REM Sleep*

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Chapter 8

Abstract

Rapid eye movement (REM) sleep was first identified by its most obvious behavior: rapid eye movements during sleep. In most adult mammals, the electroencephalogram (EEG) of the neocortex exhibits low voltage during REM sleep. The hippocampus has regular high-voltage theta waves throughout REM sleep.

The key brain structure for generating REM sleep is the brainstem, particularly the pons and adjacent portions of the midbrain. These areas and the hypothalamus contain cells that are maximally active in REM sleep, called REM-on cells, and cells that are minimally active in REM sleep, called REM-off cells. Subgroups of REM-on cells use the transmitter gamma-aminobutyric acid (GABA), acetylcholine, glutamate, or glycine. Subgroups of REM-off cells use the transmitter norepinephrine, epinephrine, serotonin, histamine, or GABA.

Destruction of large regions in the midbrain and pons can prevent the occurrence of REM sleep. Damage to portions of the brainstem can cause abnormalities in certain aspects of REM sleep. Of particular interest are manipulations that affect the regulation of muscle tone in REM sleep. Lesions of several regions in the pons and medulla can cause REM sleep to occur without the normal loss of muscle tone. In REM sleep without atonia, animals exhibit locomotor activity, appear to attack imaginary objects, and execute other motor programs during a state that otherwise resembles REM sleep. This syndrome may have some commonalties with the REM sleep behavior disorder seen in humans. Stimulation of portions of the REM sleep-controlling area of the pons can produce a loss of muscle tone in antigravity and respiratory musculature, even without eliciting all aspects of REM sleep.

Narcolepsy is characterized by abnormalities in the regulation of REM sleep. Most cases of human narcolepsy are caused by a loss of hypocretin (orexin) neurons. Hypocretin neurons, which are located in the hypothalamus, contribute to the regulation of the activity of norepinephrine, serotonin, histamine, acetylcholine, glutamate, and GABA cell groups. These transmitters have potent effects on alertness and motor control and are normally activated in relation to particular emotions.

Rapid eye movement, or REM, sleep was discovered by Aserinsky and Kleitman in 1953.[1] In a beautifully written paper that has stood the test of time, they reported that REM sleep was characterized by the periodic recurrence of rapid eye movements, linked to a dramatic reduction in amplitude from the higher-voltage activity of the prior non-REM sleep period, as seen on the electroencephalogram (EEG). They found that the EEG of subjects in REM sleep closely resembled the EEG of alert–waking subjects, and they reported that subjects awakened from REM sleep reported vivid dreams. Dement identified a similar state of low-voltage EEG with eye movements in cats.[2] Jouvet then repeated this observation, finding in addition a loss of muscle tone (i.e., atonia) in REM sleep and using the term *paradoxical sleep* to refer to this state. The paradox was that the EEG resembled that of waking, but behaviorally the animal remained asleep and unresponsive.[3-5] Subsequent authors have described this state as activated sleep, or dream sleep. More recent work in humans has shown that some mental activity can be present in non-REM sleep but has supported the original finding that linked our most vivid dreams to the REM sleep state. However, we cannot assume that any person or animal who has REM sleep also dreams. Lesions of parietal cortex and certain other regions prevent dreaming in humans, even in individuals continuing to show normal REM sleep as judged by cortical EEG, suppression of muscle tone, and rapid eye movements.[6] Children younger than 6 years, who have larger amounts of REM sleep than adults, do not typically report dream mentation, perhaps because these cortical regions have not yet developed.[7] The physiologic signs of REM sleep in both the platypus, the animal showing the most REM sleep,[8] and a related monotreme, the short-nosed echidna,[9] are largely restricted to the brainstem, in contrast to their propagation to the forebrain in adult placental and marsupial mammals. These findings make it questionable whether all or any nonhuman mammals that have REM sleep, all of which have cortical regions whose structure differs from that of adult humans, have dream mentation.

This chapter will review the following: (1) the defining characteristics of REM sleep, including its physiology and neurochemistry, (2) the techniques used to investigate the mechanisms generating REM sleep and the conclusions of such investigations, (3) the mechanisms responsible for the suppression of muscle tone during REM sleep, and the pathologic effects of the disruption of these mechanisms, (4) narcolepsy and its link to mechanisms involved in REM sleep control, and especially to the peptide hypocretin, and (5) the functions of REM sleep.

CHARACTERISTICS OF REM SLEEP

The principal electrical signs of REM sleep include a reduction in EEG amplitude, particularly in the power of its lower-frequency components (Fig. 8-1). REM sleep is also characterized by a suppression of muscle tone (atonia), visible in the electromyogram (EMG). Erections tend to occur in males.[10] Thermoregulation (e.g., sweating and shivering) largely ceases in most animals, and body temperatures drift toward environmental temperatures, as in reptiles.[11] Pupils constrict, reflecting a parasympathetic

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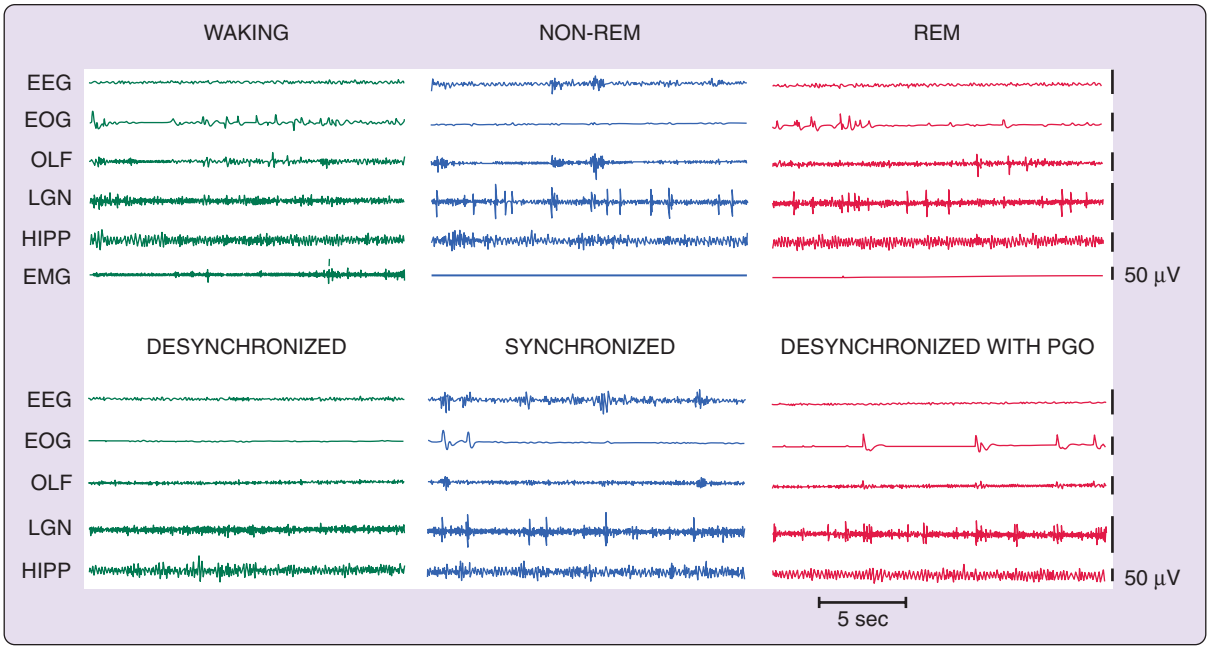

Figure 8-1 *Top,* Polygraph tracings of states seen in the intact cat. *Bottom,* States seen in the forebrain 4 days after transection at the pontomedullary junction. EEG, sensorimotor electroencephalogram; EMG, dorsal neck electromyogram; EOG, electrooculogram; HIPP, hippocampus; LGN, lateral geniculate nucleus; OLF, olfactory bulb; PGO, ponto-geniculo-occipital.

dominance in the control of the iris.[12] These changes that are present throughout the REM sleep period have been termed its tonic features.

Also visible are electrical potentials that can be most easily recorded in the lateral geniculate nucleus of the cat.[13] These potentials originate in the pons, appear after a few milliseconds in the lateral geniculate nucleus, and can be observed with further delay in the occipital cortex, leading to the name ponto-geniculo-occipital (PGO) spikes. They occur as large-amplitude, isolated potentials 30 or more seconds before the onset of REM sleep as defined by EEG and EMG criteria. After REM sleep begins, they arrive in bursts of three to ten waves, usually correlated with rapid eye movements. PGO-linked potentials can also be recorded in the motor nuclei of the extraocular muscles, where they trigger the rapid eye movements of REM sleep. They are also present in thalamic nuclei other than the geniculate and in neocortical regions other than the occipital cortex.

In humans, rapid eye movements are loosely correlated with contractions of the middle ear muscles of the sort that accompany speech generation and that are part of the protective response to loud noise.[14] Other muscles also contract during periods of rapid eye movement, briefly breaking through the muscle atonia of REM sleep. There are periods of marked irregularity in respiratory and heart rates during REM sleep, in contrast to non-REM sleep, during which respiration and heart rate are highly regular. No single pacemaker for all of this irregular activity has been identified. Rather, the signals producing twitches of the peripheral or middle ear muscles may lead or follow PGO spikes and rapid eye movements. Bursts of brainstem neuronal activity may likewise lead or follow the activity of any particular recorded muscle.[15-17] These changes that occur episodically in REM sleep have been called its phasic features.

As we will see later, certain manipulations of the brainstem can eliminate only the phasic events of REM sleep, whereas others can cause the phasic events to occur in waking; yet other manipulations can affect tonic components. These tonic and phasic features are also expressed to varying extents in different species, and not all of these features are present in all species that have been judged to have REM sleep.[18]

REM GENERATION MECHANISMS

Technical Considerations

The identification of sleep-generating mechanism can be achieved by *inactivation* or destruction of particular brain regions or neurons, by the *activation* of populations of neurons, or by *recording* the activity of neurons or measuring the release of neurotransmitters. Each approach has its advantages and limitations.

Inactivation of Neurons by Lesions, Inhibition, Antisense Administration, or Genetic Manipulation

More has been learned about brain function and about sleep control from brain damage caused by stroke, injury, or infection in patients, and by experimentally induced brain lesions in animals, than by any other technique. However, some basic principles need to be borne in mind when interpreting such data.

Brain lesions can result from ischemia, pressure, trauma, and degenerative or metabolic changes. In animals, experimental lesions are most commonly induced by aspiration, transection of the neuraxis, electrolysis, local heating by radio frequency currents, or the injection of cytotoxins.

The latter include substances such as *N*-methyl-D-aspartic acid (NMDA) and kainate that cause cell death by excitotoxicity, and targeted cytotoxins such as saporin coupled to particular ligands, which will kill only cells containing receptors for that ligand. Cytotoxic techniques have the considerable advantage of sparing axons passing through the region of damage, so that deficits will be attributable to the loss of local neurons rather than to interruption of these axons.

If damage to a brain region causes the loss of a sleep state, it cannot be concluded that this is where a center for the state resides. Lesion effects are usually maximal immediately after the lesion is created. Swelling and circulatory disruption make the functional loss larger than will be apparent from standard postmortem histologic techniques. The loss of one brain region can also disrupt functions that are organized elsewhere. For example, spinal shock, a phenomenon in which severing the spinal cord's connection to more rostral brain regions causes a loss of functions, is known to be mediated by circuits intrinsic to the spinal cord.

On the other hand, with the passage of time, this sort of denervation-induced shock dissipates. In addition, adaptive changes occur that allow other regions to take over lost functions. This is mediated by sprouting of new connections to compensate for the loss. A striking phenomenon seen after placement of lesions aimed at identifying the brain regions responsible for REM and non-REM sleep is that even massive lesions targeted at putative sleep-generating centers often produce only a transient disruption or reduction of sleep, presumably as a result of this compensation.

A particularly useful approach to the understanding of REM sleep generation has been the transection technique. In this approach, the brain is cut at the spinomedullary junction, at various brainstem levels, or at forebrain levels by passing a knife across the coronal plane of the neuraxis. Regions rostral to the cut may be left in situ or may be removed. It might be expected that such a manipulation would completely prevent sleep phenomena from appearing on either side of this cut. However, to a surprising extent this is not the case. As we will review later, REM sleep reappears within hours after some of these lesions. When both parts of the brain remain, signs of REM sleep usually appear on only one side of the cut. This kind of positive evidence is much more easily interpreted than loss of function after lesions, because we can state with certainty that the removed regions are not essential for the signs of REM sleep that survive.

It will soon be possible to acquire mutant mice in which any one, or several, of more than 20,000 genes are inactivated, to the extent that such deletions are not lethal. Investigation of two mutants[19,20] led to major insights into the etiology of human narcolepsy.[21-23] Techniques for the postnatal inactivation of genes permit investigation of gene deletions without the developmental effect of these deletions. They can also be used for investigation of the effects of gene inactivation in particular brain regions. A similar inactivation can be achieved by localized microinjections of antisense. Many if not most such mutants can be expected to have some sleep phenotype, such as increases or decreases in total sleep or REM sleep time, altered sleep rebound, altered responses of sleep to environmental variables, and altered changes in sleep with development and aging. The same interpretive considerations long appreciated in lesion studies apply to the interpretation of manipulations that inactivate genes or prevent gene expression, with the additional possibility of direct effects of genetic manipulation on tissues outside the brain.

Activation of Neurons by Electrical or Chemical Stimulation, Gene Activation, or Ion Channel Manipulation

Sites identified by lesion or anatomic studies can be stimulated to identify their roles in sleep control. Older studies used electrical stimulation and were successful in identifying the medial medulla as a region mediating the suppression of muscle tone,[24] and the basal forebrain as a site capable of triggering sleep.[25] Electrical stimulation is clearly an aphysiologic technique, involving the forced depolarization of neuronal membranes by ion flow at a frequency set by the stimulation device, rather than by the patterned afferent impulses that normally control neuronal discharge. For this reason, it has been supplanted for many purposes by administration of neurotransmitter agonists, either by direct microinjection or by diffusion from a microdialysis membrane that is placed in the target area and perfused with high concentrations of agonists.

One cannot assume that responses produced by such agonist administration demonstrate a normal role for the applied ligand. For example, many transmitter agonists and antagonists have been administered to the pontine regions thought to trigger REM sleep. In some cases, this administration has increased REM sleep. But we can only conclude from this that cells in the region of infusion have receptors for the ligand and have connections to REM sleep–generating mechanisms. Under normal conditions, these receptors may not have a role in triggering the state. Only by showing that the administration duplicates the normal pattern of release of the ligand in this area, and that blockade of the activated receptors prevents normal REM sleep, can a reasonable suspicion be raised that a part of the normal REM sleep control pathway has been identified.

Because it is far easier to inject a substance than to collect and quantify physiologically released ligands, there have been many studies implying that various substances are critical for REM sleep control based solely on microinjection. These results must be interpreted with caution. For example, hypocretin is known to depolarize virtually all neuronal types. It should therefore not be surprising to find that hypocretin microinjection into arousal systems such as the locus coeruleus produces arousal,[26] that microinjection of hypocretin into sites known to control feeding increases food intake,[27] that injection into regions known to contain cells that are waking active increase waking,[28] that injection into regions known to contain cells selectively active in REM sleep will increase the occurrence of this state,[29,30] that injection into regions known to facilitate muscle tone will increase tone, that identical injections into regions known to suppress tone will decrease tone,[31] and that intracerebroventricular injection of hypocretin can increase water intake[32] and can activate other periventricular systems.[29] Such types of findings do

not by themselves demonstrate a role for hypocretin (or any other neurotransmitter) in the observed behavior. It is necessary to obtain data on the effects of inactivation of, for example, hypocretin or hypocretin receptors, and to record evidence that indicates activity of hypocretin neurons at the appropriate times before seriously entertaining such conclusions.

Genetic manipulations enable activation of neurons or nonneuronal cells of a particular type. A recent example of a genetic approach is the insertion of a light-sensitive ion channel into hypocretin cells using a lentivirus. Fiberoptic delivery of light could then be used to activate just these cells and determine the effect on sleep–waking transitions.[33]

Observation of Neuronal Activity

Recording the activity of single neurons in vivo can provide a powerful insight into the precise time course of neuronal discharge. Unit activity can be combined with other techniques to make it even more useful. For example, electrical stimulation of potential target areas can be used to antidromically identify the axonal projections of the recorded cell. Intracellular or juxtacellular[34] labeling of neurons with dyes, with subsequent immunolabeling of their transmitter, can be used to determine the neurotransmitter phenotype of the recorded cell. Combined dialysis and unit recording or iontophoresis of neurotransmitter from multiple-barrel recording and stimulating micropipettes can be used to determine the transmitter response of the recorded cell, although it cannot be easily determined whether the effects seen are the direct result of responses in the recorded cell or are mediated by adjacent cells projecting to the recorded cell. Such distinctions can be made in in vitro studies of slices of brain tissue by blocking synaptic transmission or by physically dissociating studied cells, but in this case their role in sleep may not be easily determined.

Although the role of a neuron in fast, synaptically mediated events happening in just a few milliseconds can be traced by inspection of neuronal discharge and comparison of that discharge with the timing of motor or sensory events, such an approach may be misleading when applied to the analysis of sleep-state generation. The sleep cycle consists of a gradual coordinated change in EEG, EMG, and other phenomena over a period of seconds to minutes, as waking turns into non-REM sleep and then as non-REM sleep is transformed into REM sleep.

Despite this mismatch of time courses, the tonic latency, a measure of how long before REM sleep–onset activity in a recorded cell changes, has been computed in some studies. Neurons purported to show a "significant" change in activity many seconds or even minutes prior to REM sleep onset have been reported. However, such a measure is of little usefulness, because at the neuronal level, the activity of key cell groups can best be seen as curvilinear over the sleep cycle, rather than changing abruptly in the way that activity follows discrete sensory stimulation. A major determinant of the tonic latency, computed as defined here, is the level of noise, or variability in the cell's discharge, which affects the difficulty of detecting a significant underlying change in rate in a cell population. It is therefore not surprising that cell groups designated as executive neurons for REM sleep control on the basis of their tonic latencies were later found to have no essential role in the generation of REM sleep.[35-37] The more appropriate comparison of the unit activity cycle to state control is to compare two different cell types to see what the phase relationship of the peaks or troughs of their activity is, under similar conditions. This kind of study is difficult, involving the simultaneous long-term recording of multiple cells, and it is rarely performed. Even in this case, a phase lead does not by itself prove that the "lead" neuron is driving activity seen in the "following" neuron, but it does indicate that the reverse is not the case. However, awakening is a process that can be studied in this way, because it can be elicited by stimuli, and it appears to be preceded by abrupt changes in the activity of many neuronal groups.[38] A major advantage of unit recording approaches in the intact animal to understand sleep and other behavioral processes is their high level of temporal resolution.

Observation of the normal pattern of neurotransmitter release and neuronal activity can help determine the neurochemical correlates of sleep states. The natural release of neurotransmitters can be most easily determined by placing a tubular dialysis membrane 1 to 5 mm in length in the area of interest and circulating artificial cerebrospinal fluid through it. Neurotransmitters released outside the membrane will diffuse through the membrane and can be collected. Each sample is collected at intervals, typically ranging from 2 to 10 minutes. The collected dialysates can be analyzed by chromatography, radioimmunoassay, mass spectroscopy, or other means. The temporal resolution of this technique is typically on the order of a few minutes for each sample.[39-41]

Unit recording and dialysis approaches require a sharp research focus on a particular neurotransmitter or neuronal group. In contrast, histologic approaches can be used to measure the activity of the entire brain at cellular levels of resolution. The most popular such approach in animal studies labels the activation of immediate early genes. These genes are expressed when a neuron is highly active, and their expression is an early step in the activation of other downstream genes mobilizing the response of the cell to activation. The likelihood of such expression may vary between neuronal types and may not be detectable below some activity-dependent threshold. Activation of these genes can be detected by immunohistochemistry, most commonly by staining for the production of the Fos protein or the mRNA used to synthesize this protein.[42] Neurons can be double-labeled to determine the transmitter they express, allowing investigators to determine, for example, whether histaminergic neurons in the posterior hypothalamus were activated in a particular sleep or waking state. Metabolic labels such as 2-deoxyglucose can also provide an indication of which neurons are active.[42,43] Similar techniques using radioactive ligands in positron emission tomography (PET) studies can be used in living humans or animals. In vivo measurements of blood flow can be made throughout the brain with functional magnetic resonance imaging (fMRI). All of these techniques have in common an ability to make anatomically driven discoveries of brain region involvement in particular states, independent of specific hypotheses, thus leading to major

advances in understanding. However, another common feature of these types of recording techniques is their very poor temporal and spatial resolutions compared with neuronal recording approaches. Fos activation can take 20 minutes or more. PET takes a similar amount of time, and fMRI can be used to observe events lasting on the order of 1 to 15 seconds. It cannot be known whther areas active during a particular state caused the state or were activated because of the state.

Summary

Clearly, there is no perfect technique for determining the neuronal substrates of sleep states. Ideally, all three approaches are used in concert to reach conclusions. Next, we will explore the major findings derived from lesion, stimulation, and recording studies of REM sleep control mechanisms.

Transection Studies

The most radical types of lesion studies are those that slice through the brainstem, severing the connections between regions rostral and caudal to the cut. Sherrington[43a] discovered that animals, whose forebrain was removed after transecting the neuraxis in the coronal plane at the rostral border of the superior colliculus, showed tonic excitation of the "antigravity muscles" or extensors (Fig. 8-2, level A). This decerebrate rigidly was visible as soon as anesthesia was discontinued. Bard and Macht reported in 1958 that animals with decerebrate rigidity would show periodic limb relaxation.[44] We now know that Bard was observing the periodic muscle atonia of REM sleep.

After the discovery of REM sleep in the cat,[2] Jouvet found that this state of EEG desynchrony was normally accompanied by muscle atonia.[4] Jouvet then examined the decerebrate cat preparation used by Sherrington and Bard, now adding measures of muscle tone, eye movement and EEG. One might have expected that REM sleep originated in the forebrain, but Jouvet found something quite different. When he recorded in the forebrain after separating the forebrain from the brainstem at the midbrain level (see Fig. 8-2, levels A or B), he found no clear evidence of REM sleep. In the first few days after transection, the EEG in the forebrain always showed high voltage, but when low-voltage activity appeared, the PGO spikes that help identify REM sleep in the intact animal were absent from the thalamic structures, particularly the lateral geniculate where they can be most easily recorded. Thus it appeared that the isolated forebrain had slow-wave sleep states and possibly waking, but no clear evidence of REM sleep.

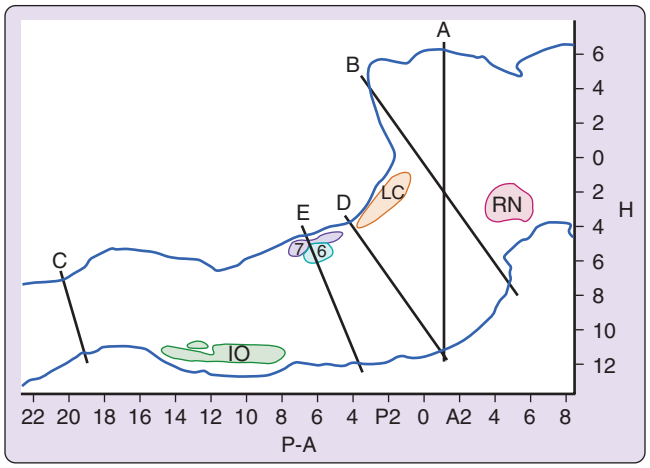

Figure 8-2 Outline of a sagittal section of the brainstem of the cat drawn from level L = 1.6 of the Berman atlas, indicating the level of key brainstem transection studies. H (horizontal) and P-A (anteroposterior) scales are drawn from the atlas. IO, inferior olive; LC, locus coeruleus; RN, red nucleus; 6, abducens nucleus; 7, genu of the facial nerve. (Scales are drawn from Berman AL. The brain stem of the cat. Madison: University of Wisconsin Press; 1968.)

In contrast, the midbrain and brainstem behind the cut showed clear evidence of REM sleep. Muscle atonia appeared with a regular periodicity and duration, like that of the intact cat's REM-sleep periods. This atonia was accompanied by PGO spikes that had a morphology similar to that seen in the intact animal. The pupils were highly constricted during atonic periods, as in REM sleep in the intact cat.

An interesting feature of REM sleep in the decerebrate animal is that its frequency and duration varied with the temperature of the animal. In the decerebrate animal, the forebrain thermoregulatory mechanisms are disconnected from their brainstem effectors. Shivering and panting do not occur at the relatively small temperature shifts that trigger them in the intact animal. For this reason, if the body temperature is not maintained by external heating or cooling, it will tend to drift toward room temperature. Arnulf and colleagues[45] found that if body temperature was maintained at a normal level, little or no REM sleep appeared. But if temperature was allowed to fall, REM sleep amounts increased to levels well above those seen in the intact animal. This suggests that REM-sleep facilitatory mechanisms are, on balance, less impaired by reduced temperature than are REM-sleep inhibitory mechanisms. Another way of looking at this phenomenon is that brainstem mechanisms are set to respond to low temperatures by triggering REM sleep, perhaps to stimulate the brainstem, and that high brainstem temperatures inhibit REM sleep. It is unclear whether this mechanism is operative in the intact animal where temperature shifts are within a much narrower range.

A further localization of the REM sleep control mechanisms can be achieved by examining the sleep of humans or animals in which the brainstem-to-spinal cord connection has been severed (see Fig. 8-2, level C). In this case, normal REM sleep in all its manifestations, except for spinally mediated atonia is present.[46] Thus we can conclude that the region between the caudal medulla and the rostral midbrain is sufficient to generate REM sleep.

This approach can be continued by separating the caudal pons from the medulla (see Fig. 8-2, level D or E). In such animals, no atonia is present in musculature controlled by the spinal cord, even though electrical or chemical stimulation of the medial medulla in the decerebrate animal suppresses muscle tone.[47] Furthermore, neuronal activity in the medulla does not resemble that seen across the REM–non-REM sleep cycle, with neuronal discharge very regular for periods of many hours, in contrast to the periodic rate modulation that is linked to the phasic events of REM sleep in the intact animal (Fig. 8-3).[48] This demonstrates

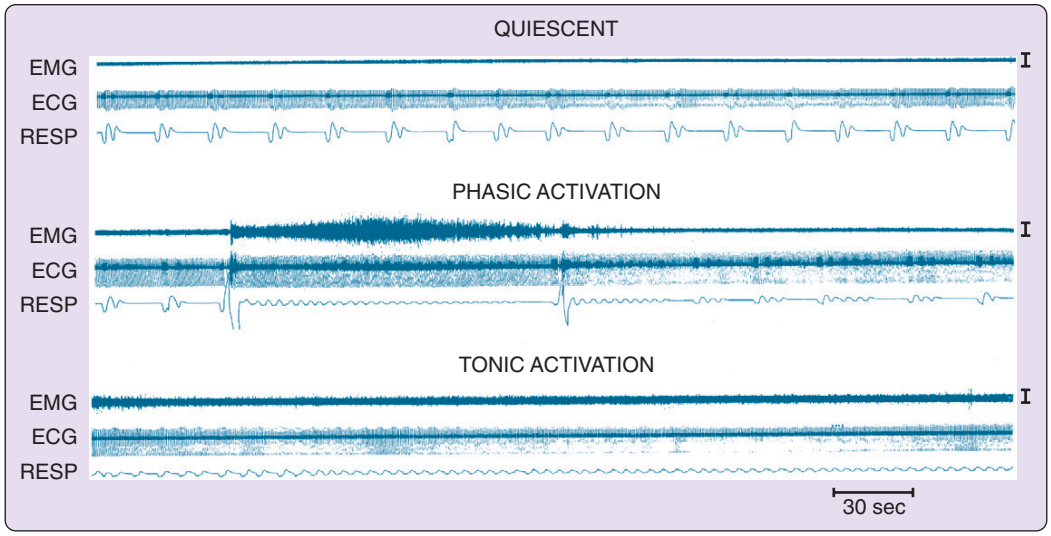

Figure 8-3 States seen in the chronic medullary cat. Note the absence of periods of atonia. Calibration, 50 μV. RESP, thoracic strain gauge measuring respiration. EMG, electromyogram, ECG, electrocardiogram. (From Siegel JM, Tomaszewski KS, Nienhuis R. Behavioral states in the chronic medullary and mid-pontine cat. Electroencephalogr Clin Neurophysiol 1986;63:274-288.)

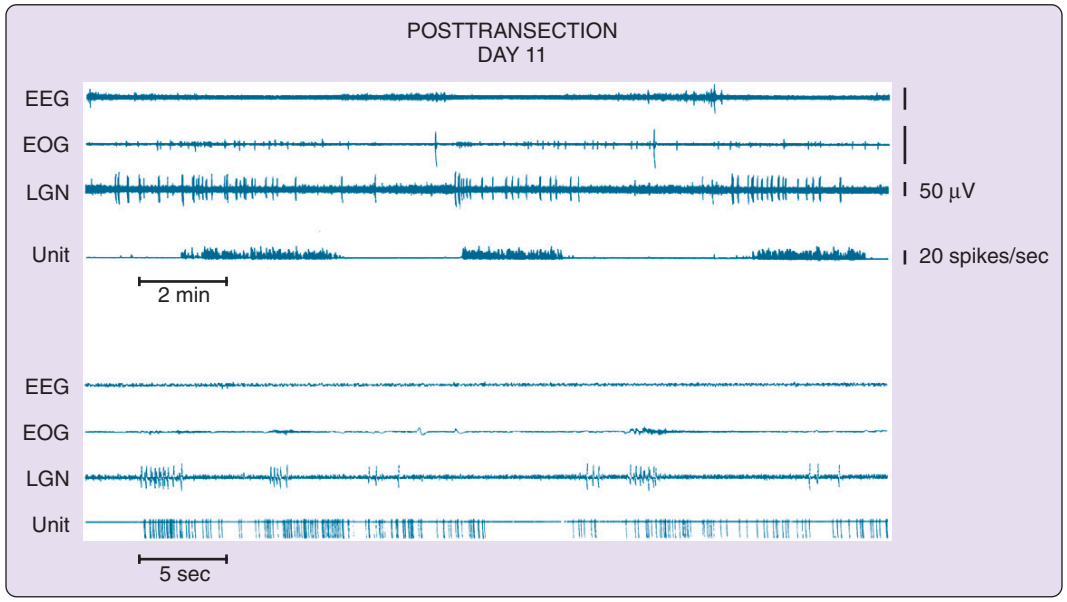

Figure 8-4 Midbrain unit: EEG, electrooculographic (EOG), and lateral geniculate nucleus (LGN) activity rostral to chronic transections at the pontomedullary junction. *Upper:* The unit channel displays the output of an integrating digital counter resetting at 1-second intervals. *Lower:* One pulse is produced for each spike by a window discriminator. (From Siegel JM. Pontomedullary interactions in the generation of REM sleep. In: McGinty DJ, Drucker-Colin R, Morrison A, et al, editors. Brain mechanisms of sleep. New York: Raven Press; 1985. pp. 157-174.)

that the medulla and spinal cord together, although they may contain circuitry whose activation can suppress muscle tone, are not sufficient to generate this aspect of REM sleep when disconnected from more rostral brainstem structures.

In contrast, the regions rostral to this cut show aspects of REM sleep (Fig. 8-4, and see Fig. 8-1, bottom).[49] In these regions, we can see the progression from isolated to grouped PGO spikes and the accompanying reduction in PGO spike amplitude that occurs in the pre-REM sleep period and the REM sleep periods in the intact animal. We also see increased forebrain unit activity, with unit spike bursts in conjunction with PGO spikes, just as in REM sleep.[48,50]

To summarize, this work shows that when pontine regions are connected to the medulla, atonia, rapid eye movements and the associated unit activity of REM sleep occur, whereas the medulla and spinal cord together, disconnected from the pons, are not sufficient to generate these local aspects of REM sleep. When the pons is connected to the forebrain, forebrain aspects of REM sleep are seen, but the forebrain without attached pons does not generate these aspects of REM sleep. Further confirmation of the importance of the pons and caudal midbrain comes

from the studies of Matsuzaki,[51] who found that when two cuts were placed, one at the junction of the midbrain and pons and the other at the junction of the pons and medulla, periods of PGO spikes could be seen in the isolated pons, but no signs of REM sleep in structures rostral or caudal to the pontine island.

These transection studies demonstrate, by positive evidence, that the pons is sufficient to generate the pontine signs of REM sleep—that is, the periodic pattern of PGO spikes and irregular neuronal activity that characterizes REM sleep. One can conclude that the pons is the crucial region for the generation of REM sleep. Later, we will consider in more detail the structures in this region that synthesize the core elements of REM sleep.

However, it is also clear that the pons alone does not generate all the phenomena of REM sleep. Atonia requires the activation of motor inhibitory systems in the medulla.[52] In the intact animal, forebrain mechanisms interact with pontine mechanisms to regulate the amplitude and periodicity of PGO spikes,[53] which in turn are linked to the twitches and rapid eye movements of REM sleep. We know from cases of human REM sleep behavior disorder that the motor activity expressed in dreams is tightly linked to the imagery of the dream.[54] Extrapolating to dream imagery in normal humans, one can hypothesize that because the structure of REM sleep results from an interaction of forebrain and brainstem mechanisms, the dream itself is not just passively driven from the brainstem but rather represents the result of a dynamic interaction between forebrain and brainstem structures.

Localized Lesion Studies

The transection studies point to a relatively small portion of the brainstem—the pons and caudal midbrain—as being critical for REM sleep generation. Further specification of the core regions can be achieved by destroying portions of the pons in an otherwise intact animal and seeing which areas are necessary and which are unnecessary for REM sleep generation. An early systematic study by Carli and Zanchetti in the cat[55] and other subsequent studies emphasized that lesions of locus coeruleus[56] and the dorsal raphe[57] nuclei, or of simultaneous lesions of locus coeruleus, forebrain cholinergic neurons, and histamine neurons,[58] did not block REM sleep. Carli and Zanchetti concluded that lesions that destroyed the region ventral to the locus coeruleus, called the nucleus reticularis pontis oralis or the subcoeruleus region, produced a massive decrease in the amount of REM sleep. In their studies, Carli and Zanchetti used the electrolytic lesion technique, in which a current is passed, depositing metal that kills cells and axons of passage. As cytotoxic techniques that allowed poisoning of cell bodies without damage to axons of passage came into use, these initial conclusions were confirmed and refined. It was shown that neurons in medial pontine regions including the giant cell region were not important in REM sleep control,[52,59,60] as near-total destruction of these cells was followed by normal amounts of REM sleep as soon as anesthesia dissipated.[36,61] However, lesions of the subcoeruleus and adjacent regions with cytotoxins did cause a prolonged reduction in the amount of REM sleep. According to one study, the extent of this loss was proportional to the percentage of cholinergic cells lost in subcoeruleus and adjacent regions of the brainstem of the cat.[62] In rats, lesion or inactivation of the same region below the locus coeruleus (called the sublaterodorsal nucleus in the terminology of Swanson[63]) has been found to reduce REM sleep.[64]

Although large lesions may eliminate all aspects of REM sleep, small bilaterally symmetrical lesions in the pons can eliminate specific aspects of REM sleep. Lesions of lateral pontine structures allow muscle atonia during REM sleep. However, PGO spikes and the associated rapid eye movements are absent when lesions include the region surrounding the superior cerebellar peduncle of the cat (Fig. 8-5, top).[65] This points to the role of this lateral region in the generation of PGO waves and some of the associated phasic activity of REM sleep.

Small lesions confined to portions of the subcoeruleus regions identified as critical for REM sleep by Carli and Zanchetti, or to the medial medulla[52] result in a very unusual syndrome. After non-REM sleep, these animals enter REM sleep, as indicated by lack of responsiveness to the environment, PGO spikes, EEG desynchrony, and pupil constriction. However, they lack the muscle atonia that normally characterizes this state (see Fig. 8-5, bottom).[5,66] During REM sleep without atonia, these animals appear to act out dreams, attacking objects that are not visible, exhibiting unusual affective behaviors and ataxic locomotion. When they are awakened, normal behavior resumes. More recent studies have demonstrated that lesions of a system extending from the ventral midbrain to the medial medulla can cause REM sleep without atonia and that activation of this system can suppress muscle tone.[52,67-69]

This subcoeruleus region is under the control of midbrain regions. A midbrain region located just beneath and lateral to the periaqueductal gray (and called the dorsocaudal central tegmental field in the cat), appears to inhibit REM sleep by inhibiting the critical REM-on subcoeruleus neurons. Muscimol, a gamma-aminobutyric acid A ($GABA_A$) receptor agonist, injected into this midbrain region, silences these cells and increases REM sleep, presumably by blocking the inhibition.[70] The same phenomena have been observed when muscimol is injected into the corresponding region of the guinea pig[71] and the rat (in the rat, this midbrain region has been called the deep mesencephalic nucleus).[72] The midbrain region of the deep mesencephalic nucleus is the heart of the classic reticular activating system, shown to induce waking when electrically stimulated,[73] and coma when lesioned.[74] Both of these manipulations affect not only intrinsic neurons but also axons of passage.

Increasing the levels of GABA in the subcoeruleus region (also called the pontine oralis nucleus in the rat and cat) produces an increase in waking, rather than the increase in REM sleep seen with GABA injection into the midbrain regions indicated previously.[75,76] This is another reminder that, despite the sleep-inducing effect of systemic administration of hypnotic medications, local manipulation shows that the effect of GABA on sleep and waking states varies across brain regions. Blocking GABA in the subcoeruleus has been reported to increase REM sleep in the cat.[77]

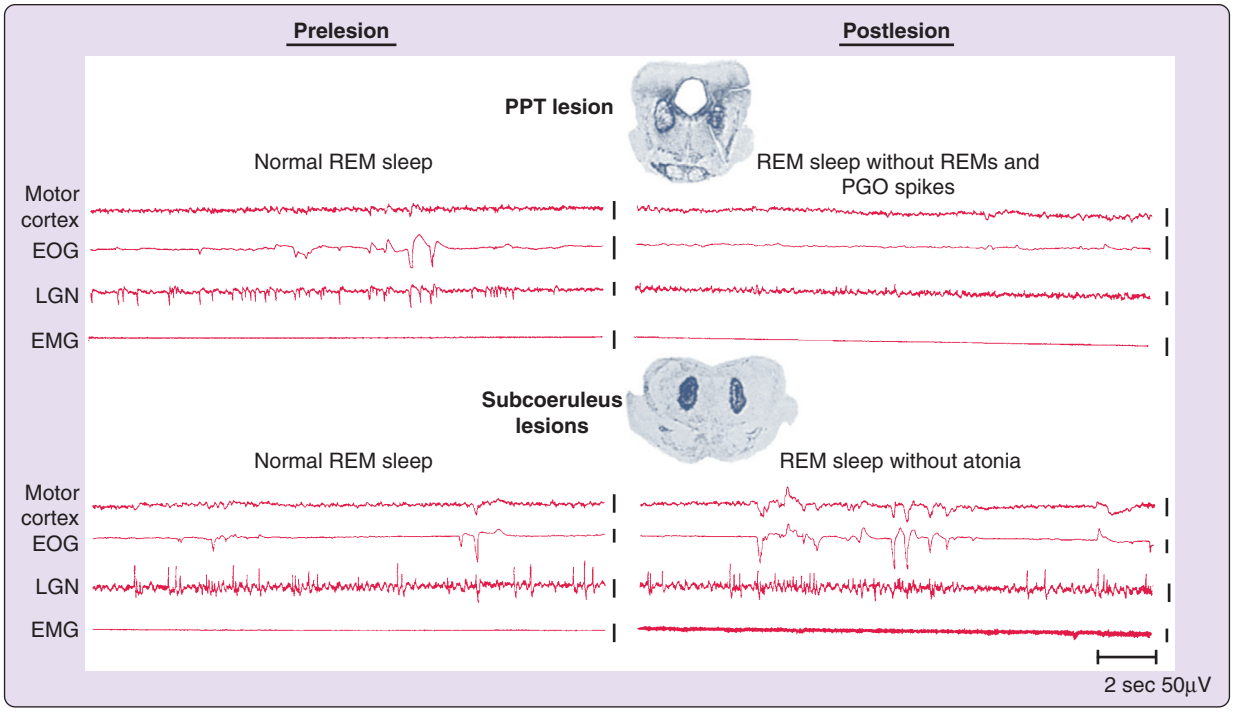

Figure 8-5 Twenty-second polygraph tracings of REM sleep before and after lesions, together with a coronal section through the center of the pontine lesions. EEG voltage reduction of REM sleep (recorded from motor cortex) was present after both lesions. *Top,* Radiofrequency lesions of the pedunculopontine region diminished ponto-geniculo-occipital (PGO) spikes and eye movement bursts during REM sleep. *Bottom,* Lesions in the region ventral to the locus coeruleus produced REM sleep without atonia without any diminution of PGO spike or REM frequency. (Reprinted from Shouse MN, Siegel JM. Pontine regulation of REM sleep components in cats: integrity of the pedunculopontine tegmentum [PPT] is important for phasic events but unnecessary for atonia during REM sleep. Brain Res 1992;571:50-63, copyright, with permission from Elsevier Science.) EEG, Electroencephalogram; EOG, electro-oculogram; LGN, lateral geniculate nucleus; EMG, dorsal neck electromyogram.

Stimulation Studies

The first study showing that stimulation could elicit REM sleep was carried our by George and colleagues.[78] They found that application of the acetylcholine agonist carbachol to specific regions of the pons ventral to the locus coeruleus could elicit REM sleep in the cat. An impressive proof that a unique REM sleep–generation mechanism was being activated was the long duration of the elicited REM sleep periods. Microinjection of acetylcholine into this region in the decerebrate cat produces an immediate suppression of decerebrate rigidity. Later studies showed that, depending on the exact site, either REM sleep or just atonia in a waking state could be triggered by such stimulation.[79-81] When stimulation was applied to the lateral regions whose lesion blocked PGO waves, continuous PGO spikes were generated even though the animal was not always behaviorally asleep.

Increased REM sleep has been reported in the rat after microinjection of cholinergic agonists into the subcoeruleus region,[82-84] although this effect is certainly not as robust as it is in the cat.[85]

The first study demonstrating a role for glutamate in the control of REM sleep was done in the cat. We found that a profound suppression of muscle tone could be elicited by the injection of glutamate into the subcoeruleus region or into the ventral medullary region.[47,86,87] Further work has demonstrated that the pontine cells in this inhibitory region receiving this cholinergic input use glutamate as their transmitter and project directly to glutamate-responsive regions of the medial medulla.[86,88-90]

Work in the rat has emphasized the strong triggering of REM sleep by glutamatergic excitation of this region.[64,91] However, glutamatergic excitation of this region in the cat also increases REM sleep,[92] suggesting that the difference in response in the two species does not indicate a fundamental difference in control features, although it does indicate species differences in the relative potency of these transmitters or perhaps in the pattern of distribution of receptors for them.

Neuronal Activity, Transmitter Release

The transection, lesion, and stimulation studies all point to the same regions of the pons and caudal midbrain as the critical region for the generation of the state of REM sleep as a whole, and smaller subregions in the brainstem and forebrain in the control of its individual components. The pons contains a complex variety of cells differing in their neurotransmitter, receptors, and axonal projections. Unit recording techniques allow an analysis of the interplay between these cell groups and their targets to further refine our dissection of REM sleep mechanisms.

Medial Brainstem Reticular Formation

Most cells in the medial brainstem reticular formation are maximally active in waking, greatly reduce discharge rate in non-REM sleep, and increase discharge rate back to

waking levels in REM sleep.[15,16,60,93,94] Discharge is most regular in non-REM sleep and is relatively irregular in both waking and REM sleep. The similarity of the waking and REM sleep discharge patterns suggests a similar role for these cells in both states. Indeed, most of these cells have been shown to be active in waking in relation to specific lateralized movements of the head, neck, tongue, face, or limbs. For example, a cell may discharge only with extension of the ipsilateral forelimb or abduction of the tongue. The twitches that are normally visible in facial and limb musculature during REM sleep and the phenomenon of REM sleep without atonia suggest that these cells command movements that are blocked by the muscle tone suppression of REM sleep. Lesion of these cells has little or no effect on REM sleep duration or periodicity,[36,37] but it does dramatically prevent movements of the head and neck in waking.[95]

Cholinergic Cell Groups

Cholinergic cell groups have an important role in REM sleep control in the cat. As was pointed out earlier, microinjection of cholinergic agonists into the pons of the cat reliably triggers long REM sleep periods that can last for minutes or hours. Microdialysis studies show that pontine acetylcholine release is greatly increased during natural REM sleep when compared with either non-REM sleep or waking.[96] Recordings of neuronal activity in the cholinergic cell population demonstrate the substrates of this release. Certain cholinergic cells are maximally active in REM sleep (REM-on cells). Others are active in both waking and REM sleep.[97] Presumably, the REM sleep–on cholinergic cells project to the acetylcholine-responsive region in the subcoeruleus area.[98]

Cells with Activity Selective for REM Sleep

Cells with activity selective for REM sleep can be identified in the subcoeruleus area in both cats[99] and rats.[72] Anatomic studies using Fos labeling and tract tracing suggest that these neurons are glutamatergic, and that some of them project to the ventral medullary region involved in the triggering of the muscle atonia of REM sleep.[47,64,72,86,88-90]

Monoamine-Containing Cells

Monoamine-containing cells have a very different discharge profile. Most if not all noradrenergic[100,101] and serotonergic[102] cells of the midbrain and pontine brainstem, and histaminergic[103] cells of the posterior hypothalamus are continuously active during waking, decrease their activity during non-REM sleep, and further reduce or cease activity during REM sleep (Fig. 8-6). As pointed out earlier, these cell groups are not critical for REM sleep generation, but it is likely that they modulate the expression of REM sleep. The cessation of discharge in monoaminergic cells during REM sleep appears to be caused by the release of GABA onto these cells,[104-107] presumably by REM sleep–active GABAergic brainstem neurons.[108,109] Administration of a GABA agonist to the raphe cell group increases REM sleep duration,[105] demonstrating a modulatory role for this cell group in REM sleep control. Some studies indicate that dopamine cells do not change discharge across sleep states,[41,110,111] other work suggests that there is increased release of dopamine in REM sleep,[112,113] other work shows decreased release in REM sleep,[114] and still other work shows selective waking activity in these neurons.[115] These findings may reflect heterogeneity of firing of different dopamine cell groups and presynaptic control of release in dopamine terminals.

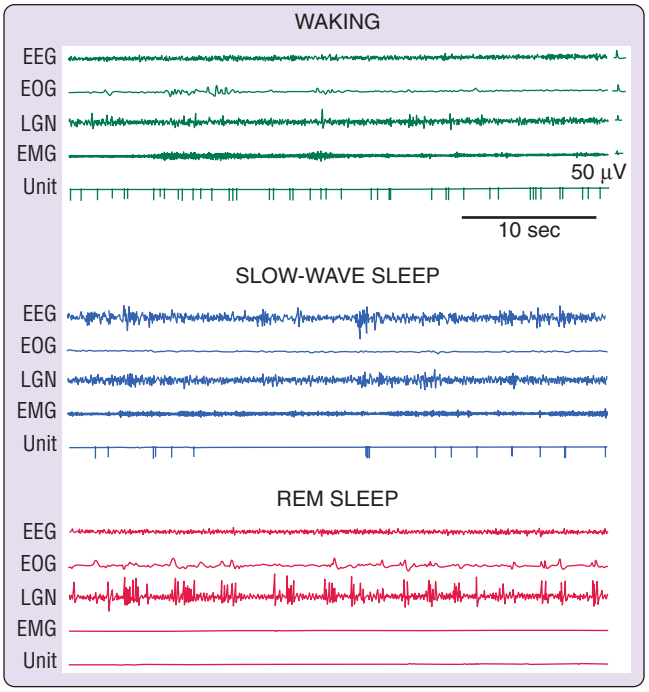

Figure 8-6 Activity of an REM sleep-off cell recorded in the locus coeruleus. EEG, sensorimotor electroencephalogram; EOG, electro-oculogram; LGN lateral geniculate activity; EMG, electromyogram; Unit, pulses triggered by locus coeruleus cell.

Other Cholinergic Cells in Lateral Pontine Regions

Other cholinergic cells in lateral pontine regions discharge in bursts before each ipsilateral PGO wave.[116,117] These cells may therefore participate in the triggering of these waves. We know from other studies that PGO waves are tonically inhibited in waking by serotonin input.[118-120] Therefore, it is likely that certain groups of cholinergic cells receive direct or perhaps indirect serotonergic inhibition in waking, and that the decrease of this inhibition in non-REM sleep and REM sleep facilitates PGO wave and REM sleep generation.

Fos Labeling

A more global mapping of neurons active in REM sleep can be achieved by using the Fos labeling to identify neurons active within the 20 minutes or more before sacrifice. Quattrochi and colleagues demonstrated that microinjections of the cholinergic agonist carbachol triggered episodes of continuous PGO waves in waking-activated neurons in the laterodorsal and pedunculopontine nuclei. Destruction of these nuclei blocks these waves.[120-122]

More extensive Fos mapping has been done to identify neurons activated during REM sleep in the rat. Verret and associates[123] found that only a few cholinergic neurons from the laterodorsal and pedunculopontine tegmental

nuclei were Fos-labeled after REM sleep. In contrast, a large number of noncholinergic Fos-labeled cells were observed in the laterodorsal tegmental nucleus, the subcoeruleus region, and the lateral, ventrolateral, and dorsal periaqueductal gray of the midbrain. In addition, other regions outside the brainstem regions critical for REM sleep control were labeled. These included the alpha and ventral gigantocellular reticular nuclei of the medulla, dorsal, and lateral paragigantocellular reticular[124] nuclei and the nucleus raphe obscurus. Half of the cells in the latter nucleus were cholinergic, suggesting that these neurons might be a source of acetylcholine during REM sleep. In a second study, an effort was made to identify the source of the GABAergic input thought to cause the cessation of discharge in locus coeruleus cells during REM sleep.[106] Verret and colleagues[87] found that the dorsal and lateral paragigantocellular reticular nuclei of the medulla and regions of the periaqueductal gray of the midbrain—regions with large percentages of GABAergic cells—are active in REM sleep. Maloney and associates[108] found GABAergic cells adjacent to the locus coeruleus that expressed Fos during periods of high REM sleep. Because the critical phenomena of REM sleep do not appear to require the medulla, it seems likely that the periaqueductal gray GABAergic neurons and GABAergic neurons adjacent to locus coeruleus and raphe nuclei are sufficient to suppress the activity of noradrenergic and serotonergic neurons,[105,125] although medullary neurons may participate in the intact animal.

Fos mapping has also been used to identify forebrain regions likely to control REM sleep. The preoptic region, important in non-REM sleep control (see Chapter 7), contains neurons that express Fos maximally in REM sleep–deprived animals, suggesting that these neurons may be related to the triggering or duration of REM sleep by brainstem systems.[126] Fos studies also indicate that melanin-concentrating-hormone neurons, which are located in the hypothalamus, express Fos during periods with large amounts of REM sleep and that intracerebroventricular administration of melanin-concentrating hormone increases the amount of subsequent REM sleep.[127,128] These results suggest that melanin-concentrating-hormone neurons are an additional source of forebrain modulation of REM sleep.

It should be emphasized that the identity of the cells involved in triggering and controlling REM sleep is not easily determined. The Fos studies do not necessarily identify all the cells active during REM sleep, only those of a phenotype that allows them to express Fos during the tested manipulations. Certain cell types do not readily express Fos even when very active. In other words, cells not expressing Fos during periods of REM sleep may be involved and may even have a critical role in REM sleep control. On the other hand, cells expressing Fos because of their activity during REM sleep may be responding to the motor and autonomic changes characteristic of this state, rather than causing these changes. With neuronal activity recording, the identification of the cells responsible for starting the process of REM sleep triggering cannot be easily be determined without a complete profile of discharge across the sleep cycle and a direct comparison of candidate cell groups, for the reasons just reviewed. Finally, recording from neurons in head-restrained animals, while easier than in freely moving animals, can be misleading because it can lower the activity of movement-related cells in waking, making them appear to be selectively active in REM sleep.[35] Nevertheless by comparing the results of multiple recording and stimulation techniques, with those of lesions, we gather evidence that helps identify the brainstem and forebrain neuronal groups that are the best candidates for controlling the REM sleep state.

CONTROL OF MUSCLE TONE

Abnormalities of muscle tone control underlie many sleep disorders. During REM sleep, central motor systems are highly active, whereas motoneurons are hyperpolarized.[129] The normal suppression of tone in the tongue and laryngeal muscles is a major contributing factor in sleep apnea (see Chapter 100). The failure of muscle tone suppression in REM sleep causes REM sleep behavior disorder (see Chapter 95). Triggering of the REM sleep muscle tone control mechanism in waking is responsible for cataplexy (see Chapter 100).[130]

Early work using intracellular recording and microiontophoresis had shown that motoneuron hyperpolarization during REM sleep was accompanied by the release of glycine onto motoneurons.[129,131] Microdialysis sampling showed that both GABA and glycine are released onto motoneurons during atonia induced by carbachol in the cat.[40] This release occurs in ventral horn motoneurons as well as in hypoglossal motoneurons. The glycinergic inhibition during a carbachol-elicited REM sleeplike state was investigated with immunohistochemistry and found to be caused by the activation of glycinergic neurons in the nucleus reticularis gigantocellularis and nucleus magnocellularis in the rostroventral medulla and the ventral portion of the nucleus paramedianus reticularis,[131] regions whose activation has been shown to suppress muscle tone in the unanesthetized decerebrate animal.[86] A second population was located in the caudal medulla adjacent to the nucleus ambiguus; these neurons may be responsible for the REM sleep–related inhibition of motoneurons that innervate the muscles of the larynx and pharynx.

In related work, it has been shown that norepinephrine and serotonin release onto motoneurons is decreased during atonia.[132] Because these monoamines are known to excite motoneurons, and GABA and glycine are known to inhibit them, it appears that the coordinated activity of these cell groups produces motoneuron hyperpolarization and hence atonia in REM sleep by a combination of inhibition and disfacilitation.

The inhibitory and facilitatory systems are strongly and reciprocally linked. Electrical stimulation of the pontine inhibitory area (or PIA, located in the subcoeruleus region[86]) produces muscle tone suppression. Even though the pontine inhibitory area is within a few millimeters of the noradrenergic locus coeruleus, electrical stimulation in the pontine inhibitory area that suppresses muscle tone will always cause a cessation of activity in the noradrenergic neurons of the locus coeruleus and other facilitatory cell groups.[133] Cells that are maximally active in REM sleep (REM-on cells) are present in the pontine inhibitory area

and also in the region of the medial medulla that receives pontine inhibitory area projections (Fig. 8-7).

The release of GABA and glycine onto motoneurons during REM sleep atonia is most likely mediated by a pathway from the pontine inhibitory area to the medial medulla.[89,90] The pontine region triggering this release not only is sensitive to acetylcholine but also responds to glutamate (Fig. 8-8).[86,88] The medullary region with descending projections to motoneurons can be subdivided into a rostral portion responding to glutamate and a caudal portion responding to acetylcholine (see Fig. 8-8).[47,134] The medullary interaction with pontine structures is critical for muscle tone suppression, because inactivation of pontine regions greatly reduces the suppressive effects of medullary stimulation on muscle tone.[135,136] This ascending pathway from the medulla to the pons may mediate the inhibition of locus coeruleus during atonia and may also help recruit other active inhibitory mechanisms. Thus, damage anywhere in the medial pontomedullary region can block muscle atonia by interrupting ascending and descending portions of the pontomedullary inhibitory system, as can muscimol injection into the pons,[135] again indicating that pontine activation is a key component of motor inhibition.

The studies just reviewed focused largely on ventral horn and hypoglossal motoneurons. However, the control of jaw muscles is also a critical clinical issue. The success of jaw appliances indicates that reduced jaw muscle activity can contribute to closure of the airway in sleep apnea (see Chapter 109). Jaw muscle relaxation is a common initial sign of cataplexy, and tonic muscle activation underlies bruxism. Investigation of the control of masseter motor neurons allows analysis of the regulation of muscle tone on one side of the face, while using the other side as a control for changes in behavioral state caused by application of neurotransmitter agonists and antagonists.[137] Using this model, it was determined that tonic glycine release reduces muscle tone in both waking and non-REM sleep. However, blockade of glycine receptors did not prevent the suppression of muscle tone in REM sleep. In a similar manner, blockade of GABA receptors alone or in combination with glycine receptors increased tone in waking and non-REM sleep but did not prevent the suppression of masseter tone[138] or of genioglossus tone in REM sleep.[139]

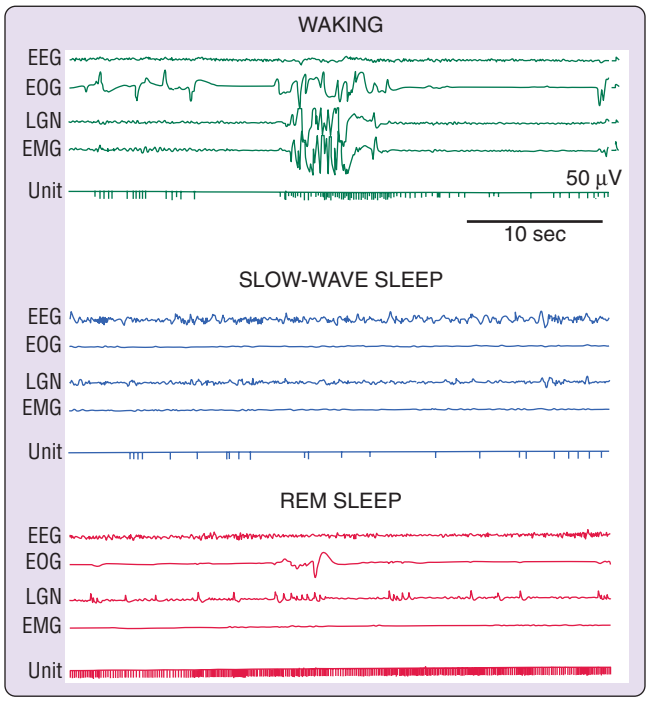

Figure 8-7 Activity of medullary REM sleep-on cell. Note the tonic activity during REM sleep. In waking, activity is generally absent even during vigorous movement. However, some activity is seen during movements involving head lowering and postural relaxation. EEG, electroencephalogram; EOG, electrooculogram; LGN, lateral geniculate nucleus; EMG, dorsal neck electromyogram; Unit, pulses triggered by an REM-on cell.

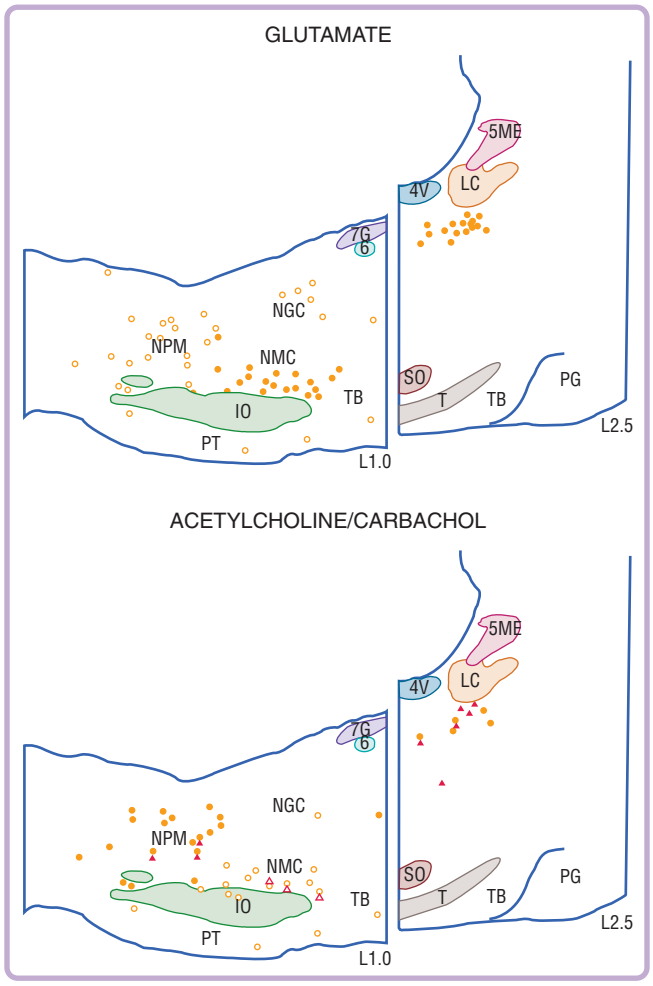

Figure 8-8 Sagittal map of pontomedullary inhibitory areas. Electrical stimulation produced atonia at all the points mapped. All electrically defined inhibitory sites were microinjected with glutamate or cholinergic agonists. *Filled symbols* represent points at which microinjections decreased muscle tone (to less than 30% of baseline values or to complete atonia). *Open circles* indicate points at which injections increased or produced no change in baseline values. *Top,* Glutamate injections. *Bottom,* Acetylcholine (ACh; *circles*) and carbachol (Carb; *triangles*) injections. IO, inferior olivary nucleus; LC, locus coeruleus nucleus, NGC, nucleus gigantocellularis; NMC, nucleus magnocellularis; NPM, nucleus paramedianus; PG, pontine gray; PT, pyramid tract; SO, superior olivary nucleus; T, nucleus of the trapezoid body; TB, trapezoid body; 4V, fourth ventricle; 5ME, mesencephalic trigeminal tract; 6, abducens nucleus; 7G, genu of the facial nerve. (From Lai YY, Siegel JM. Medullary regions mediating atonia. J Neurosci 1988;8:4790-4796.)

However, both of these manipulations increased phasic masseter muscle activity in REM sleep.

Further studies showed that a blockade of glutamate receptors reduces the normal enhancement of muscle tone in waking relative to the level in non-REM sleep. Glutamate also contributes to the phasic motor activity during REM sleep. However, reduction in glutamate alone is not sufficient to account for the suppression of muscle tone in REM sleep, because stimulation of NMDA and non-NMDA glutamate receptors does not appear to restore muscle tone in REM sleep.[140]

A study in the anesthetized rat suggested that activation of norepinephrine receptors, in combination with the activation of glutamate receptors, was sufficient to potently increased muscle tone in the masseter muscles.[141] A study of the hypoglossal motor nucleus in the unanesthetized rat concluded that the suppression of muscle tone in REM sleep was mediated to a large extent by a reduction in norepinephrine release, but not by reduced serotonin release.[142] Thus, this work in the context of prior microdialysis analysis of transmitter release suggests that the reduction of norepinephrine release may be a key factor regulating muscle tone, along with the changes in amino acid release described earlier. These conclusions are consistent with prior work indicating that cataplexy was linked to a reduction in the activity of noradrenergic neurons (see later).[143] Although the current literature suggests that trigeminal, hypoglossal, and ventral horn motoneurons are subjected to similar neurochemical control across the sleep cycle, direct comparison of these systems has not been made, and it is likely that some aspects of control differ across systems as well as species.

The role of reduced serotonin release in the suppression of muscle tone has been investigated in the hypoglossal nucleus of the rat. It was found that the modulation of genioglossus activity across natural sleep-wake states was not greatly affected by endogenous input from serotonergic neurons, although prior studies in vagotomized and anesthetized rats had shown an effect of serotonin on muscle tone under these aphysiologic conditions.[144-146] Although same glycinergic inhibition clearly acts, at the level of the motoneuronal membrane, it remains to be determined to what extent norepinephrine, GABA, and glutamate effects are exerted directly at the motoneuronal membrane level. Some of the effects of these neurotransmitters on motoneurons may be mediated polysynaptically.

Recent work suggests that inhibition of motor output is accompanied by a neurochemically similar inhibition of sensory relays during REM sleep.[147] Such sensory inhibition may be important in preserving sleep and, in particular, in blocking the sensory input produced by twitches breaking through the motor inhibition of REM sleep. The failure of this inhibition may contribute to sleep disruption and increased motor activity in sleep in pathologic states.

In contrast to norepinephrine, serotonin, and histamine cell groups, it was reported that mesencephalic dopaminergic neurons do not appear to alter their discharge rate across the sleep cycle.[110] Dopamine release in the amygdala measured by dialysis does not significantly vary across the sleep cycle.[148] In disagreement with this finding, a Fos study indicted that dopaminergic neurons in the ventral portion of the mesencephalic tegmentum were activated during periods of increased REM sleep.[149] A unit recording study indicated that dopaminergic neurons in the ventral tegmental area of the midbrain show maximal burst firing in both waking and REM sleep.[112] Other work using the Fos labeling technique identified a wake-active dopaminergic cell population in the ventral periaqueductal gray.[115] In dialysis measurements of dopamine release, we have seen reduced dopamine release in the dorsal horn of the spinal cord during the REM sleeplike state triggered by carbachol. We did not see such a decrease in the ventral horn or hypoglossal nucleus.[132] These data suggest either heterogeneity in the behavior of sleep cycle activity of dopaminergic neurons or presynaptic control of dopamine release independent of action potentials in the cell somas.

Figure 8-9 illustrates some of the anatomic and neurochemical substrates of the brainstem generation of REM sleep.

NARCOLEPSY AND HYPOCRETIN

Narcolepsy has long been characterized as a disease of the REM sleep mechanism. Narcoleptic patients often have REM sleep within 5 minutes of sleep onset, in contrast to normal individuals, who rarely show such sleep-onset REM sleep. Most narcoleptic patents experience cataplexy, a sudden loss of muscle tone with the same reflex suppression that is seen in REM sleep. High-amplitude theta activity in the hippocampus, characteristic of REM sleep, is also prominent in cataplexy, as observed in dogs.[143] Further evidence for links between narcolepsy and REM sleep comes from studies of neuronal activity during cataplexy. Many of the same cell populations in the pons and medulla that are tonically active only during REM sleep in normal subjects, become active during cataplexy in narcoleptic animals.[17,150] Likewise, cells in the locus coeruleus, which cease discharge only in REM sleep in normal animals, invariably cease discharge in cataplexy.[151] However, just as cataplexy differs behaviorally from REM sleep in its maintenance of consciousness, not all neuronal aspects of REM sleep are present during cataplexy. As was noted earlier, in the normal animal, noradrenergic, serotonergic, and histaminergic cell are all tonically active in waking, reduce discharge in non-REM sleep, and cease discharge in REM sleep.[143,151] However, unlike noradrenergic cells, serotonergic cells do not cease discharge during cataplexy, reducing discharge only to quiet waking levels. Histaminergic cells actually increase discharge in cataplexy relative to quiet waking levels (Fig. 8-10).[152] These findings allow us to identify some of the cellular substrates of cataplexy. Medullary inhibition and noradrenergic disfacilitation are linked to cataplexy's loss of muscle tone. In contrast, the maintained activity of histamine neurons is a likely substrate for the maintenance of consciousness during cataplexy that distinguishes cataplexy from REM sleep. Thus, the study of neuronal activity in the narcoleptic animal provides an insight into both narcolepsy and the normal role of these cell groups across the sleep cycle.

In 2001, it was discovered that most human narcolepsy was caused by a loss of hypothalamic cells containing the peptide hypocretin (Fig. 8-11).[22,23] On average, 90% of these cells are lost in narcolepsy. Subsequently, it was discovered that a lesser reduction in the number of hypocretin cells was seen in Parkinson's disease, with a loss of up to 60% of hypocretin cells.[153,154] It was found that

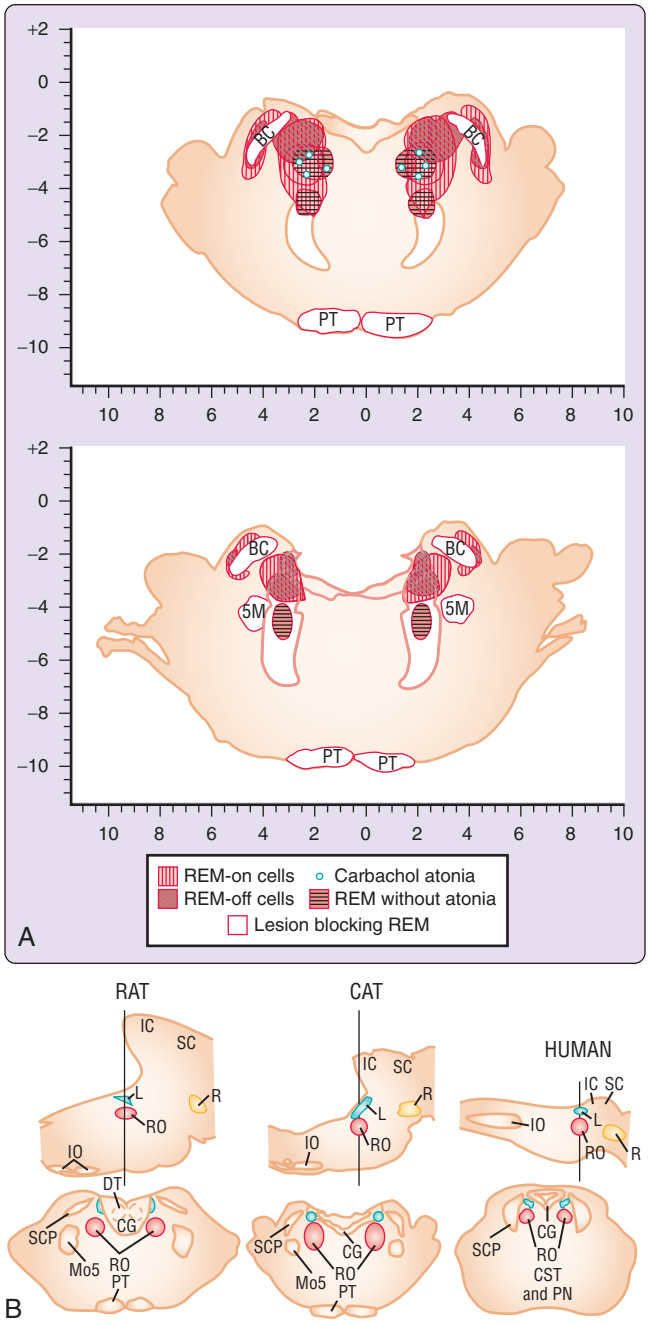

Figure 8-9 A, Anatomic relationship of REM sleep-on and sleep-off cells, carbachol-induced atonia sites, lesions blocking atonia but not preventing REM sleep, and lesions completely blocking REM sleep. **B,** Anatomic locations of REM on areas in cats, rat, and projected location in human, in sagittal and coronal views. (**A** from Siegel JM, Rogawski MA. A function for REM sleep: regulation of noradrenergic receptor sensitivity. Brain Res 1988;13:213-233; **B** from Siegel JM. The stuff dreams are made of: anatomical substrates of REM sleep. Nat Neurosci 2006;9:721-722.) CG, central gray; CST, corticospinal tract; DT, dorsal tegmental; IO, inferior olive; IC, inferior colliculus; L, locus colliculus; Mo5, motor nucleus of the trigeminal nerve (5M); PN, pontine nuclei; R, red nucleus; RO, reticularis oralis nucleus; SC, superior colliculus; SCP, superior cerebellar peduncle (brachium conjunctivum).

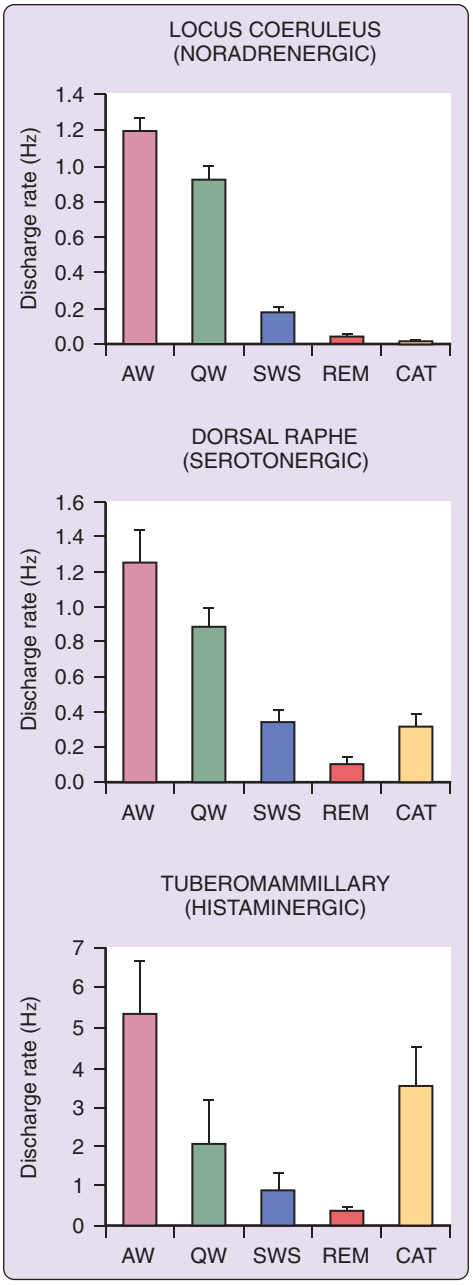

Figure 8-10 Comparison of mean discharge rates in sleep-waking states and cataplexy of REM-off cells recorded from three brain regions. Posterior hypothalamic histaminergic neurons remain active, whereas dorsal raphe serotonergic neurons reduced discharge, and locus coeruleus noradrenergic neurons cease discharge during cataplexy. All of these cell types were active in waking, reduced discharge in NREM sleep, and were silent or nearly silent in REM sleep. (From John J, Wu M-F, Boehmer LN, Siegel JM. Cataplexy-active neurons in the posterior hypothalamus: implications for the role of histamine in sleep and waking behavior. Neuron 2004;42:619-634.) AW, active waking; QW, quiet waking; SWS, slow wave (non-REM) sleep; REM, REM sleep; CAT, cataplexy.

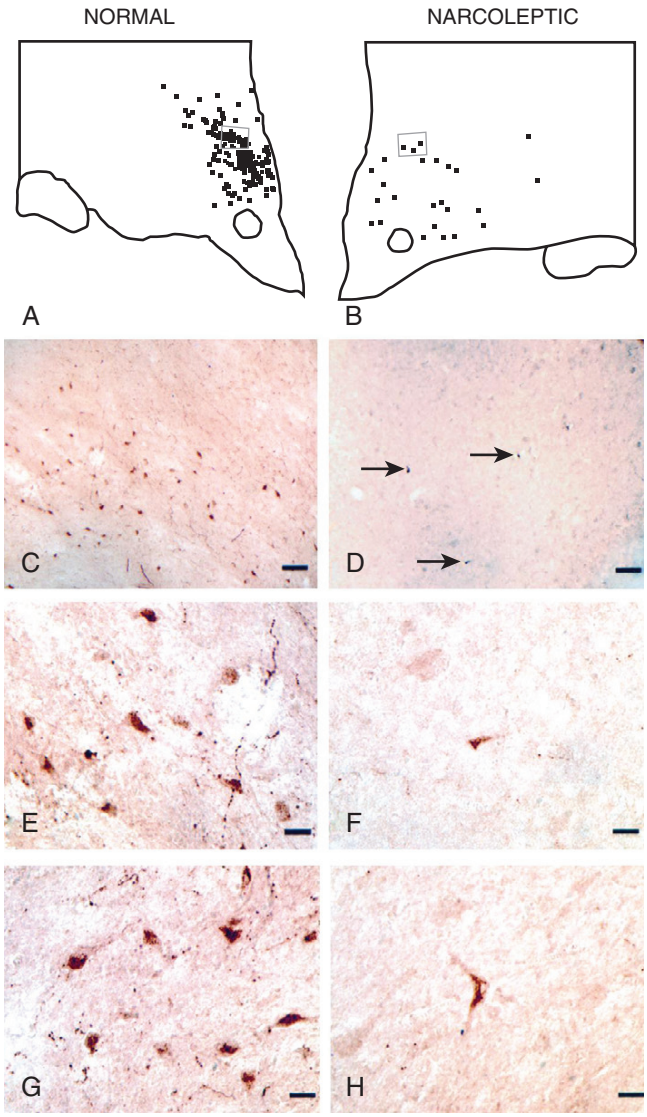

Figure 8-11 Loss of hypocretin cells in human narcolepsy. Distribution of cells in perifornical and dorsomedial hypothalamic regions of normal and narcoleptic humans. (From Thannickal TC, Moore RY, Nienhuis R, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000;27:469-474.)

administration of the peptide to genetically narcoleptic dogs reversed symptoms of the disorder,[155] and that nasal administration reversed sleepiness in monkeys,[156] suggesting that similar treatment could be uniquely effective for narcolepsy and perhaps for other disorders characterized by sleepiness.

In further work in normal animals, it was determined that identified hypocretin neurons are maximally active during active waking.[34,34a] This discharge was reduced or absent during aversive waking situations, even if the EEG indicated high levels of alertness (Fig. 8-12).[34] This is consistent with the hypothesis that release of hypocretin facilitates motor activity during emotionally charged activities of the sort that trigger cataplexy in narcoleptics, such as laughter.[157-159] Even normal individuals experience weakness at these times, seen in the "doubling over" that often accompanies laughter, or the weakness that can result from other sudden-onset, strong emotions. Mice engineered to lack hypocretin cannot maintain alertness when working for positive reinforcement, but they are unimpaired when working to avoid aversive stimuli.[160] Studies of hypocretin release in the cat[161] and preliminary studies in humans are also consistent with this hypothesis.[162] In the absence of the hypocretin-mediated motor facilitation, muscle tone is lost at these times. Hypocretin cells also send ascending projections to cortical and basal forebrain regions. In the absence of hypocretin-mediated facilitation of forebrain arousal centers, waking periods are truncated, resulting in the sleepiness of narcolepsy.[158]

The functions of hypocretin have been investigated in knockout animals that do not have the peptide, using operant reinforcement tasks. Hypocretin knockout mice were deficient in the performance of bar presses to secure food or water reinforcement. However, they did not differ from their normal littermates in their performance when trained to bar press to avoid foot shock. Periods of poor performance on the positive reinforcement tasks were characterized by EEG deactivation. Fos labeling of normal mice showed that the positive reinforcement task used in this study was characterized by activation of hypocretin neurons. However, hypocretin neurons were not activated in the negative reinforcement task despite high levels of EEG activation, indicating that nonhypocretin systems mediate arousal during this behavior. This study led to the conclusion that hypocretin neurons are critically involved in arousal linked to positive reinforcement, and that in their absence such behaviors are impaired. However, they are not required to maintain arousal in conditions of negative reinforcement, indicating that other brain systems subserve this role.

Hypocretin appears to act largely by modulating the release of amino acid neurotransmitters.[163] Systemic injection of hypocretin causes a release of glutamate in certain hypocretin-innervated regions, producing a potent postsynaptic excitation.[137,164] In other regions, it facilitates GABA release, producing postsynaptic inhibition.[161,165] The loss of these competing inhibitory and facilitatory influences in narcolepsy appears to leave brain motor regulatory and arousal systems less stable than the tightly regulated balance that can be maintained in the presence of hypocretin (Fig. 8-13). According to this hypothesis, this loss of stability is the underlying cause of narcolepsy, with the result being inappropriate loss of muscle tone in waking and inappropriate increases of muscle tone during sleep resulting in a striking increased incidence of REM sleep behavior disorder in narcoleptics (see Chapter 84). In the same manner, although a principal symptom of narcolepsy is intrusions of sleep into the waking period, narcoleptics sleep poorly at night, with frequent awakenings.[166-168] In other words, narcoleptics are not simply weaker and sleepier than normal subjects. Rather, their muscle tone and sleep-waking state regulation is less stable than that in normal subjects as a result of the loss of hypocretin function.

THE FUNCTIONS OF REM SLEEP

Research into the control of REM sleep turns into a seemingly infinite regression, with REM-on cells inhibited

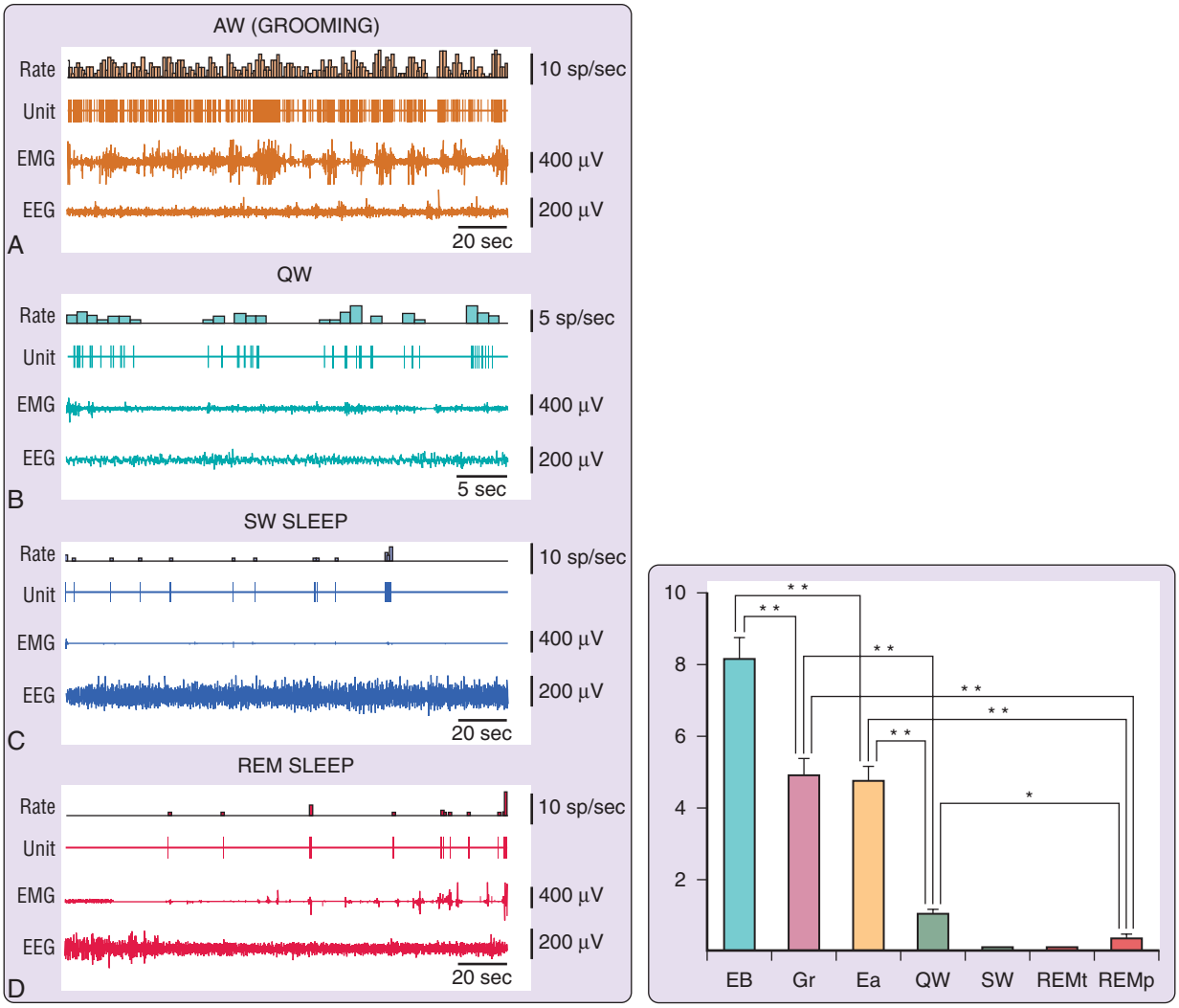

Figure 8-12 Firing rate of hypocretin cells in waking and sleep behaviors in freely moving rats. *Left:* The discharge pattern of a representative hypocretin neuron across the sleep-waking cycle in the freely moving rat. **A,** High firing rates are seen during AW (active waking—grooming). **B,** Reduced firing rate or cessation of activity is seen in QW (quiet waking) and drowsiness. **C,** A further decrease or cessation of firing is seen during slow wave [nonREM] sleep (SW) sleep. **D,** Minimal firing rate is seen during the tonic phase of REM sleep. Brief hypocretin (Hcrt) cell discharge bursts are correlated with muscle twitches during the phasic events of REM sleep. *Right:* Summary data from identified Hcrt cells: exploratory behavior (EB), grooming (Gr), eating (Ea), quiet wake (QW), slow wave sleep (SW), and tonic (REMt) and phasic (REMp) sleep. Maximal discharge is seen during exploration-approach behavior, sec, seconds. (From Mileykovskiy BY, Kiyashchenko LI, Siegel JM. Behavioral correlates of activity in identified hypocretin [orexin] neurons. Neuron 2005;46:787-798.)

by REM-off cells, which in turn may be inhibited by other REM-on cells. It is in fact very difficult to identify the sequence in which these cell groups are normally activated because the axonal condition and synaptic delays could not be more than a few milliseconds between these cell groups, yet REM sleep onset occurs over a period of minutes in man and cat and at least 30 or more seconds in the rat. It also does not completely enlighten us with respect to the ultimate functional question; what is REM sleep for? To answer this question, we need to determine what if any physiologic process is altered over REM sleep periods. Is some toxin excreted or some protein synthesized? If so, how do we account for the widely varying durations of the typical REM sleep? In the human, REM sleep typically lasts from 5 to 30 minutes, whereas in the mouse it typically lasts 90 seconds.[169] What can be accomplished in 90 seconds in the mouse but requires an average of approximately 15 minutes in humans? If a vital process is accomplished, why do drug treatments that abolish REM sleep have no discernable effect on any vital process, even when such drugs are taken continuously for many years? Why do some marine mammals have no apparent REM sleep (see Chapter 7)? Why is REM sleep present in homeotherms (i.e., birds and mammals) but apparently absent in the reptilian ancestors of homeotherms?

Great progress has been made in localizing the mechanisms that generate REM sleep. As described earlier, we know many of the key neurotransmitters and neurons involved. The recent discovery of the role of hypocretin in narcolepsy serves as a reminder that there may still be key cell groups that need to be identified before we can gain fundamental insights into the generation, mechanism, and functions of REM sleep. Yet, despite this caveat, we already understand a substantial amount about what goes on in the brain during REM sleep.

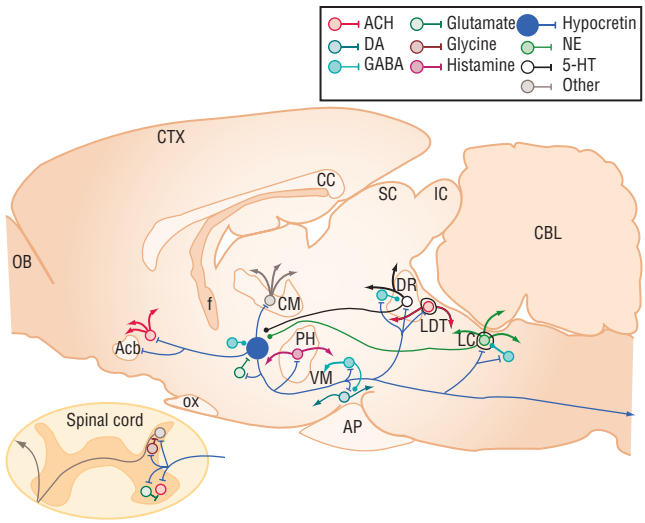

Figure 8-13 Major identified synaptic interactions of hypocretin neurons. *Lines terminated by perpendicular lines* denote excitation; *circular terminations* indicate inhibition. Acb, nucleus accumbens; ACH, acetylcholine; AP, anterior pituitary; CBL, cerebellum; CC, corpus callosum; CM, centromedian nucleus of the thalamus; DA, dopamine; DR, dorsal raphe; f, fornix; GABA, gamma-aminobutyric acid; 5-HT, serotonin; IC, inferior colliculus; LC, locus coeruleus; LDT, laterodorsal tegmental and pedunculopontine; NE, norepinephrine; OB, olfactory bulb; OX, optic chiasm; PH, posterior hypothalamus; SC, superior colliculus; VM, ventral midbrain.

However, the mystery exposed by the discovery of REM sleep remains. We do not know the biological need that initiates REM sleep. We do not know the source of the REM sleep debt that accumulates during REM sleep deprivation.[170]

What is clear is that increased brain activity in REM sleep consumes considerable amounts of metabolic energy. The intense neuronal activity shown by most brain neurons, similar to or even more intense than that seen during waking, extracts a price in terms of energy consumption and wear and tear on the brain. It is unlikely that such a state would have produced a Darwinian advantage and remained so ubiquitous among mammals if it did not have benefits compensating for these costs. But what might the benefits be?

One idea that has received much media attention is that REM sleep has an important role in memory consolidation. However, the evidence for this is poor.[171] Although early animal work suggested that REM sleep deprivation interfered with learning, subsequent studies showed that it was the stress of the REM sleep deprivation procedure, rather than the REM sleep loss itself, that was critical. A leading proponent of a sleep and memory consolidation relationship has concluded that sleep has no role in the consolidation of declarative memory,[172] which would exclude a role for sleep in rote memory, language memory, and conceptual memory, leaving only the possibility of a role in procedural memory, the sort of memory required for learning to ride a bicycle or play a musical instrument. However, studies supporting a role for sleep in the consolidation of human procedural learning have made contradictory claims about similar learning tasks, with some concluding that REM but not non-REM sleep is important, others stating just the reverse, and yet others claiming that both sleep states are essential.[171] Millions of humans have taken monoamine oxidase (MAO) inhibitors or tricyclic antidepressants, often for 10 to 20 years. These drugs profoundly depress, or in many cases completely eliminate, all detectable aspects of REM sleep. However, there is not a single report of memory deficits attributable to such treatment. Likewise, well-studied individuals with permanent loss of REM sleep resulting from pontine damage show normal learning abilities; the best-studied such individual completed law school after his injury[173] and was last reported to be the puzzle editor of his city newspaper. Humans with multiple-system atrophy can have a complete loss of slow-wave sleep and disruption of REM sleep without manifesting any memory deficit.[174] A recent well-controlled study showed that REM sleep suppression with selective serotonin reuptake inhibitors or serotonin-norepinephrine reuptake inhibitors produced no significant decrement in memory consolidation on any task and even produced a small significant improvement in a motor learning task.[175]

Another idea that has been repeatedly suggested is that REM sleep serves to stimulate the brain.[45,176,177] According to this theory, the inactivity of non-REM sleep causes metabolic processes to slow down to an extent that the animal would be unable to respond to a predator, capture prey, or meet other challenges on awakening. This would leave mammals functioning like reptiles, with slow response after periods of inactivity. This hypothesis explains the appearance of REM sleep after non-REM sleep under most conditions. It also explains the well-documented increased proportion of sleep time in REM sleep as the sleep period nears its end in humans and other animals. Humans are more alert when aroused from REM sleep than non-REM sleep, as are rats,[178] consistent with this idea. The very low amounts or absence of REM sleep in dolphins, whose brainstem is continuously active and which never have bilateral EEG synchrony, can be explained by this hypothesis. If one hemisphere is always active, there is no need for the periodic stimulation of REM sleep to maintain the ability to respond rapidly. However, the brain-stimulation hypothesis of REM sleep function does not explain why waking does not substitute for REM sleep in terrestrial mammals. REM sleep–deprived individual have a REM sleep rebound even if they are kept in an active waking state for extended periods.

One phenomenon that may explain REM sleep rebound is the cessation of activity of histamine, norepinephrine, and serotonin neurons during REM sleep. This cessation does not occur during waking, so waking would not be expected to substitute for this aspect of REM sleep.[179] Therefore, REM sleep rebound may be caused by an accumulation of a need to inactivate these aminergic cell groups. Several cellular processes might benefit from the cessation of activity in aminergic cells. Synthesis of these monoamines and their receptors might be facilitated during this period of reduced release. The receptors for these substances might be resensitized in the absence of their agonist. The metabolic pathways involved in the reuptake and inactivation of these transmitters might also benefit from periods of inactivity. Some but not all studies have supported this hypothesis.[180-184]

Further investigation at the cellular level may lead to an inside-out explanation of REM sleep function, deriving a

functional explanation from a better understanding of the neuronal basis of REM sleep control.

Further relevant literature can be found at http://www.semel.ucla.edu/sleepresearch.

❖ Clinical Pearls

The loss of hypocretin neurons is responsible for most human narcolepsy. It is thought that this cell loss may be the result of an immune system attack on these neurons, but convincing evidence for this is lacking. Administration of hypocretin is a promising future avenue for the treatment of narcolepsy. Because the hypocretin system has potent effects on arousal systems, including the norepinephrine, serotonin, acetylcholine, and histamine systems, manipulation of the hypocretin system with agonists and antagonists is likely to be important in further pharmacotherapies for narcolepsy, insomnia, and other sleep disorders.

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