effects seen in the brain. A unique type of cell, the NREM-on cell, localized to this area is likely to be responsible for these effects.[22,23] These cells are maximally active in NREM sleep and are inactive in both waking and REM sleep. The preoptic NREM-on cells are also thermosensitive, most increasing discharge when heated. These cells may mediate the somnogenic effects of heat. Thus, the investigation of NREM sleep generation has led us to one possible function of sleep, a homeostatic reduction in activity in response to brain heating. The chemical nature of the basal forebrain sleep-active cells is not yet established.

However, they may be GABAergic. Cholinergic cells in the same regions may discharge in reciprocal relation to the NREM sleep-on cells, like pontine cholinergic cells. This is likely to be the mechanism underlying the increased release of acetylcholine seen in the cortex in waking and REM sleep.

In conclusion, NREM sleep results from coordinated activity in a number of brain regions. Hypnogenic influences from the medulla and basal forebrain are balanced against arousing influences from the posterior hypothalamus and midbrain. The functional role of each of these inputs remains to be determined, but the evidence suggests that the basal forebrain portion of the circuit may have a thermoregulatory role.

Although REM and NREM sleep mechanisms can be separated, they are not independent. Most manipulations that reduce NREM sleep time also reduce REM sleep time. For example, basal forebrain lesions that reduce NREM sleep below approximately 20% of total time in the cat (versus the normal 60%) completely block REM sleep.[24-26] This is due to a forebrain inhibition of brainstem mechanisms generating REM sleep, because total removal of the forebrain does not reduce REM sleep to a comparable extent.

REM Sleep Generation

Although the normal expression of REM sleep depends on many neural systems, the principal generator mechanisms for REM sleep are localized to the rostral pons and caudal midbrain, as indicated by transection, lesion, stimulation, and unit recording studies.

Transection Studies. Michel Jouvet[27] found that transection of the brainstem in front of the caudal midbrain did not prevent the appearance of REM sleep behind the transection. REM sleep may be recognized by muscle atonia, rapid eye movements, and PGO spikes recordable from what is now known to be their mesencephalic-pontine origins. Whereas cats transected between the brainstem and forebrain showed brainstem signs of REM sleep, forebrain regions in these animals did not show REM sleep.

Because animals and humans with spinal cord transections have REM sleep, and decerebrate animals have brainstem REM sleep, it is apparent that REM sleep generation mechanisms reside in the pontomedullary brainstem. To further localize the regions critical for REM sleep, Siegel, Tomaszewski, and Nienhuis[28] placed transections at the pontomedullary junction. In the brain behind the transection, that is, the medulla and spinal cord, no signs of REM sleep occurred.

A very different picture emerged in regions in front of transections at the pontomedullary junction. There were periods with EEG activity resembling waking and NREM. There were also periods with characteristic EEG, PGO, and neuronal activity patterns of REM sleep. However, unlike normal REM sleep, these periods may continue for hours and rapid eye movements did not consistently accompany the PGO activity.[29]

To summarize the transection work, when the pons and caudal midbrain are left attached to the medulla, a REM sleep-like state appears in the brainstem. When the pons and caudal midbrain are left attached to the forebrain, many aspects of REM sleep appear in the forebrain. The pons and caudal midbrain contain structures necessary and sufficient for many aspects of REM sleep.

Lesion Studies. Localized lesions of the brain-stem have further delimited the regions critical for REM sleep. Bilateral destruction of the dorsal pontine and caudal midbrain reticular formation can completely eliminate REM sleep for extended periods.[30] Small lesions within this region can disrupt specific features of REM sleep. Destruction of the PPN eliminates PGO spike activity and correlated eye movements, even while the "tonic" signs of REM sleep continue. Destruction limited to the region just ventral to the locus coeruleus does not diminish phasic events, but produces REM sleep without atonia, a kind of sleep walking. In this syndrome the cats engage in vigorous motor behaviors while PGO, EEG, and autonomic indicators are those of REM sleep.[31]

Stimulation Studies. George, Haslett, and Jen-den[32] found that microinjection of the acetylcholine agonist carbachol in the pons elicits extended periods of REM sleep. Subsequent studies have found that smaller injections into the Pedunculopontine region produce only PGO and eye movement activity or only muscle atonia without the other aspects of REM sleep.[33] Thus, the dorsal pons contains a cluster of acetylcholine sensitive mechanisms capable of inducing components of REM sleep or the entire state of REM

sleep, depending on the portion of the region activated.

Neuronal Recording. Most brainstem neurons are maximally active in REM sleep, with slightly lower rates in active waking and minimal rates in non-REM sleep.[34,35] Some pontine and medullary reticular cells with this discharge pattern have projections to motoneurons in the spinal cord and extraocular muscle nuclei. Burst discharge in these neurons mediates head, neck, and eye movement in waking and the rapid eye movements and muscle twitches that break through the peripheral motor inhibition of REM sleep.

Many presumably cholinergic neurons in the LDT/PPN region have an REM-on, waking-on discharge pattern, with some discharging in a regular tonic pattern in both states. Such cells with ascending projections help produce the EEG desynchrony of REM sleep and waking as discussed previously.[36]

A subgroup of presumptive cholinergic cells in the LDT/PPN region fires in a burst before PGO spikes and is likely to represent the generator for these waves. It has been hypothesized by Steriade, Pare, Datta, et al[36] that the burst firing in LDT/PPN cells is the result of a rebound calcium spike following a phasic GABAergic inhibition. The mechanism may, therefore, be similar to that involved in slow-wave generation in the thalamus.

REM sleep-on cells, which are silent in active and quiet waking, are thought to control the REM sleep state and generate physiologic changes unique to REM sleep, such as muscle atonia. Some REM-on cells are cholinergic. REM-on cells are localized to the pons and hypothalamus. It is likely that pontine cholinergic REM sleep-on cells have a role in coordinating the neuronal activity changes that trigger the REM sleep state.[37]

Many REM sleep-on cells are clearly not cholinergic and probably use glutamate, GABA, and other neurotransmitters. It is likely that most of these noncholinergic REM sleep-on cells are cholinoceptive.

Descending pathways from pontine REM-on cells are responsible for the suppression of muscle tone that keeps humans from acting out their dreams. Chase and Morales[38] have established that the descending projections of REM sleep-on neurons ultimately excite neurons that release glycine onto motoneurons. The resulting hyperpolarization largely prevents most action potentials in motoneurons, even though motoneurons receive powerful barrages of EPSPs during REM sleep.

Serotonergic neurons, localized to the raphe nuclei, have a REM sleep-off discharge pattern, roughly opposite to that of the REM sleep-on cells.[39] These serotonergic cells have an important role in regulating the discharge of the cholinergic cells responsible for PGO spikes. During waking, the tonic activity of raphe cells releases serotonin on to the cholinergic EDT/PPN cells responsible for PGO wave generation. Serotonin hyperpolarizes the cells, blocking their burst-firing mode. In the transition from NREM sleep to REM sleep, the cessation of activity in serotonergic cells allows the LDT/PPN neurons to begin discharging in bursts mediated by calcium spikes.[4]

Locus coeruleus neurons have an REM sleep-off discharge pattern similar to that of serotonergic cells.[40,41] These cells may also have a similar direct or indirect role in PGO spike suppression, because locus coeruleus lesions, like raphe lesions, produce a release of PGO spikes into waking.[42]

The tonic activity of noradrenergic, serotonergic, and histaminergic cells in waking combines with acetylcholine release to maintain forebrain arousal and EEG desynchrony in waking. These cells also facilitate motoneuron activity in waking.[43] Cessation of activity in norepineph-rinergic and serotonergic cells may cause some of the tonic changes unique to REM sleep, such as the reduction in sympathetic tone.

The cessation of activity in noradrenergic locus coeruleus, serotonergic raphe, and histaminergic posterior hypothalamic neurons is due to the release of GABA onto these neurons by a population of GABAergic REM sleep-on neurons.[15,44,45] Blockade of GABA receptors in the raphe prevents REM sleep, whereas microinjection of GABA agonists produces prolonged REM sleep periods.[45]

To summarize, I have presented evidence that there are several populations of brainstem neurons that are selectively "on" in REM sleep. It is likely that these include neurons using acetylcholine, glutamate, or GABA as their transmitter. I have also pointed out that norepinephrine, serotonin, and histamine cells are selectively "off" in REM sleep. There is good evidence for intercon-

nections between REM active and REM inactive cell populations. However, it remains unclear how the time course of the changes in activity in these cell populations is determined. Hypocretin and other transmitters, as well as intracellular mechanisms, are likely to be involved in coordinating the activity changes in the cells that control REM sleep. Are any of the REM sleep-on or REM sleep-off cells being driven by some biochemical "debt" or "excess" analogous to the changes detected by the neurons that drive the respiratory or feeding systems? Scientists do not yet know the answer to this fundamental mystery of sleep.

• An Overall View

Neurons in the basal forebrain hypothalamus and medulla participate in the generation of NREM sleep. REM sleep is largely controlled by the neurons in the pons and caudal brainstem. Acetylcholine, glutamate, and GABA are widely used in REM sleep circuits, whereas serotonin and norepinephrine block REM sleep phenomena. Thus, sleep is actively generated by an interaction of several neuronal populations and neuro transmitters.

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