Chapter 8

Rapid Eye Movement Sleep

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Chapter Highlights

- Rapid eye movement (REM) sleep was first identified by its most obvious feature: rapid eye movements occurring during sleep. In most adult mammals the electroencephalogram (EEG) of the neocortex is low in voltage during REM sleep. The hippocampus exhibits 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 caudal midbrain. The isolated brainstem can generate REM sleep, including rapid eye movements, spike potentials linked to eye movements called ponto-geniculo-occipital (PGO) waves, muscle tone suppression (atonia), and autonomic variability. The structures rostral to the caudal midbrain–pontine brainstem cannot generate the forebrain aspects of REM sleep, such as PGO waves or rapid eye movements. The brainstem 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 each use a specific transmitter—gamma-aminobutyric acid (GABA), acetylcholine, glutamate, or glycine. Subgroups of REM-off cells use the transmitters norepinephrine, epinephrine, serotonin, histamine, and GABA.
- Destruction of large regions within the midbrain and pons can prevent the occurrence of REM sleep. More limited 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 within REM sleep. Early animal work found that 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. Subsequent work found a similar syndrome in humans that has been termed the REM sleep behavior disorder. Stimulation of portions of the REM sleep–controlling area of the pons can produce a loss of muscle tone in antigravity and respiratory musculature during waking, 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. Hypocretin neurons have potent effects on alertness and motor control and normally are activated in relation to particular, generally positive, emotions in humans as well as in animals.

OVERVIEW

Rapid eye movement (REM) sleep was discovered by Aserinsky and Kleitman in 1953.[1] These workers reported that REM sleep was characterized by the periodic recurrence of rapid eye movements, linked to a dramatic reduction in the amplitude of the electroencephalogram (EEG) from that of the higher-voltage activity of the previous NREM sleep period. In their study, the EEG pattern in REM sleep closely resembled that in alert waking; and those subjects awakened from REM sleep reported vivid dreams. Dement identified a similar state of low-voltage EEG with eye movements in cats.[2] Jouvet repeated this observation, finding in addition a loss of muscle tone (i.e., atonia) in REM sleep. He used the term *paradoxical sleep* to refer to this state. The "paradox" was that the EEG resembled that recorded during waking, while 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 NREM sleep but has supported the original finding linking the most vivid dreaming to the REM sleep state. Lesions of parietal cortex and certain other regions prevent dreaming in humans, even in persons continuing to show normal REM sleep as judged

by cortical EEG activity and suppression of muscle tone and rapid eye movements.[6] Children younger than 6 years of age, in whom the amount of REM sleep is greater than in adults, do not typically report dream mentation, perhaps because the cortical regions involved have not yet fully 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 with their propagation to the forebrain in adult placental and marsupial mammals. These findings make it questionable whether all or any nonhuman mammals that apparently experience REM sleep, all of which have cortical regions whose structure differs from that in adult humans, have dream mentation.

Surveyed in this chapter are (1) the defining characteristics of REM sleep, including its physiology and neurochemistry; (2) the techniques used to investigate the mechanisms generating REM sleep; (3) the mechanisms responsible for the suppression of muscle tone during REM sleep and the pathologic effects of 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 RAPID EYE MOVEMENT SLEEP

The principal electrical signs of REM sleep include a reduction in EEG amplitude, particularly in the power of its lower-frequency components (as shown in Figure 8-1, *top*). REM sleepalso is characterized by a suppression of muscle tone, called *atonia*, visible in the electromyogram (EMG). In males, erections are more likely to occur.[10] Thermoregulation (e.g., sweating, shivering) largely ceases in most animals, and body temperatures drift toward environmental temperatures, as in reptiles.[11] Pupils constrict, reflecting a parasympathetic dominance in the control of the iris.[12] These changes, which 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 designation *ponto-geniculo-occipital* (PGO) *spikes*. They occur as large-amplitude, isolated potentials arising 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 3 to 10 waves, usually correlated with rapid eye movements. PGO-linked potentials also can be recorded in the motor nuclei of the extraocular muscles, where they trigger the rapid eye movements of REM sleep. They also are 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. Periods of marked irregularity in respiratory and heart rates are characteristic of REM sleep, in contrast with NREM sleep, during which respiration and heart rate are 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]

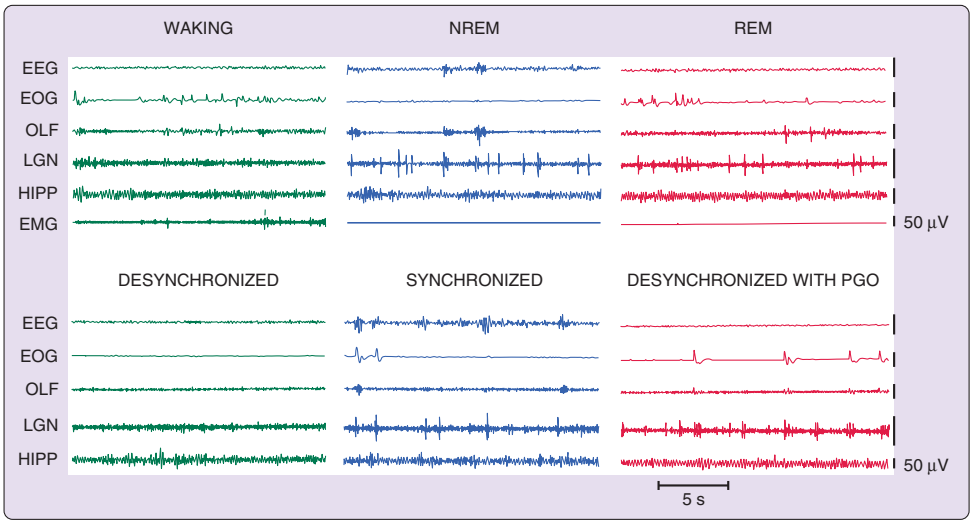

Figure 8-1 *Top*, Polygraph tracings of states seen in the intact cat. *Bottom*, Polygraph tracings of states seen in the forebrain 4 days after transection at the pontomedullary junction. EEG, Sensorimotor electroencephalogram; EMG, dorsal neck electromyogram; EOG, electrooculogram; HIPP, hippocampus;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

These changes that occur episodically in REM sleep have been called its "phasic" features.

As described further on, 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 also are expressed to various extents in different species, and not all of these features are present in all species that have been judged to have REM sleep.[18]

The distribution of REM sleep in the animal kingdom is discussed in Chapter 10.

RAPID EYE MOVEMENT 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 *observation* of the activity of neurons or measurement of the release of neurotransmitters. Each approach has its advantages and limitations.

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

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 most commonly are induced by aspiration, transection of the neuraxis, electrolysis, local heating by radio frequency currents, or the injection of cytotoxins. These substances include certain amino acids, such as *N*-methyl-D-aspartate (NMDA) and kainate, that cause cell death by excitotoxicity, and targeted cytotoxins, such as saporin coupled to a particular ligand, 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 interruption of these axons. Injection of inhibitory neurotransmitters, such as muscimol, allows reversible inactivation of neurons in the injection region. DREADDs ("designer receptors exclusively activated by designer drugs") also can be used to inactivate or activate groups of neurons. Viral vectors or transgenic mouse models can be used to express the receptors in the desired populations, which can then be manipulated by the locally or systemically applied "designer drug."

If damage to or inactivation of a brain region causes the loss of a sleep state, that region cannot be assumed to be where a "center" for the state resides. Lesion effects usually are 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 also can disrupt functions that are organized elsewhere. For example, so-called spinal shock is a well-known phenomenon in which severing the spinal cord's connection to more rostral brain regions causes a loss of functions known to be mediated by circuits intrinsic to the spinal cord.

On the other hand, this sort of denervation-induced shock dissipates with the passage of time. In addition, adaptive changes occur that allow other regions to take over lost functions. This process 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 NREM sleep is that with even massive lesions targeted at putative sleep-generating "centers," often only a transient disruption or reduction of sleep occurs, presumably as a result of this compensation.[19]

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. Such a manipulation might be expected to completely prevent sleep phenomena from appearing on either side of this cut. To a surprising extent, however, this is not the case. As reviewed further on, REM sleep reappears within hours after introduction of 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 undoubtedly the removed regions are not essential for the signs of REM sleep that survive.

It is increasingly possible to acquire mutant mice in which any one or several of more than 20,000 genes are inactivated. Investigation of two mutants[20,21] led to major insights into the etiology of human narcolepsy.[22-24] 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 within 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, or altered changes in sleep with to development and aging. The same interpretive constraints 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, Insertion of Messenger RNAs, or Optogenetic Stimulation

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[25] and basal forebrain as a site capable of triggering sleep.[26] Electrical stimulation is an obviously 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 placed in the target area and perfused with high concentrations of agonists, and most recently by optogenetic activation.

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. The only permissible conclusion from this finding, however, is 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, many reported studies have implicated various substances as critical for REM sleep control solely on the basis of microinjection techniques. 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,[27] that microinjection of hypocretin into sites known to control feeding increases food intake,[28] that injection into regions known to contain cells that are waking-active increase waking,[29] that injection into regions known to contain cells selectively active in REM sleep will increase the occurrence of this state,[30,31] that injection into regions known to facilitate muscle tone will increase tone, that identical injections into regions known to suppress tone will decrease tone,[32] and that intracerebroventricular injection of hypocretin can increase water intake[33] and can activate other periventricular systems.[30] 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 recording 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.[34] The interpretation of optogenetics responses is discussed elsewhere.[26]

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"[35] 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 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 cannot easily be determined. 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 readily ascertained.

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 and EMG activity and other phenomena over a period of seconds to minutes, as the awake state turns into NREM sleep and then as NREM 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 calculated in some studies. Neurons purported to show a "significant" change in activity many seconds or even minutes before REM sleep onset have been reported. Such a measure is of little utility, however, 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, calculated as defined earlier, 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 initially 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.[36-38] The more appropriate method of assessment of the unit activity cycle relative to state control is to compare two different cell types to observe the specific phase relation of the peaks or troughs of their activity under similar conditions. This kind of study is difficult, involving the simultaneous long-term recording of multiple cells, and 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. Awakening, however, is a process that can be studied in this way, because it can be elicited by stimuli and appears to be preceded by abrupt changes in the activity of many neuronal groups.[39] A major advantage of unit recording approaches in the intact animal to investigating 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 long in the area of interest and circulating artificial cerebrospinal fluid through it. Neurotransmitters released outside the membrane will diffuse through the membrane and then can be collected.

Each sample is obtained 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 typically is on the order of a few minutes for each sample.[40-42]

Unit recording and dialysis approaches require a sharp research focus on a particular neurotransmitter or neuronal group. By 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 in the nucleus 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. Activation of these genes can be detected by immunohistochemistry techniques, most commonly staining for the production of the Fos protein or the mRNA used to synthesize this protein.[43] Neurons can be double-labeled to identify 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 also can provide an indication of which neurons are active.[43,44] 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 their ability to make anatomically driven discoveries of brain regions that are active in particular states, independent of specific hypotheses, thereby leading to major advances in understanding. However, another common feature of these types of "recording" techniques is their very poor temporal and spatial resolutions in comparison to neuronal recording approaches. Fos activation can take 20 minutes or more. PET takes a similar amount of time, and fMRI can observe events lasting on the order of 1 to 15 seconds. Accordingly, whether areas active during a particular state caused the state or were activated because of the state cannot be determined with certainty.

Summary of Technical Considerations

Clearly there is no perfect technique for determining the neuronal substrates of sleep states. Ideally, all three approaches should be used in concert to reach conclusions. Explored next are the major findings derived from lesion (transection), 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 discovered that animals in which the forebrain is removed after transection of the neuraxis in the coronal plane at the rostral border of the superior colliculus showed tonic excitation of the "antigravity muscles" or extensors (Figure 8-2, level A). This decerebrate rigidity was visible as soon as anesthesia was discontinued. Bard and Macht reported in 1958 that animals with decerebrate rigidity would show periodic limb relaxation.[45] It is now known that these researchers were observing the periodic muscle atonia of REM sleep.

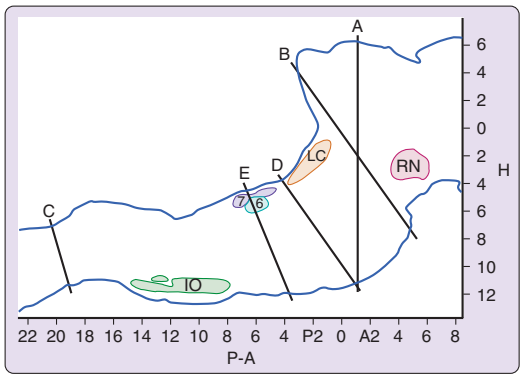

Figure 8-2 Outline of a sagittal section of the brainstem of the cat (level L =1.6) indicating the level of key brainstem transection studies. 6, Abducens nucleus; 7, genu of the facial nerve; H, horizontal (x-axis scale); IO, inferior olive; LC, locus coeruleus; P-A, posterior-anterior (y-axis scale); RN, red nucleus. (Modified from Berman AL. *The brain stem of the cat.* Madison [Wisc.]: University of Wisconsin Press; 1968.)

After the discovery of REM sleep in the cat,[2] Jouvet found that this state of EEG desynchrony normally was accompanied by muscle atonia.[4] Jouvet then examined the decerebrate cat preparation used by Sherrington and by Bard and Macht, with the addition of measures of muscle tone, eye movement, and EEG. One might have expected that REM sleep originates 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 (Figure 8-2, level 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 activity, but when low-voltage activity appeared, the PGO spikes that help identify REM sleep in the intact animal were absent in traces from the thalamic structures, particularly the lateral geniculate, where they can be most easily recorded. Thus it appeared that activity in the isolated forebrain included slow wave sleep states and possibly waking, but with no clear evidence of REM sleep.

By contrast, the midbrain and brainstem behind the cut showed clear evidence of REM sleep. Muscle atonia appeared with a regular periodicity and duration, similar to that of the intact cat's REM sleep periods. This atonia was accompanied by PGO spikes with a morphology resembling that 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 body temperature. 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[46] found that if body temperature was maintained at a normal level, little or no REM sleep was seen. But if temperature was allowed to fall, REM sleep amounts increased to levels well above those seen in the intact animal. This observation 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, in which 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–spinal cord connection has been severed (Figure 8-2, level C). In this case, normal REM sleep in all its manifestations, except for spinally mediated atonia is present.[47] These findings support the conclusion that the region between the caudal medulla and rostral midbrain is sufficient to generate REM sleep.

This approach can be continued by separating the caudal pons from the medulla (Figure 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.[48] Furthermore, neuronal activity in the medulla does not resemble that seen across the REM-NREM sleep cycle, with neuronal discharge very regular for periods of many hours, in contrast with the periodic rate modulation that is linked to the phasic events of REM sleep in the intact animal[49] (Figure 8-3). This observation demonstrates 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 the pons and more rostral brainstem structures.

By contrast, the regions *rostral* to this cut show aspects of REM sleep[50] (Figure 8-4; see also Figure 8-1, *bottom*). Within these regions can be seen 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. Also evident is increased forebrain unit activity, with unit spike bursts in conjunction with PGO spikes, just as in REM sleep.[49,51]

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 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 and associates.[52] These workers 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 were seen in the isolated pons, but no signs of REM sleep were evident 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. The structures within this region that synthesize the core elements of REM sleep are considered in greater detail further on.

Also clear, however, is that the pons alone does not generate all of the phenomena of REM sleep. Atonia requires the activation of motor inhibitory systems in the medulla.[53] In the intact animal, forebrain mechanisms interact with pontine mechanisms to regulate the amplitude and periodicity of PGO spikes,[54] which in turn are linked to the twitches and rapid eye movements of REM sleep. As documented in cases of human REM sleep behavior disorder, the motor activity expressed in dreams is linked to the imagery of the dream.[55]

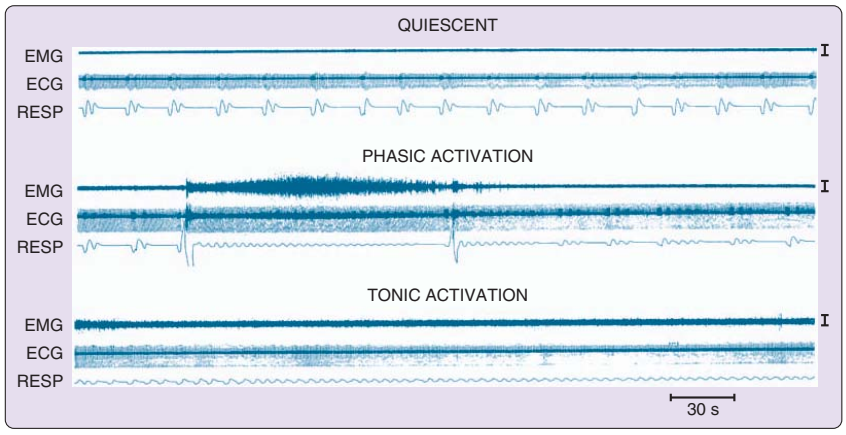

Figure 8-3 States seen caudal to chronic transection at the pontomedullary junction in the cat. Note the absence of periods of atonia. ECG, Electrocardiogram; EMG, electromyogram; RESP, thoracic strain gauge (i.e., respiratory movements). Calibration, 50 μV. (From Siegel JM, Tomaszewski KS, Nienhuis R. Behavioral states in the chronic medullary and mid-pontine cat. *Electroencephalogr Clin Neurophysiol* 1986;63:274-88.)

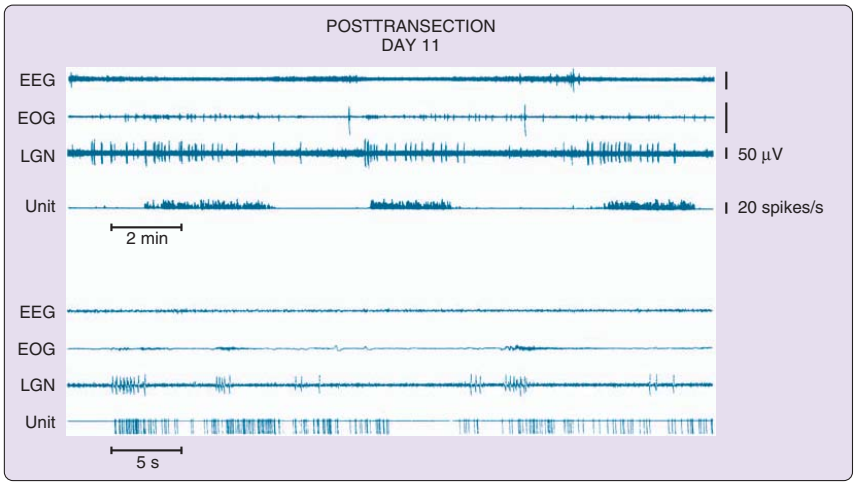

Figure 8-4 States seen rostral to chronic transection at the pontomedullary junction in the cat. Note the presence of ponto-geniculo-occipital (PGO) spikes and associated increases in unit activity triggered by the pons. Midbrain unit: electroencephalogram (EEG), electrooculogram (EOG), and lateral geniculate nucleus (LGN) activity rostral to chronic transections at the pontomedullary junction. *Top traces:* The unit channel displays the output of an integrating digital counter resetting at 1-sec intervals. *Bottom traces:* 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, p. 157-74.)

With extrapolation to dream imagery in normal humans, it can be hypothesized 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 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 then seeing which areas are necessary and which are unnecessary for REM sleep generation. An early systematic study by Carli and Zanchetti in the cat[56] and other subsequent studies emphasized that lesions of locus coeruleus[57] and the dorsal raphe[58] nuclei or of simultaneous lesions of locus coeruleus, forebrain cholinergic neurons and histamine neurons[19] did not block REM sleep. These investigators concluded that lesions that destroyed the region ventral to the locus coeruleus, called the "nucleus reticularis pontis oralis" or "subcoeruleus region," produced a massive decrease in the amount of REM sleep. These studies used the electrolytic lesion technique, in which a current is passed through brainstem tissue, depositing metal that kills cells and axons of passage. As cytotoxic techniques that allowed poisoning of cell bodies without the 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,[53,59,60] because near-total destruction of these cells was followed by normal amounts of REM sleep as soon as anesthesia dissipated.[37,61] However, lesions of the subcoeruleus and adjacent regions with cytotoxins were associated with 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, lesioning 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 lead to elimination of all aspects of REM sleep, introduction of small, bilaterally symmetric lesions within the pons can eliminate specific aspects of REM sleep. With lesions of lateral pontine structures, muscle atonia during REM sleep is seen. However, PGO spikes and the associated rapid eye movements are absent when lesions include the region surrounding the superior cerebellar peduncle of the cat[65] (Figure 8-5, *top*). This observation points to a role for this lateral region in the generation of PGO waves and 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,[53] result in a very unusual syndrome. After NREM sleep, affected 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[5,66] (Figure 8-5, *bottom*). 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

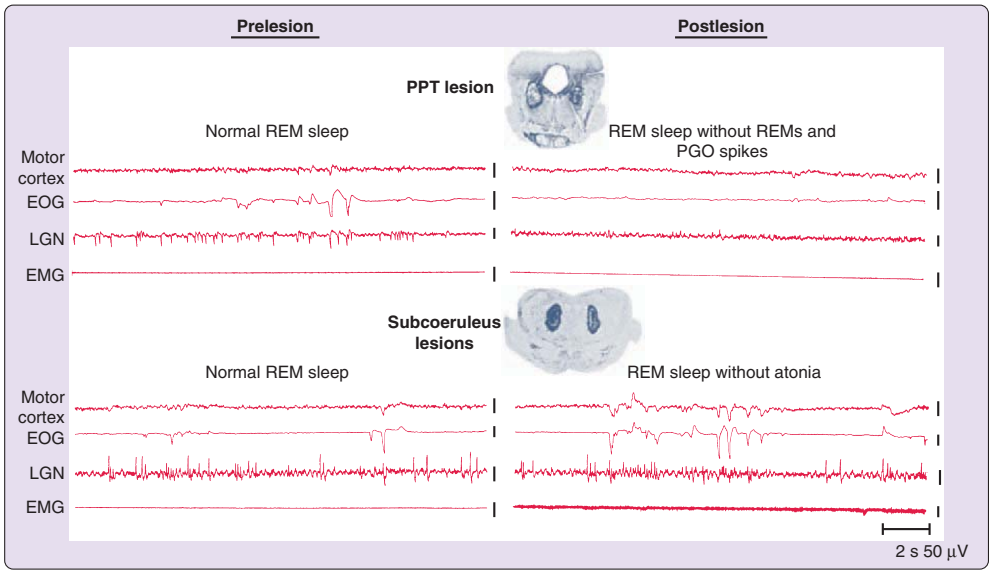

Figure 8-5 Disruption of phasic or tonic aspects of REM sleep by lesions. Twenty-second polygraph tracings during REM sleep before and after introduction of lesions, together with a coronal section through the center of the pontine lesions. Electroencephalographic voltage reduction of REM sleep (recorded from motor cortex) was associated with both lesion locations: pediculopontine tegmental [PPT] and subcoeruleus. *Top traces:* Radiofrequency lesions of the PPT region diminished ponto-geniculo-occipital (PGO) spikes and eye movement bursts during REM sleep. *Bottom traces:* 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 1992, with permission from Elsevier Science.)

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.[53,67-69]

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 $GABA_A$ 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 in the guinea pig[71] and the rat[72] (in the rat, this midbrain region has been called the deep mesencephalic nucleus.) 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]

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 as previously indicated.[75,76] This finding is another reminder that despite the sleep-inducing effect of systemic administration of GABAergic hypnotic medications (such as benzodiazepines), the effect of GABA on sleep and waking states induced by local manipulation varies across brain regions. Blocking GABA in the subcoeruleus has been reported to increase REM sleep in the cat.[77]

Stimulation Studies

The first study showing that stimulation could elicit REM sleep was carried out by George and colleagues.[78] These investigators 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, which could last hours. 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 with lesions blocking 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. The investigators 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.[48,86,87] Further work has demonstrated that the pontine cells in this inhibitory region receiving 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] Glutamatergic excitation of this region in the cat also increases REM sleep,[92] suggesting that both cholinergic and glutamatergic mechanisms are intimately involved in the triggering of REM sleep, although the evidence points tospecies differences in the relative potency of the effect of microinjection of these two neurotransmitters.

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 REM sleep state as a whole, and to 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 dissection of REM sleep mechanisms.

Medial Brainstem Reticular Formation

Most cells within the medial brainstem reticular formation are maximally active in waking, greatly reduce discharge rate in NREM sleep, and increase discharge rate back to waking levels in REM sleep.[15,16,60,93,94] Discharge is most regular in NREM sleep and is relatively irregular in both waking and REM sleep. The similarity of the waking and REM sleep discharge patterns suggests similar roles 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 normally are 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. Lesioning of these cells has little or no effect on REM sleep duration or periodicity[37,38] but 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 above, 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 NREM sleep or waking.[96] Recordings of neuronal activity within 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.[97,98] Presumably the REM sleep-on cholinergic cells project to the acetylcholine responsive region in the subcoeruleus area.[99]

Cells with Activity Selective for REM Sleep

Cells with activity selective for REM sleep can be identified within the subcoeruleus area in both cats[100] and rats.[72] Anatomic studies using Fos labeling and tract tracing and unit recording studies indicate that these neurons are glutamatergic and GABAergic[98] and that some of them project to the ventral medullary region involved in the triggering of the muscle atonia of REM sleep.[48,64,72,86,88-90]

Monoamine-Containing Cells

Monoamine-containing cells have a very different discharge profile. Most if not all noradrenergic,[101,102] and serotoninergic[103] cells of the midbrain and pontine brainstem and histaminergic[104] cells of the posterior hypothalamus are continuously active during waking, decrease their activity during NREM sleep, and further reduce or cease activity during REM sleep (Figure 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,[105-108] presumably by REM sleep–active GABAergic brainstem neurons.[109,110] Administration of a GABA agonist to target the raphe cell group increases REM sleep duration,[106] 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,[42,111,112] other work suggests an increased release of dopamine in REM sleep[113,114] or

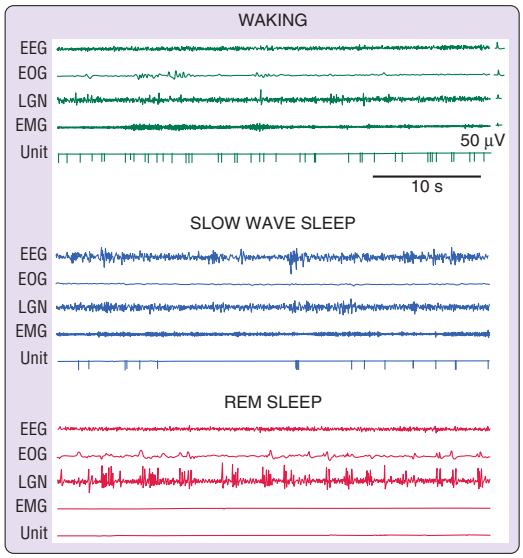

Figure 8-6 Activity of a "REM sleep–off" cell recorded in the locus coeruleus. (Slow wave sleep = NREM sleep.) EEG, Sensorimotor electroencephalogram; EMG, neck electromyogram; EOG, electrooculogram (eye movements); LGN, lateral geniculate activity; Unit, pulses triggered by the locus coeruleus cell.

shows decreased release in REM sleep, and still other work shows selective waking activity in these neurons.[116] These findings may reflect heterogeneity of firing of different dopamine cell groups and presynaptic control of release in dopamine terminals.

Other Cholinergic Cells in Lateral Pontine Regions

Other cholinergic cells in lateral pontine regions discharge in bursts before each ipsilateral PGO wave.[117,118] These cells may therefore participate in the triggering of these waves. As shown in other studies, PGO waves are tonically inhibited in waking by serotonin input.[119-121] It is likely, therefore, that certain groups of cholinergic cells receive direct or perhaps indirect serotoninergic inhibition in waking and that the decrease of this inhibition in NREM 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-minute (or longer) period before sacrifice. Quattrochi and associates demonstrated that microinjections of the cholinergic agonist carbachol that triggered episodes of continuous PGO waves in waking activated neurons within the laterodorsal and pedunculopontine nuclei. Destruction of these nuclei blocks these waves.[121-123]

More extensive Fos mapping has been done to identify neurons activated during REM sleep in the rat. Verret and colleagues[124] found that only a few cholinergic neurons from the laterodorsal and pedunculopontine tegmental nuclei were Fos-labeled after REM sleep. By contrast, a large number of noncholinergic Fos-labeled cells was observed in the laterodorsal tegmental nucleus, subcoeruleus region and lateral, ventrolateral, and dorsal periaqueductal gray of the midbrain. In addition, other regions outside of 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[125] 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 thou ght to cause the cessation of discharge in locus coeruleus cells during REM sleep.[107] Verret and coworkers[87] also showed 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[109] 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 serotoninergic neurons,[106,126] although medullary neurons may participate in the intact animal.

Fos mapping also has been used to identify forebrain regions likely to control REM sleep. The preoptic region, important in NREM sleep control (see Chapter ••) 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.[127] 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.[128,129] These results suggest that melanin-concentrating hormone neurons also contribute to forebrain modulation of REM sleep.

Of note, the identity of the cells involved in triggering and controlling REM sleep is not easily determined. The Fos studies do not necessarily identify all cells active during REM sleep; only those of a phenotype that allows them to express Fos during the tested manipulations are so labeled. 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. Conversely, 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, identification of the cells responsible for starting the process of REM sleep triggering cannot be easily be achieved 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, although easier than in freely moving animals, can be misleading, because such restraint can lower the activity of movement-related cells in waking, making them appear to be selectively active in REM sleep.[36] Nevertheless, by comparing the results of multiple recording and stimulation techniques with data on lesions, the evidence thus obtained 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.[130] The normal suppression of tone in the tongue and laryngeal muscles in REM sleep is a major contributing factor in sleep apnea (see Chapter ••). The failure of muscle tone suppression in REM sleep causes REM sleep behavior disorder[131] (Chapter ••). Triggering of the REM sleep muscle tone control mechanism in waking is responsible for cataplexy.[132]

Early work using intracellular recording and microiontophoresis had shown that motoneuron hyperpolarization during REM sleep was accompanied by the release of glycine onto motoneurons.[130,133] Microdialysis sampling showed that both GABA and glycine are released onto motoneurons during atonia induced by carbachol in the cat.[41] This release occurs in ventral horn motoneurons as well as in hypoglossal motoneurons. The glycinergic inhibition during a carbachol elicited REM sleep–like state was investigated with immunohistochemistry and found to be due to 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,[133] 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.[134] Because these monoamines are known to excite motoneurons and because GABA and glycine are known to inhibit motoneurons, 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, located in the subcoeruleus region,[86] produces muscle tone suppression. Even though the pontine inhibitory area is situated 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.[135] 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 which receives pontine inhibitory area projections (Figure 8-7).

The release of GABA and glycine onto motoneurons during REM sleep atonia is most likely to be mediated by a pathway from the pontine inhibitory area to the medial medulla.[89,90] The pontine region triggering this release is not only sensitive to acetylcholine but also responds to glutamate[88] (Figure 8-8).[86] 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[48,136] (Figure 8-8). The medullary interaction with pontine structures evidently is critical for muscle tone suppression, because inactivation of pontine regions greatly reduces the suppressive effects of medullary stimulation on muscle tone.[137,138] This ascending pathway from the medulla to the pons may mediate the inhibition of locus coeruleus during atonia and also may 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,[137]

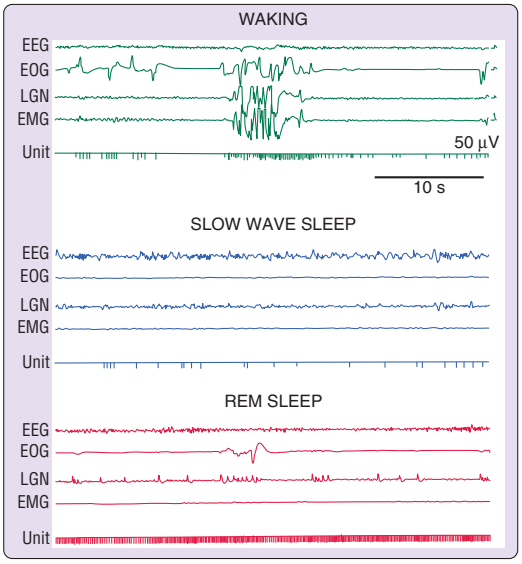

Figure 8-7 Activity of medullary "REM sleep–on" cell. Note the tonic activity during REM sleep. (Slow wave sleep = NREM sleep.) In waking, activity generally is absent even during vigorous movement. Some activity, however, is seen during movements involving head lowering and postural relaxation. EEG, Sensorimotor electroencephalogram; EMG, neck electromyogram; EOG, electrooculogram (eye movements); LGN, lateral geniculate activity; Unit, pulses triggered by the locus coeruleus cell.

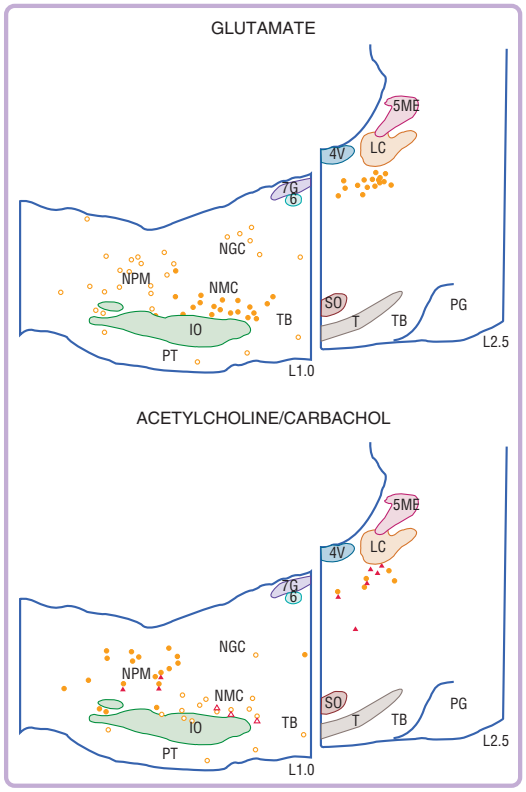

Figure 8-8 Sagittal map of pontomedullary inhibitory areas. Electrical stimulation produced atonia at all points mapped. All electrically defined inhibitory sites were microinjected with glutamate or cholinergic agonists. *Filled symbols* represent points at which microinjections decreased muscle tone (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. Data for glutamate injections are shown at the top; data for acetylcholine (ACh) and carbachol (Carb) injections at the bottom, with *circles* and *triangles* representing ACh and Carb injections, respectively. 4V, Fourth ventricle; 5ME, mesencephalic trigeminal tract; 6, abducens nucleus; 7G, genu of the facial nerve; 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. (From Lai YY, Siegel JM. Medullary regions mediating atonia. *J Neurosci* 1988;8:4790–6.)

again indicating that the pons is a key component of the circuit producing motor inhibition.

The studies just reviewed focused largely on ventral horn and hypoglossal motoneurons. However, the control of jaw muscles also is 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 ••). 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, with use of the other side as a control for changes in behavioral state caused by application of neurotransmitter agonist and antagonists.[139] Using this model, it was determined that tonic glycine release reduces muscle tone in both waking and NREM sleep. Blockade of glycine receptors, however, 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 NREM sleep but did not prevent the suppression of masseter tone[140] or of genioglossus tone in REM sleep.[141] Both of these manipulations, however, 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 NREM sleep. Glutamate also contributes to the phasic motor activity during REM sleep. Reduction in glutamate alone, however, 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.[142]

A study in the anesthetized rat suggested that activation of norepinephrine receptors, in combination with the activation of glutamate receptors was sufficient to potently increase muscle tone in the masseter muscles.[143] 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.[144] In the context of previous microdialysis analysis of transmitter release, these studies suggest that the reduction of norepinephrine release may a key factor regulating muscle tone, along with the aforementioned changes in amino acid release. These conclusions are consistent with earlier work indicating that cataplexy was linked to a reduction in the activity of noradrenergic neurons (see further on).[145] 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 may 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 serotoninergic neurons, although earlier studies in vagotomized and anesthetized rats had shown an effect of serotonin on muscle tone under these aphysiologic conditions.[146-148]

Subsequent work suggested that inhibition of motor output is accompanied by a neurochemically similar inhibition of sensory relays during REM sleep.[149] 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 with the 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.[111] Dopamine release in the amygdala measured by dialysis does not significantly vary across the sleep cycle.[150] In disagreement with this finding, a Fos study indicted that dopaminergic neurons within the ventral portion of the mesencephalic tegmentum were activated during periods of increased REM sleep.[151] 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.[113] Other work using the Fos labeling technique identified a wake-active dopaminergic cell population in the ventral periaqueductal gray.[116] In dialysis measurements of dopamine release, dopamine release was reduced in the dorsal horn of the spinal cord during the REM sleep–like state triggered by carbachol. Such a decrease was not seen in the ventral horn or hypoglossal nucleus.[134] 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 enter REM sleep within 5 minutes of sleep onset, in contrast with normal persons, who rarely show such "sleep-onset REM sleep." Most narcoleptics experience cataplexy,[152] 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.[145] 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 normals, become active during cataplexy in narcoleptics including cells in the medial medullary inhibitory region that are selectively active in relation to the atonia of REM sleep.[17,132] Likewise, cells in the locus coeruleus, which cease discharge only in REM sleep in normal animals, invariably cease discharge in cataplexy.[153] 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 noted previously, in the normal animal, noradrenergic, serotoninergic, and histaminergic cells are all tonically active in waking, reduce discharge in NREM sleep, and cease discharge in REM sleep.[145,153] Unlike noradrenergic cells, however, serotoninergic cells do not cease discharge during cataplexy, only reducing discharge to quiet waking levels. Histaminergic cells actually increase discharge in cataplexy relative to quiet waking levels[154] (Figure 8-10). These findings allow identification of some of

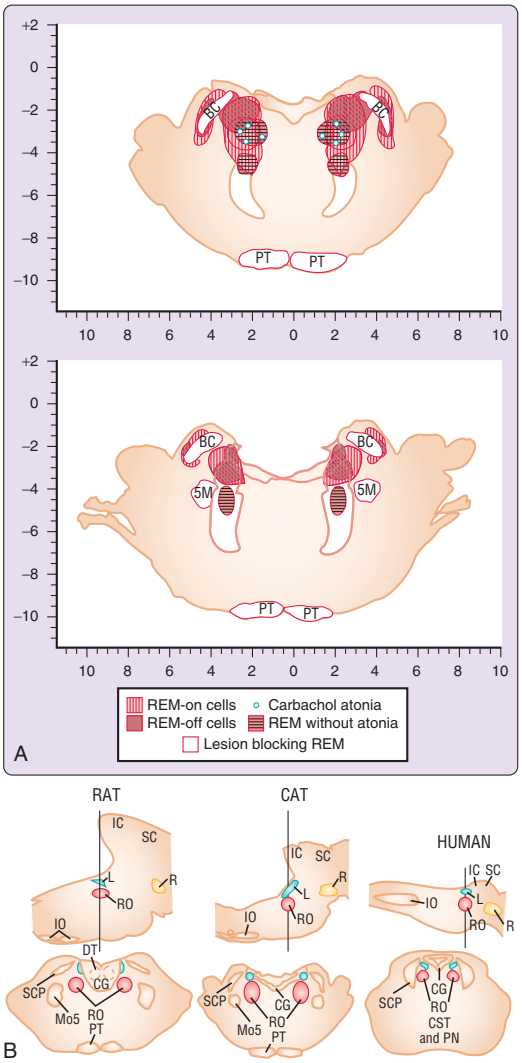

Figure 8-9 A, B, Anatomic relation of "REM sleep–on" and "REM sleep–off" cells, carbachol-induced atonia sites, lesions blocking atonia but not preventing REM sleep, and lesions completely blocking REM sleep. **A,** ••. BC, ••; 5M, ••; PT, ••. **B** shows anatomic locations of REM on areas in cat and rat brains and projected location in the human in sagittal and coronal views. CG, Central gray; CST, corticospinal tract; DT, dorsal tegmental; IC, inferior colliculus; IO, inferior olive; L, locus coeruleus; Mo5, motor nucleus of the trigeminal nerve (5M); PN, pontine nuclei, PT, ••; R, red nucleus; RO, reticularis oralis nucleus; SC, superior colliculus; SCP, superior cerebellar peduncle (brachium conjunctivum). (**A** from Siegel JM, Rogawski MA. A function for REM sleep: regulation of noradrenergic receptor sensitivity. *Brain Res* 1988;13:213-33. **B** from Siegel JM. The stuff dreams are made of: anatomical substrates of REM sleep. *Nat Neurosci* 2006;9:721–2.)

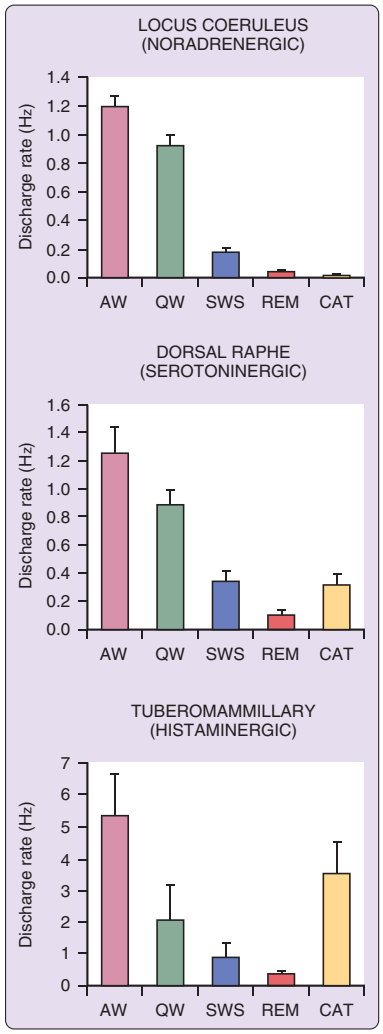

Figure 8-10 Comparison of mean discharge rates in sleep-waking states and cataplexy for REM-off cells recorded from three brain regions. Posterior hypothalamic histaminergic neurons remain active, whereas dorsal raphe serotoninergic neurons reduced discharge, and locus coeruleus noradrenergic neurons ceased 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. AW, Active waking; CAT, cataplexy; QW, quiet waking; REM, REM sleep; SWS, slow wave (NREM) sleep. (From John J, Wu MF, Boehmer LB, SiegelJM. Cataplexy-active neurons in the posterior hypothalamus: implications for the role of histamine in sleep and waking behavior. *Neuron* 2004;42:619–34.).

the cellular substrates of cataplexy. Medullary inhibition and noradrenergic disfacilitation are linked to cataplexy's loss of muscle tone. By 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 insight into both narcolepsy and the normal role of these cell groups in maintaining consciousness and muscle tone.

In 2001 it was discovered that most human narcolepsy was caused by a loss of hypothalamic cells containing the peptide hypocretin[23,24] (Figure 8-11). 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 disease, with a loss of up to 60% of hypocretin cells.[155,156] It was found that administration of the peptide to genetically narcoleptic dogs reversed symptoms of the disorder[157] and that nasal administration reversed sleepiness in monkeys,[158] suggesting that similar treatment could be uniquely effective for narcolepsy and perhaps for other disorders characterized by sleepiness.[159-161] Recently it also has been found that in human narcoleptics, the number of detectable histamine cells is increased more than 65%.[162,163] It has been speculated that because this change is not seen in any of four different animal genetic models of narcolepsy, the increase may be related to the presumed immune activation that causes human narcolepsy.[162]

In further work in normal animals, it was determined that identified hypocretin neurons discharge at their highest rates during active waking[35,164] (Figure 8-12). This discharge was reduced or absent during aversive waking situations, even if the EEG indicated high levels of alertness.[35] The hypocretin level in normal dogs is nearly doubled when they are let out into a yard to play with other dogs. By contrast, when these same dogs run at maximal speed on a treadmill, hypocretin levels are unchanged, demonstrating that motor activity and associated changes in respiratory rate, heart rate, and body temperature do not by themselves determine the release of hypocretin. Findings in studies of hypocretin release in the cat[165] also are consistent with this hypothesis. Hypocretin cells send ascending projections to cortical and basal forebrain regions, in addition to their descending projection to locus coeruleus and other brainstem regions. In the absence of hypocretin-mediated facilitation of forebrain arousal centers, waking periods are truncated, resulting in the sleepiness of narcolepsy.[166]

The functions of hypocretin have been investigated in genetic knockout animals lacking the peptide and in their wild-type littermates, using operant reinforcement tasks. Hypocretin-knockout mice are deficient in the performance of bar presses to secure food or water reinforcement. However, they do 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 are characterized by EEG deactivation.[167] This deficit is restricted to the light phase, suggesting that hypocretin neurons mediate the arousing and mood-elevating effects of light,[167] effects that are central to the current understanding of depression. Fos labeling studies in normal littermates showed that the positive reinforcement task used in this study is characterized by activation of hypocretin neurons. However, hypocretin neurons are not activated in the negative reinforcement task or during the same positively motivated task in the dark phase, despite high levels of EEG activation, indicating that nonhypocretin systems mediate arousal during these behaviors.

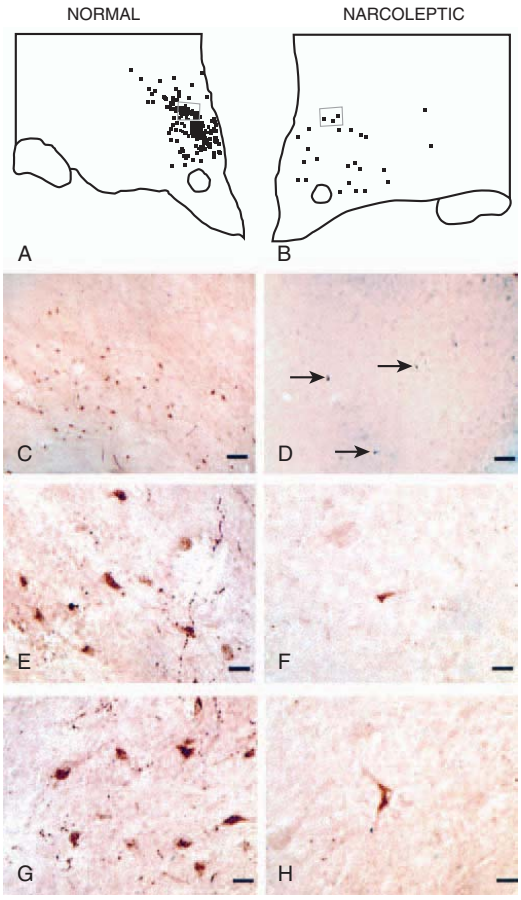

Figure 8-11 Loss of hypocretin cells in human narcolepsy. Distribution of cells in perifornical and dorsomedial hypothalamic regions of normal (**A, C, E, G**) and narcoleptic (**B, D, F, H**) humans. (From Thannickal TC, Moore RY, Nienhuis R, et al. Reduced number of hypocretin neurons in human narcolepsy. *Neuron* 2000;27:469-74.)

The conclusions of these animal studies were extended in the first study of hypocretin release within the human brain. Hypocretin levels were shown to be maximal during positive emotion, social interaction, and anger, behaviors that induce cataplexy in human narcoleptics. This finding is consistent with the hypothesis that release of hypocretin facilitates motor activity during emotionally charged activities of the sort that trigger cataplexy in narcoleptics.[166,168,169] Even normal persons experience weakness at these times, seen in the "doubling over" that often accompanies laughter or the weakness that can result from other strong emotions of sudden onset. In the absence of the hypocretin-mediated motor facilitation of locus coeruleus and other brainstem regions, muscle tone is lost at

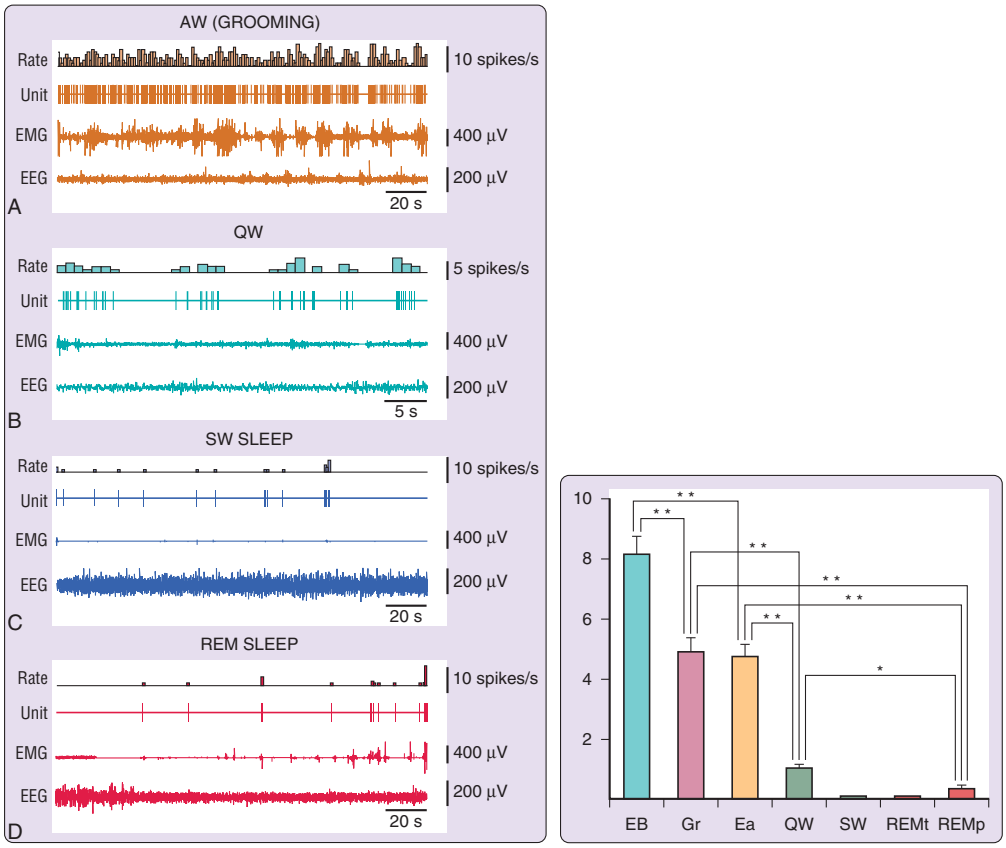

Figure 8-12 Firing rate of hypocretin cells in waking and sleep behaviors in freely moving rats. *Left:* The discharge pattern of a representative hypocretin neuron across the sleep-waking cycle in the freely moving rat. **A,** High firing rates are seen during active waking (AW) withgrooming. **B,** Reduced firing rate or cessation of activity is seen in quiet waking (QW) and drowsiness. **C,** A further decrease or cessation of firing is seen during slow wave (NREM) sleep. **D,** Minimal firing rate is seen during the tonic phase of REM sleep. Brief hypocretin cell discharge bursts are correlated with muscle twitches during the phasic events of REM sleep. *Right:* Summary data from identified hypocretin cells: exploratory behavior (EB), grooming (Gr), eating (Ea), QW, SW sleep (SW), and tonic (REMt) and phasic (REMp) sleep. Maximal discharge is seen during exploration-approach behavior. (From Mileykovskiy BY, Kiyashchenko LI, Siegel JM. Behavioral correlates of activity in identified hypocretin (orexin) neurons. *Neuron* 2005;46:787-98.)

these times. By contrast, the release in humans of melanin-concentrating hormone, a peptide produced by neurons intermixed in the hypothalamus with the hypocretin neurons, is minimal during social interaction but is increased after eating. Both peptides are at minimal levels during periods of postoperative pain despite high levels of arousal. Melanin-concentrating hormone levels increase at sleep onset, consistent with a role in sleep induction,[170] whereas hypocretin-1 levels increase at wake onset, consistent with a role in wake induction. Levels of these two peptides in humans are not simply linked to arousal but rather are correlated with specific emotions and state transitions[171] (Figure 8-13).

The findings that hypocretin is released and hypocretin neurons are active only during arousal linked to certain emotions suggests a new approach to the understanding of arousal systems. Hypocretin is clearly related to arousal linked to certain generally positive emotions. Other arousal systems must mediate arousal during aversive situations. An analysis of the differential activation of arousal systems as a function of emotion, light level, and other variables might provide important clinical and basic science insights into the unique roles of each arousal system.

Hypocretin appears to act largely by modulating the release of amino acid neurotransmitters.[172] Systemic injection of hypocretin causes a release of glutamate in certain hypocretin-innervated regions, producing a potent postsynaptic excitation.[139,173] In other regions it facilitates GABA release, producing postsynaptic inhibition.[165,174] The loss of these competing inhibitory and facilitatory influences in narcolepsy appears to leave brain motor regulatory and arousal systems

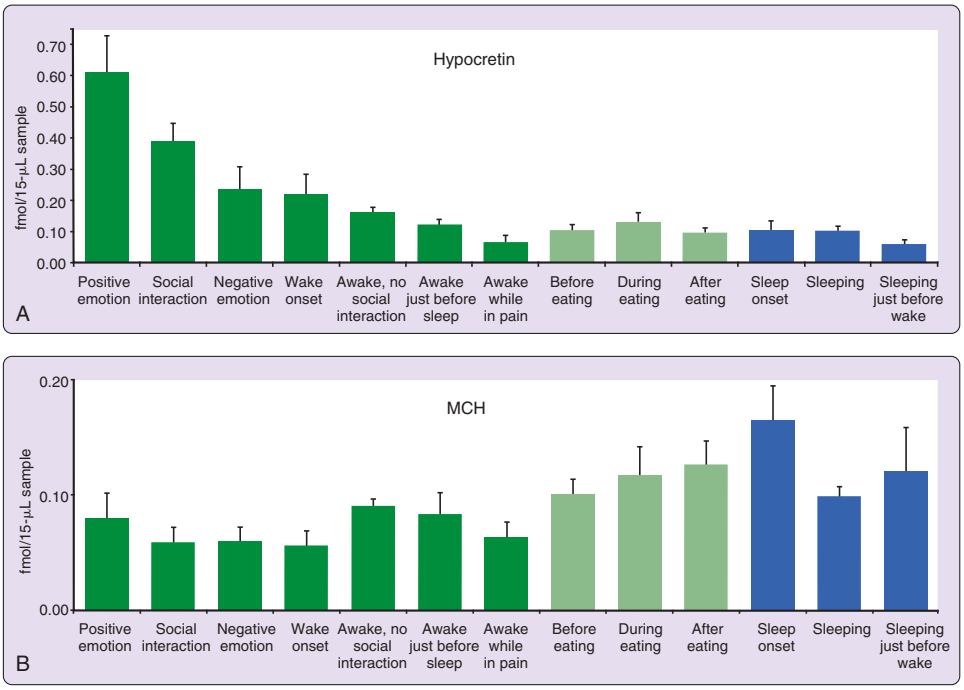

Figure 8-13 Hypocretin and melanin-concentrating hormone (MCH) levels across waking and sleep activities in humans. **A,** Maximal hypocretin levels in waking are seen during positive emotions and social interactions and on awakening; minimal levels are seen before sleep and in alert waking associated with reported pain. Changes during and after eating are smaller than those during monitored non–eating-related activities. Waking values are shown in *shades of green*; sleep values, in *blue*. For all awake samples, subjects were awake but were not exhibiting social interaction or reporting emotion. **B,** Maximal MCH levels are seen at sleep onset and after eating. Minimal levels are seen during wake onset, social interaction, and pain. Error bars represent ±S.E.M. (From Blouin AM, Fried I, Wilson CL, et al. Human hypocretin and melanin-concentrating hormone levels are linked to emotion and social interaction. *Nat Commun* 2013;4:1547.)

less stable than the tightly regulated balance that can be maintained in the presence of hypocretin (Figure 8-14). 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 in muscle tone during sleep, resulting in a striking *increased* incidence of REM sleep behavior disorder in narcoleptics (Chapter ••). In the same manner, although a principal symptom of narcolepsy is intrusions of sleep into the waking period, narcoleptic persons sleep poorly at night, with frequent awakenings.[175-177] 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 in normal persons as a result of the loss of hypocretin function.

THE FUNCTIONS OF RAPID EYE MOVEMENT SLEEP

Research into the control of REM sleep turns into a seemingly infinite regression, with REM-on cells inhibited by REM-off cells, which in turn may be inhibited by other REM-on cells. It is very difficult to identify the sequence in which these cell groups normally are 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 the human and cat and at least 30 or more seconds in the rat. It also does not completely enlighten researchers with respect to the ultimate functional question: What is REM sleep for? To answer this question requires determining what if any physiologic process is altered over REM sleep periods. Is some toxin excreted or some protein synthesized? If so, how can the widely varying durations of the typical REM sleep be accounted for? In the human, REM sleep typically lasts from 5 to 30 minutes, whereas in the mouse, it typically lasts 90 seconds.[178] 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? The biologic need that initiates REM sleep remains unknown, as well as the source of the REM sleep "debt" that accumulates during REM sleep deprivation.[179] Why do some marine mammals have no apparent REM sleep (see Chapter 10). 17

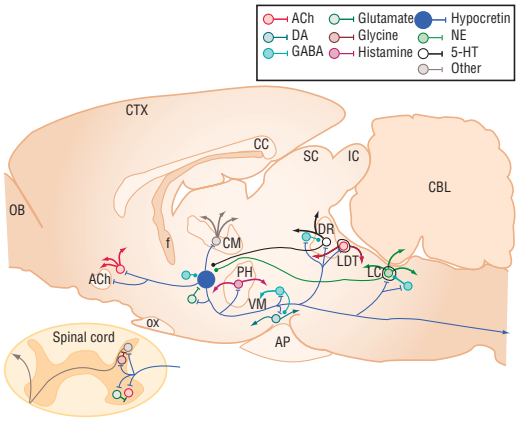

Figure 8-14 Major identified synaptic interactions of hypocretin neurons. Lines terminated by *perpendicular* lines denote excitation; *circular* terminations indicate inhibition. Acb, Nucleus accumbens; ACh, acetylcholine; AP, anterior pituitary; CBL, cerebellum; CC, ••; CM, centromedian nucleus of the thalamus; CTX, cortex; DA, dopamine; DR, dorsal raphe; f, fornix; 5-HT, 5-hydroxytryptamine (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.

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 previously, many of the key neurotransmitters and neurons involved have been identified. The discovery of the role of hypocretin in narcolepsy serves as a reminder that key cell groups may still need to be identified before fundamental insights can be gainto the generation mechanism and functions of REM sleep can be gained. Yet despite this caveat, a substantial amount of information about what goes on in the brain during REM sleep has already been accumulated.

Clearly, 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. Such a state would be unlikely to have produced a Darwinian advantage and remained so ubiquitous among mammals if it did not have benefits compensating for its obvious costs. But what might these 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 such a role is poor.[180] 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.[181] A leading proponent of a sleep and memory consolidation relationship has concluded that sleep has no role in the consolidation of declarative memory,[182] 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 NREM sleep is important and others stating just the reverse, and still others claiming that both sleep states are essential.[180] Millions of people 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. Of note, however, not a single report of memory deficits attributable to such treatment has emerged. Likewise, well-studied patients with permanent loss of REM sleep resulting from pontine damage show normal learning abilities; the best-studied of these patients completed law school after his injury[183] and was last reported to be the puzzle editor of his city newspaper. People with multiple systems atrophy can have a complete loss of slow wave sleep and disruption of REM sleep without manifesting any substantial memory deficit.[184] A recent well-controlled study showed that REM sleep suppression with selective serotonin reuptake inhibitors or serotonin-norepinephrine reuptake inhibitors was associated with no significant decrement in memory consolidation on any task and even produced a small but significant improvement in a motor learning (i.e., procedural) task.[185]

Another idea that has been suggested repeatedly is that REM sleep serves to stimulate the brain.[186-188] According to this theory, the inactivity of NREM 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 upon awakening. Such alterations would leave mammals functioning like reptiles, with slow response after periods of inactivity. This hypothesis explains the appearance of REM sleep after NREM 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 from NREM sleep, as are rats[189]—findings consistent with this idea. The very low amounts or absence of REM sleep in dolphins, in which the brainstem is continuously active and which never show 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 cannot substitute for REM sleep in terrestrial mammals. REM sleep–deprived persons experience a REM sleep rebound even if they are kept in an active waking state for extended periods, although this effect may be a result of stress, rather than REM sleep loss.[181]

A 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 the awake state, so waking would not be expected to substitute for this aspect of REM sleep.[190] REM sleep rebound may therefore be due to 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 also may potentially benefit from periods of inactivity. Some but not all studies have supported this hypothesis.[191-195]

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.

CLINICAL PEARL

The loss of hypocretin neurons is responsible for most cases of 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 explanation 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, as well as for depression.

SUMMARY

REM sleep was first identified by its most obvious behavior: rapid eye movements during sleep. In most adult mammals the EEG of the neocortex is low in voltage during REM sleep. The hippocampus has regular high-voltage theta waves throughout REM sleep. The tone of the postural muscles is greatly reduced or abolished during this state.

The key brain structure for generating REM sleep is the brainstem, particularly the pons and adjacent portions of the midbrain. Considerable progress has been made in identifying the neurons most closely linked to REM sleep within these regions and the transmitters that they employ. Massive damage to the REM-generating region can abolish REM sleep. Small lesions can cause REM sleep without atonia in animals or REM sleep behavior disorder in humans

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, a cell group whose somas are localized to the hypothalamus. Hypocretin neurons have potent effects on alertness and motor control and normally are activated in relation to particular, generally positive emotions in humans as well as in animals. In the absence of this cell group, cataplexy, a REM sleep–like loss of muscle tone, occurs.

ACKNOWLEDGEMENTS

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Selected Readings

Rasch B, Pommer J, Diekelmann S, Born J. Pharmacological REM sleep suppression paradoxically improves rather than impairs skill memory. *Nat Neurosci* 2009;**12**:396–7.

Schenck CH, Montplaisir JY, Frauscher B, et al. REM sleep behavior disorder (RBD): devising controlled active treatment studies for symptomatic and neuroprotective therapy—a consensus statement by the International RBD Study Group. *Sleep Med* 2013;**14**:795–806.

Siegel JM. The REM sleep-memory consolidation hypothesis. *Science* 2001;**294**(5544):1058–63.

Siegel JM. Clues to the functions of mammalian sleep. *Nature* 2005;**437**:1264–71.

A complete reference list can be found online at https://ExpertConsult.inkling.com/.

References

1. Aserinsky E, Kleitman N. Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. *Science* 1953;**118**:273–4.
2. Dement WC. The occurrence of low voltage, fast, electroencephalogram patterns during behavioral sleep in the cat. *Electroencephalogr Clin Neurophysiol* 1958;**10**:291–6.
3. Jouvet M, Michael F, Courjon J. Sur un stade d'activite electrique cerebrale rapide au cours du sommeil physiologique. *C R Soc Biol* 1959;**153**: 1024–8.
4. Jouvet M. Recherches sur les structures nerveuses et les mechanismes responsables des differentes phases du sommeil physiologique. *Arch Ital Biol* 1962;**100**:125–206.
5. Jouvet M, Delorme F. Locus coeruleus et sommeil paradoxal. *C R Soc Biol* 1965;**159**:895–9.
6. Solms M. Dreaming and REM sleep are controlled by different brain mechanisms. *Behav Brain Sci* 2000;**23**(6):843–50.
7. Foulkes D. Home and laboratory dreams: four empirical studies and a conceptual reevaluation. *Sleep* 1979;**2**(2):233–51.
8. Siegel JM, Manger PR, Nienhuis R, et al. Sleep in the platypus. *Neuroscience* 1999;**91**(1):391–400.
9. Siegel JM, Manger P, Nienhuis R, et al. The echidna Tachyglossus aculeatus combines REM and nonREM aspects in a single sleep state: implications for the evolution of sleep. *J Neurosci* 1996;**16**:3500–6.
10. Hirshkowitz M, Schmidt MH. Sleep-related erections: clinical perspectives and neural mechanisms. *Sleep Med Rev* 2005;**9**(4):311–29.
11. Parmeggiani PL. Thermoregulation and sleep. *Front Biosci* 2003;**8**: s557–67.
12. Villablanca J. Behavioral and polygraphic study of "sleep" and "wakefulness" in chronic decerebrate cats. *Electroencephalogr Clin Neurophysiol* 1966;**21**:562–77.
13. Morrison AR, Bowker RM. The biological significance of PGO spikes in the sleeping cat. *Acta Neurobiol Exp (Wars)* 1975;**35**:821–40.
14. De Gennaro L, Ferrara M. Sleep deprivation and phasic activity of REM sleep: independence of middle-ear muscle activity from rapid eye movements. *Sleep* 2000;**23**(1):81–5.
15. Siegel JM, Tomaszewski KS. Behavioral organization of reticular formation: studies in the unrestrained cat. I. Cells related to axial, limb, eye, and other movements. *J Neurophysiol* 1983;**50**:696–716.
16. Siegel JM, Tomaszewski KS, Wheeler RL. Behavioral organization of reticular formation: studies in the unrestrained cat: II. Cells related to facial movements. *J Neurophysiol* 1983;**50**:717–23.
17. Siegel JM, Nienhuis R, Fahringer HM, et al. Activity of medial mesopontine units during cataplexy and sleep-waking states in the narcoleptic dog. *J Neurosci* 1992;**12**:1640–6.
18. Siegel JM. Do all animals sleep? *Trends Neurosci* 2008;**31**(4):208–13.
19. Blanco-Centurion C, Gerashchenko D, Shiromani PJ. Effects of saporin-induced lesions of three arousal populations on daily levels of sleep and wake. *J Neurosci* 2007;**27**(51):14041–8.
20. Chemelli RM, Willie JT, Sinton CM, et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* 1999;**98**(4): 437–51.
21. Lin L, Faraco J, Kadotani H, et al. The REM sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor gene. *Cell* 1999;**98**:365–76.
22. Nishino S, Ripley B, Overeem S, et al. Hypocretin (orexin) deficiency in human narcolepsy. *Lancet* 2000;**355**:39–41.
23. Peyron C, Faraco J, Rogers W, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat Med* 2000;**6**:991–7.
24. Thannickal TC, Moore RY, Nienhuis R, et al. Reduced number of hypocretin neurons in human narcolepsy. *Neuron* 2000;**27**(3):469–74.
25. Magoun HW. Bulbar inhibition and facilitation of motor activity. *Science* 1944;**100**:549–50.
26. Sterman MB, Clemente CD. Forebrain inhibitory mechanisms: sleep patterns induced by basal forebrain stimulation in the behaving cat. *Exp Neurol* 1962;**6**:103–17.
27. Bourgin P, Huitron-Resendiz S, Spier AD, et al. Hypocretin-1 modulates rapid eye movement sleep through activation of locus coeruleus neurons. *J Neurosci* 2000;**20**:7760–5.
28. Sakurai T, Amemiya A, Ishii M, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 1998;**92**(4):573–85.
29. Espana RA, Baldo BA, Kelley AE, Berridge CW. Wake-promoting and sleep-suppressing actions of hypocretin (orexin): basal forebrain sites of action. *Neuroscience* 2001;**106**(4):699–715.
30. Kuru M, Ueta Y, Serino R, et al. Centrally administered orexin/hypocretin activates HPA axis in rats. *Neuroreport* 2000;**11**(9): 1977–80.
31. Xi MC, Fung SJ, Yamuy J, et al. Induction of active (REM) sleep and motor inhibition by hypocretin in the nucleus pontis oralis of the cat. *J Neurophysiol* 2002;**87**(6):2880–8.
32. Kiyashchenko LI, Mileykovskiy BY, Lai YY, Siegel JM. Increased and decreased muscle tone with orexin (hypocretin) microinjections in the locus coeruleus and pontine inhibitory area. *J Neurophysiol* 2001;**85**: 2008–16.
33. Kunii K, Yamanaka A, Nambu T, et al. Orexins/hypocretins regulate drinking behaviour. *Brain Res* 1999;**842**:256–61.
34. Adamantidis AR, Zhang F, Aravanis AM, et al. Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature* 2007;**450**(7168):420–4.
35. Mileykovskiy BY, Kiyashchenko LI, Siegel JM. Behavioral correlates of activity in identified hypocretin/orexin neurons. *Neuron* 2005;**46**(5): 787–98.
36. Siegel JM, McGinty DJ. Pontine reticular formation neurons and motor activity. *Science* 1978;**199**:207–8.
37. Sastre JP, Sakai K, Jouvet M. Are the gigantocellular tegmental field neurons responsible for paradoxical sleep? *Brain Res* 1981;**229**: 147–61.
38. Drucker-Colin RR, Pedraza JG. Kianic acid lesions of gigantocellular tegmental field (THG) neurons does not abolish REM sleep. *Brain Res* 1983;**272**:367–91.
39. Takahashi K, Lin JS, Sakai K. Neuronal activity of orexin and non-orexin waking-active neurons during wake-sleep states in the mouse. *Neuroscience* 2008;**153**(3):860–70.
40. Kodama T, Lai YY, Siegel JM. Enhanced glutamate release during REM sleep in the rostromedial medulla as measured by in vivo microdialysis. *Brain Res* 1998;**780**:178–81.
41. Kodama T, Lai YY, Siegel JM. Changes in inhibitory amino acid release linked to pontine-induced atonia: an in vivo microdialysis study. *J Neurosci* 2003;**23**(4):1548–54.
42. Lai YY, Kodama T, Siegel JM. Changes in monoamine release linked to pontine inhibition of muscle tone: An in vivo microdialysis study. *J Neurosci* 2001;**21**:7384–91.
43. Sagar SM, Sharp FR, Curran T. Expression of c-fos protein in brain: metabolic mapping at the cellular level. *Science* 1988;**240**(4857): 1328–31.
44. Sharp FR, Sagar SM, Swanson RA. Metabolic mapping with cellular resolution: c-fos vs. 2-deoxyglucose. *Crit Rev Neurobiol* 1993;**7**(3–4): 205–28.
45. Bard P, Macht MB. The behavior of chronically decerebrate cats. In: Wolstenholme GE, O'Conner CM, editors. *Neurological basis of behavior*. London: Churchhill; 1958. p. 55–75.
46. Arnulf I, Sastre JP, Buda C, Jouvet M. Hyperoxia increases paradoxical sleep rhythm in the pontine cat. *Brain Res* 1998;**807**(1–2):160–6.
47. Adey WR, Bors E, Porter RW. EEG sleep patterns after high cervical lesions in man. *Arch Neurol* 1968;**19**:377–83.
48. Lai YY, Siegel JM. Medullary regions mediating atonia. *J Neurosci* 1988;**8**:4790–6.
49. Siegel JM, Tomaszewski KS, Nienhuis R. Behavioral states in the chronic medullary and mid-pontine cat. *Electroencephalogr Clin Neurophysiol* 1986;**63**:274–88.
50. Siegel JM, Nienhuis R, Tomaszewski KS. REM sleep signs rostral to chronic transections at the pontomedullary junction. *Neurosci Lett* 1984;**45**:241–6.
51. Siegel JM. Pontomedullary interactions in the generation of REM sleep. In: McGinty DJ, Drucker-Colín R, Morrison A, Parmeggiani PL, editors. *Brain mechanisms of sleep*. New York: Raven Press; 1985. p. 157–74.
52. Matsuzaki M. Differential effects of sodium butyrate and physostigmine upon the activities of para-sleep in acute brain stem preparations. *Brain Res* 1969;**13**:247–65.
53. Schenkel E, Siegel JM. REM sleep without atonia after lesions of the medial medulla. *Neurosci Lett* 1989;**98**:159–65.
54. Gadea-Ciria M. Tele-encephalic versus cerebellar control upon pontogeniculo-occipital waves during paradoxical sleep in the cat. *Experientia* 1976;**32**(7):889–90.
55. Mahowald MW, Schenck CH, Bornemann MA. Pathophysiologic mechanisms in REM sleep behavior disorder. *Curr Neurol Neurosci Rep* 2007;**7**(2):167–72.
56. Carli G, Zanchetti A. A study of pontine lesions suppressing deep sleep in the cat. *Arch Ital Biol* 1965;**103**:725–50.

57. Jones BE, Harper ST, Halaris AE. Effects of locus coeruleus lesions upon cerebral monoamine content, sleep wakefulness states and the response to amphetamine in the cat. *Brain Res* 1977;**124**:473–96.
58. Juvancz P. The effect of raphe lesion on sleep in the rat. *Brain Res* 1980;**194**:371–6.
59. Siegel JM, McGinty DJ. Pontine reticular formation neurons: relationship of discharge to motor activity. *Science* 1977;**196**:678–80.
60. Siegel JM, McGinty DJ, Breedlove SM. Sleep and waking activity of pontine gigantocellular field neurons. *Exp Neurol* 1977;**56**:553–73.
61. Drucker-Colín R, Pedraza JG. Kainic acid lesions of gigantocellular tegmental field (FTG) neurons does [*sic*] not abolish REM sleep. *Brain Res* 1983;**272**:387–91.
62. Webster HH, Jones BE. Neurotoxic lesions of the dorsolateral pontomesencephalic tegmentum-cholinergic cell area in the cat II. Effects upon sleep-waking states. *Brain Res* 1988;**458**:285–302.
63. Swanson LW. *Brain maps: structure of the rat brain*. Amsterdam: Elsevier; 1992.
64. Lu J, Sherman D, Devor M, Saper CB. A putative flip-flop switch for control of REM sleep. *Nature* 2006;**441**(7093):589–94.
65. 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.
66. Hendricks JC, Morrison AR, Mann GL. Different behaviors during paradoxical sleep without atonia depend on pontine lesion site. *Brain Res* 1982;**239**:81–105.
67. Lai YY, Shalita T, Hajnik T, et al. Neurotoxic *N*-methyl-D-aspartate lesion of the ventral midbrain and mesopontine junction alters sleep-wake organization. *Neuroscience* 1999;**90**:469–83.
68. Lai YY, Hsieh KC, Nguyen D, et al. Neurotoxic lesions at the ventral mesopontine junction change in sleep time and muscle activity in sleep: an animal model of motor disorders in sleep. *Neuroscience* 2008;**154**(2): 431–43.
69. Holmes CJ, Mainville LS, Jones BE. Distribution of cholinergic, GABAergic and serotonergic neurons in the medial medullary reticular formation and their projections studied by cytotoxic lesions in the cat. *Neuroscience* 1994;**62**(4):1155–78.
70. Crochet S, Onoe H, Sakai K. A potent non-monoaminergic paradoxical sleep inhibitory system: a reverse microdialysis and single-unit recording study. *Eur J Neurosci* 2006;**24**(5):1404–12.
71. Vanini G, Torterolo P, McGregor R, et al. GABAergic processes in the mesencephalic tegmentum modulate the occurrence of active (rapid eye movement) sleep in guinea pigs. *Neuroscience* 2007;**145**(3): 1157–67.
72. Luppi PH, Gervasoni D, Verret L, et al. Paradoxical (REM) sleep genesis: the switch from an aminergic-cholinergic to a GABAergic-glutamatergic hypothesis. *J Physiol (Paris)* 2006;**100**(5–6):271–83.
73. Moruzzi G, Magoun HW. Brain stem reticular formation and activation of the EEG. *Electroencephalogr Clin Neurophysiol* 1949;**1**(4): 455–73.
74. Moruzzi G. The sleep-waking cycle. *Ergebn Physiol* 1972;**64**:1–165.
75. Xi MC, Morales FR, Chase MH. Interactions between GABAergic and cholinergic processes in the nucleus pontis oralis: neuronal mechanisms controlling active (rapid eye movement) sleep and wakefulness. *J Neurosci* 2004;**24**(47):10670–8.
76. Watson CJ, Soto-Calderon H, Lydic R, Baghdoyan HA. Pontine reticular formation (PnO) administration of hypocretin-1 increases PnO GABA levels and wakefulness. *Sleep* 2008;**31**(4):453–64.
77. Xi MC, Morales FR, Chase MH. Interactions between GABAergic and cholinergic processes in the nucleus pontis oralis: neuronal mechanisms controlling active (rapid eye movement) sleep and wakefulness. *J Neurosci* 2004;**24**(47):10670–8.
78. George R, Haslett WL, Jenden DJ. A cholinergic mechanism in the brainstem reticular formation: Induction of paradoxical sleep. *Int J Neuropharmacol* 1964;**3**:541–52.
79. Vanni-Mercier G, Sakai K, Lin JS, Jouvet M. Mapping of cholinoceptive brainstem structures responsible for the generation of paradoxical sleep in the cat. *Arch Ital Biol* 1989;**127**:133–64.
80. Katayama Y, DeWitt DS, Becker DP, Hayes RL. Behavioral evidence for cholinoceptive pontine inhibitory area: Descending control of spinal motor output and sensory input. *Brain Res* 1984;**296**:241–62.
81. Mitler MM, Dement WC. Cataplectic-like behavior in cats after microinjection of carbachol in the pontine reticular formation. *Brain Res* 1974;**68**:335–43.
82. Gnadt JW, Pegram V. Cholinergic brainstem mechanisms of REM sleep in the rat. *Brain Res* 1986;**384**:29–41.
83. Shiromani PJ, Fishbein W. Continuous pontine cholinergic microinfusion via mini-pump induces sustained alterations in rapid eye movement (REM) sleep. *Pharmacol Biochem Behav* 1986;**25**:1253–61.
84. Velazquez-Moctezuma J, Gillin JC, Shiromani PJ. Effect of specific M1, M2 muscarinic receptor agonists on REM sleep generation. *Brain Res* 1989;**503**:128–31.
85. Deurveilher S, Hars B, Hennevin E. Pontine microinjection of carbachol does not reliably enhance paradoxical sleep in rats. *Sleep* 1997;**20**(8): 593–607.
86. Lai YY, Siegel JM. Pontomedullary glutamate receptors mediating locomotion and muscle tone suppression. *J Neurosci* 1991;**11**:2931–7.
87. Verret L, Fort P, Gervasoni D, et al. Localization of the neurons active during paradoxical (REM) sleep and projecting to the locus coeruleus noradrenergic neurons in the rat. *J Comp Neurol* 2006;**495**(5):573–86.
88. Lai YY, Siegel JM. Muscle tone suppression and stepping produced by stimulation of midbrain and rostral pontine reticular formation. *J Neurosci* 1990;**10**:2727–38.
89. Lai YY, Clements J, Siegel J. Glutamatergic and cholinergic projections to the pontine inhibitory area identified with horseradish peroxidase retrograde transport and immunohistochemistry. *J Comp Neurol* 1993; **336**:321–30.
90. Lai YY, Clements JR, Wu XY, et al. Brainstem projections to the ventromedial medulla in cat: retrograde transport horseradish peroxidase and immunohistochemical studies. *J Comp Neurol* 1999;**408**:419–36.
91. Boissard R, Gervasoni D, Schmidt MH, et al. The rat ponto-medullary network responsible for paradoxical sleep onset and maintenance: a combined microinjection and functional neuroanatomical study. *Eur J Neurosci* 2002;**16**(10):1959–73.
92. Onoe H, Sakai K. Kainate receptors: a novel mechanism in paradoxical sleep generation. *Neuroreport* 1995;**6**:353–6.
93. Siegel JM. Behavioral functions of the reticular formation. *Brain Res* 1979;**1**:69–105.
94. Siegel JM, Wheeler RL, McGinty DJ. Activity of medullary reticular formation neurons in the unrestrained cat during waking and sleep. *Brain Res* 1979;**179**:49–60.
95. Suzuki SS, Siegel JM, Wu MF. Role of pontomedullary reticular formation neurons in horizontal head movements: an ibotenic acid lesion study in the cat. *Brain Res* 1989;**484**:78–93.
96. Kodama T, Takahashi T, Honda Y. Enhancement of acetylcholine release during paradoxical sleep in the dorsal tegmental field of the cat brain stem. *Neurosci Lett* 1990;**114**:277–82.
97. Steriade M, Datta S, Pare D, et al. Neuronal activities in brain-stem cholinergic nuclei related to tonic activation processes in thalamocortical systems. *J Neurosci* 1990;**10**:2541–59.
98. Boucetta S, Cisse Y, Mainville L, et al. Discharge profiles across the sleepwaking cycle of identified cholinergic, GABAergic, and glutamatergic neurons in the pontomesencephalic tegmentum of the rat. *J Neurosci* 2014;**34**(13):4708–27.
99. Greene RW, Gerber U, McCarley RW. Cholinergic activation of medial pontine reticular formation neurons in vitro. *Brain Res* 1989;**476**(1): 154–9.
100. Sakai K, Crochet S, Onoe H. Pontine structures and mechanisms involved in the generation of paradoxical (REM) sleep. *Arch Ital Biol* 2001;**139**(1–2):93–107.
101. Hobson JA, McCarley RW, Wyzinski PW. Sleep cycle oscillation: reciprocal discharge by two brainstem neuronal groups. *Science* 1975;**189**: 55–8.
102. Fenik V, Marchenko V, Janssen P, et al. A5 cells are silenced when REM sleep-like signs are elicited by pontine carbachol. *J Appl Physiol* 2002; **93**(4):1448–56.
103. McGinty DJ, Harper RM. Dorsal raphe neurons: depression of firing during sleep in cats. *Brain Res* 1976;**101**:569–75.
104. Steininger TL, Alam MN, Gong H, et al. Sleep-waking discharge of neurons in the posterior lateral hypothalamus of the albino rat. *Brain Res* 1999;**840**:138–47.
105. Nitz D, Siegel JM. GABA release in the posterior hypothalamus of the cat as a function of sleep/wake state. *Am J Physiol* 1996;**40**:R1707–12.
106. Nitz D, Siegel JM. GABA release in the dorsal raphe nucleus: role in the control of REM sleep. *Am J Physiol* 1997;**273**:R451–5.
107. Nitz D, Siegel JM. GABA release in the cat locus coeruleus as a function of the sleep/wake state. *Neuroscience* 1997;**78**:795–801.
108. Gervasoni D, Darracq L, Fort P, et al. Electrophysiological evidence that noradrenergic neurons of the rat locus coeruleus are tonically inhibited by GABA during sleep. *Eur J Neurosci* 1998;**10**:964–70.
109. Maloney K, Mainville L, Jones B. Differential c-Fos expression in cholinergic, monoaminergic, and GABAergic cell groups of the

pontomesencephalic tegmentum after paradoxical sleep deprivation and recovery. *J Neurosci* 1999;**19**(8):3057–72.
110. Torterolo P, Yamuy J, Sampogna S, et al. GABAergic neurons of the cat dorsal raphe nucleus express c-fos during carbachol-induced active sleep. *Brain Res* 2000;**884**(1–2):68–76.
111. Miller JD, Farber J, Gatz P, et al. Activity of mesencephalic dopamine and non-dopamine neurons across stages of sleep and waking in the rat. *Brain Res* 1983;**273**:133–41.
112. Steinfels GF, Heym J, Strecker RE, Jacobs BL. Behavioral correlates of dopaminergic unit activity in freely moving cats. *Brain Res* 1983;**258**: 217–28.
113. Dahan L, Astier B, Vautrelle N, et al. Prominent burst firing of dopaminergic neurons in the ventral tegmental area during paradoxical sleep. *Neuropsychopharmacology* 2007;**32**(6):1232–41.
114. Lena I, Parrot S, Deschaux O, et al. Variations in extracellular levels of dopamine, noradrenaline, glutamate, and aspartate across the sleep-wake cycle in the medial prefrontal cortex and nucleus accumbens of freely moving rats. *J Neurosci Res* 2005;**81**(6):891–9.
115. Taepavarapruk N, Taepavarapruk P, John J, et al. State-dependent changes in glutamate, glycine, GABA, and dopamine levels in cat lumbar spinal cord. *J Neurophysiol* 2008;**100**:598–608.
116. Lu J, Jhou TC, Saper CB. Identification of wake-active dopaminergic neurons in the ventral periaqueductal gray matter. *J Neurosci* 2006;**26**(1): 193–202.
117. Datta S, Siwek DF. Single cell activity patterns of pedunculopontine tegmentum neurons across the sleep-wake cycle in the freely moving rats. *J Neurosci Res* 2002;**70**(4):611–21.
118. Steriade M, Pare D, Datta S, et al. Different cellular types in mesopontine cholinergic nuclei related to ponto-geniculo-occipital waves. *J Neurosci* 1990;**10**:2560–79.
119. Ruch-Monachon MA, Jaffre M, Haefely W. Drugs and PGO waves in the lateral geniculate body of the curarized cat. IV. The effects of acetylcholine, GABA and benzodiazepines on PGO wave activity. *Arch Int Pharmacodyn Ther* 1976;**219**:308–25.
120. Wu MF, Siegel JM. Facilitation of the acoustic startle reflex by ponto-geniculo-occipital waves: effects of PCPA. *Brain Res* 1990;**532**: 237–41.
121. Brooks DC, Gershon MD. An analysis of the effect of reserpine upon ponto-geniculo-occipital wave activity in the cat. *Neuropharmacology* 1972;**11**:499–510.
122. Quattrochi JJ, Bazalakova M, Hobson JA. From synapse to gene product: prolonged expression of c-fos induced by a single microinjection of carbachol in the pontomesencephalic tegmentum. *Brain Res Mol Brain Res* 2005;**136**(1–2):164–76.
123. Datta S, Li G, Auerbach S. Activation of phasic pontine-wave generator in the rat: a mechanism for expression of plasticity-related genes and proteins in the dorsal hippocampus and amygdala. *Eur J Neurosci* 2008;**27**(7):1876–92.
124. Verret L, Leger L, Fort P, Luppi PH. Cholinergic and noncholinergic brainstem neurons expressing Fos after paradoxical (REM) sleep deprivation and recovery. *Eur J Neurosci* 2005;**21**(9):2488–504.
125. Goutagny R, Luppi PH, Salvert D, et al. Role of the dorsal paragigantocellular reticular nucleus in paradoxical (rapid eye movement) sleep generation: a combined electrophysiological and anatomical study in the rat. *Neuroscience* 2008;**152**(3):849–57.
126. Pompeiano O, Hoshino K. Tonic inhibition of dorsal pontine neurons during the postural atonia produced by an anticholinesterase in the decerebrate cat. *Arch Ital Biol* 1976;**114**:310–40.
127. Gvilia I, Xu F, McGinty D, Szymusiak R. Homeostatic regulation of sleep: a role for preoptic area neurons. *J Neurosci* 2006;**26**(37): 9426–33.
128. Hanriot L, Camargo N, Courau AC, et al. Characterization of the melanin-concentrating hormone neurons activated during paradoxical sleep hypersomnia in rats. *J Comp Neurol* 2007;**505**(2):147–57.
129. Verret L, Goutagny R, Fort P, et al. A role of melanin-concentrating hormone producing neurons in the central regulation of paradoxical sleep. *BMC Neurosci* 2003;**4**:19.
130. Chase MH, Morales FR. Control of motoneurons during sleep. In: Kryger MH, Roth T, Dement WC, editors. *Principles of sleep medicine*. 4th ed. Philadelphia: Saunders; 2005. p. 154–68.
131. Hsieh KC, Nguyen D, Siegel JM, Lai YY. New pathways and data on rapid eye movement sleep behaviour disorder in a rat model. *Sleep Med* 2013;**14**(8):719–28.
132. Siegel JM, Nienhuis R, Fahringer H, et al. Neuronal activity in narcolepsy: identification of cataplexy related cells in the medial medulla. *Science* 1991;**252**:1315–18.
133. Morales FR, Sampogna S, Rampon C, et al. Brainstem glycinergic neurons and their activation during active (rapid eye movement) sleep in the cat. *Neuroscience* 2006;**142**(1):37–47.
134. Lai YY, Kodama T, Siegel JM. Changes in monoamine release in the ventral horn and hypoglossal nucleus linked to pontine inhibition of muscle tone: an in vivo microdialysis study. *J Neurosci* 2001;**21**: 7384–91.
135. Mileykovskiy BY, Kiyashchenko LI, Siegel JM. Cessation of activity in red nucleus neurons during stimulation of the medial medulla in decerebrate rats. *J Physiol* 2002;**545**(Pt 3):997–1006.
136. Kodama T, Lai YY, Siegel JM. Enhancement of acetylcholine release during REM sleep in the caudomedial medulla as measured by in vivo microdialysis. *Brain Res* 1992;**580**:348–50.
137. Kohyama J, Lai YY, Siegel JM. Inactivation of the pons blocks medullary-induced muscle tone suppression in the decerebrate cat. *Sleep* 1998;**21**:695–9.
138. Siegel JM, Nienhuis R, Tomaszewski KS. Rostral brainstem contributes to medullary inhibition of muscle tone. *Brain Res* 1983;**268**: 344–8.
139. Peever JH, Lai YY, Siegel JM. Excitatory effects of hypocretin-1 (orexin-A) in the trigeminal motor nucleus are reversed by NMDA antagonism. *J Neurophysiol* 2003;**89**(5):2591–600.
140. Brooks PL, Peever JH. Glycinergic and $GABA_A$-mediated inhibition of somatic motoneurons does not mediate rapid eye movement sleep motor atonia. *J Neurosci* 2008;**28**(14):3535–45.
141. Morrison JL, Sood S, Liu H, et al. $GABA_A$ receptor antagonism at the hypoglossal motor nucleus increases genioglossus muscle activity in NREM but not REM sleep. *J Physiol* 2003;**548**(Pt 2):569–83.
142. Burgess C, Lai D, Siegel J, Peever J. An endogenous glutamatergic drive onto somatic motoneurons contributes to the stereotypical pattern of muscle tone across the sleep-wake cycle. *J Neurosci* 2008;**28**(18): 4649–60.
143. Schwarz PB, Mir S, Peever JH. Noradrenergic modulation of masseter muscle activity during natural rapid eye movement sleep requires glutamatergic signalling at the trigeminal motor nucleus. *J Physiol* 2014;**592**(16):3597–609.
144. Chan E, Steenland HW, Liu H, Horner RL. Endogenous excitatory drive modulating respiratory muscle activity across sleep-wake states. *Am J Respir Crit Care Med* 2006;**174**(11):1264–73.
145. John J, Wu MF, 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**:619–34.
146. Fenik VB, Davies RO, Kubin L. Noradrenergic, serotonergic and GABAergic antagonists injected together into the XII nucleus abolish the REM sleep-like depression of hypoglossal motoneuronal activity. *J Sleep Res* 2005;**14**(4):419–29.
147. Jelev A, Sood S, Liu H, et al. Microdialysis perfusion of 5-HT into hypoglossal motor nucleus differentially modulates genioglossus activity across natural sleep-wake states in rats. *J Physiol* 2001;**532**(Pt 2): 467–81.
148. Sood S, Raddatz E, Liu X, et al. Inhibition of serotonergic medullary raphe obscurus neurons suppresses genioglossus and diaphragm activities in anesthetized but not conscious rats. *J Appl Physiol* 2006;**100**(6): 1807–21.
149. Taepavarapruk N, Taepavarapruk P, John J, et al. State-dependent release of glycine in Clarke's column of the upper lumbar spinal cord. *Sleep* 2003;**26**:A11–12.
150. Shouse MN, Staba RJ, Saquib SF, Farber PR. Monoamines and sleep: microdialysis findings in pons and amygdala. *Brain Res* 2000;**860**(1–2): 181–9.
151. Maloney KJ, Mainville L, Jones BE. c-Fos expression in dopaminergic and GABAergic neurons of the ventral mesencephalic tegmentum after paradoxical sleep deprivation and recovery. *Eur J Neurosci* 2002;**15**(4): 774–8.
152. Dauvilliers Y, Siegel JM, Lopez R, et al. Cataplexy: clinical aspects, pathophysiology and management strategy. *Nat Rev Neurol* 2014;**10**(7): 386–95.
153. Wu MF, Gulyani S, Yao E, et al. Locus coeruleus neurons: cessation of activity during cataplexy. *Neuroscience* 1999;**91**:1389–99.
154. John J, Wu MF, Maidment N, et al. Increased activity reduces cataplexy in canine narcolepsy: possible role of elevated hypocretin level. *Sleep* 2004;**27**:049.
155. Fronczek R, Overeem S, Lee SY, et al. Hypocretin (orexin) loss in Parkinson's disease. *Brain* 2007;**130**(Pt 6):1577–85.
156. Thannickal TC, Lai YY, Siegel JM. Hypocretin (orexin) cell loss in Parkinson's disease. *Brain* 2007;**130**(6):1586–95.

157. John J, Wu MF, Siegel JM. Hypocretin-1 reduces cataplexy and normalizes sleep and waking durations in narcoleptic dogs. *Sleep* 2000;**23**(A11): A12.
158. Deadwyler SA, Porrino L, Siegel JM, Hampson RE. Systemic and nasal delivery of orexin-A (hypocretin-1) reduces the effects of sleep deprivation on cognitive performance in nonhuman primates. *J Neurosci* 2007;**27**(52):14239–47.
159. Baier PC, Hallschmid M, Seeck-Hirschner M, et al. Effects of intranasal hypocretin-1 (orexin A) on sleep in narcolepsy with cataplexy. *Sleep Med* 2011;**12**(10):941–6.
160. Baier PC, Weinhold SL, Huth V, et al. Olfactory dysfunction in patients with narcolepsy with cataplexy is restored by intranasal orexin A (hypocretin-1). *Brain* 2008;**131**:2734–41.
161. Weinhold SL, Seeck-Hirschner M, Nowak A, et al. The effect of intranasal orexin-A (hypocretin-1) on sleep, wakefulness and attention in narcolepsy with cataplexy. *Behav Brain Res* 2014;**262**:8–13.
162. John J, Thannickal TC, McGregor R, et al. Greatly increased numbers of histamine cells in human narcolepsy with cataplexy. *Ann Neurol* 2013;**74**(6):786–93.
163. Valko PO, Gavrilov YV, Yamamoto M, et al. Increase of histaminergic tuberomammillary neurons in narcolepsy. *Ann Neurol* 2013;**74**(6): 794–804.
164. Lee MG, Hassani OK, Jones BE. Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. *J Neurosci* 2005;**25**(28): 6716–20.
165. Kiyashchenko LI, Mileykovskiy BY, Maidment N, et al. Release of hypocretin (orexin) during waking and sleep states. *J Neurosci* 2002;**22**: 5282–6.
166. Siegel JM. Hypocretin (orexin): role in normal behavior and neuropathology. *Annu Rev Psychol* 2004;**55**:125–48.
167. McGregor R, Wu MF, Barber G, et al. Highly specific role of hypocretin (orexin) neurons: differential activation as a function of diurnal phase, operant reinforcement vs. operant avoidance and light level. *J Neurosci* 2011;**31**(43):15455–67.
168. Gulyani S, Wu MF, Nienhuis R, et al. Cataplexy-related neurons in the amygdala of the narcoleptic dog. *Neuroscience* 2002;**112**(2):355–65.
169. Siegel JM, Boehmer LN. Narcolepsy and the hypocretin system-where motion meets emotion. *Nat Clin Pract Neurol* 2006;**2**(10):548–56.
170. Konadhode RR, Pelluru D, Blanco-Centurion C, et al. Optogenetic stimulation of MCH neurons increases sleep. *J Neurosci* 2013;**33**(25): 10257–63.
171. Blouin AM, Fried I, Wilson CL, et al. Human hypocretin and melanin-concentrating hormone levels are linked to emotion and social interaction. *Nat Commun* 2013;**4**:1547.
172. van den Pol AN, Gao XB, Obrietan K, et al. Presynaptic and postsynaptic actions and modulation of neuroendocrine neurons by a new hypothalamic peptide, hypocretin/orexin. *J Neurosci* 1998;**18**(19): 7962–71.
173. John J, Wu MF, Kodama T, Siegel JM. Intravenously administered hypocretin-1 alters brain amino acid release: an in vivo microdialysis study in rats. *J Physiol* 2003;**548**(2):557–62.
174. Liu RJ, van den Pol AN, Aghajanian GK. Hypocretins (orexins) regulate serotonin neurons in the dorsal raphe nucleus by excitatory direct and inhibitory indirect actions. *J Neurosci* 2002;**22**(21):9453–64.
175. Siegel JM. Narcolepsy: a key role for hypocretins (orexins). *Cell* 1999;**98**:409–12.
176. Guilleminault C. Anognos A. Narcolepsy. In: Kryger MH, Roth T, Dement WC, editors. *Principles and practice of sleep medicine*. 3rd ed. Philadelphia: WB Saunders; 2000. p. 676–86.
177. Siegel JM. Narcolepsy. *Sci Am* 2000;**282**:76–81.
178. Zhang S, Zeitzer JM, Sakurai T, et al. Sleep/wake fragmentation disrupts metabolism in a mouse model of narcolepsy. *J Physiol* 2007; **581**(2):649–63.
179. Siegel JM. Why we sleep. *Sci Am* 2003;**289**(5):92–7.
180. Siegel JM. The REM sleep-memory consolidation hypothesis. *Science* 2001;**294**(5544):1058–63.
181. Mueller AD, Parfyonov M, Pavlovski I, et al. The inhibitory effect of sleep deprivation on cell proliferation in the hippocampus of adult mice is eliminated by corticosterone clamp combined with interleukin-1 receptor 1 knockout. *Brain Behav Immun* 2014;**35**:182–8.
182. Smith C. Sleep states and memory processes in humans: procedural versus declarative memory systems. *Sleep Med Rev* 2001;**5**(6):491–506.
183. Lavie P, Pratt H, Scharf B, et al. Localized pontine lesion: nearly total absence of REM sleep. *Neurology* 1984;**34**:118–20.
184. Vetrugno R, Alessandria M, D'Angelo R, et al. Status dissociatus evolving from REM sleep behaviour disorder in multiple system atrophy. *Sleep Med* 2009;**10**(2):247–52.
185. Rasch B, Pommer J, Diekelmann S, Born J. Pharmacological REM sleep suppression paradoxically improves rather than impairs skill memory. *Nat Neurosci* 2009;**12**(4):396–7.
186. Arnulf I, Sastre JP, Buda C, Jouvet M. Hyperoxia increases paradoxical sleep rhythm in the pontine cat. *Brain Res* 1998;**807**(1–2):160–6.
187. Ephron HS, Carrington P. Rapid eye movement sleep and cortical homeostasis. *Psychol Rev* 1966;**73**(6):500–26.
188. Wehr TA. A brain-warming function for REM sleep. *Neurosci Biobehav Rev* 1992;**16**:379–97.
189. Horner RL, Sanford LD, Pack AI, Morrison AR. Activation of a distinct arousal state immediately after spontaneous awakening from sleep. *Brain Res* 1997;**778**(1):127–34.
190. Mallick BN, Siegel JM, Fahringer H. Changes in pontine unit activity with REM sleep deprivation. *Brain Res* 1989;**515**:94–8.
191. Tsai L, Bergman B, Perry B, Rechtschaffen A. Effects of chronic total sleep deprivation on central noradrenergic receptors in rat brain. *Brain Res* 1993;**602**:221–7.
192. Hipolide DC, Tufik S, Raymond R, Nobrega JN. Heterogeneous effects of rapid eye movement sleep deprivation on binding to alpha- and beta-adrenergic receptor subtypes in rat brain. *Neuroscience* 1998;**86**(3): 977–87.
193. Hipolide DC, Wilson AA, Barlow K, et al. Effects of paradoxical sleep deprivation on serotonin transporter (SERT) binding. *Sleep* 2003;**26**: A176.
194. Troncone LR, Braz S, Benedito MAC, Tufik S. REM sleep deprivation induces a decrease in norepinephrine-stimulated ^{3}H-cyclic AMP accumulation in slices from rat brain. *Pharmacol Biochem Behav* 1986;**25**: 223–5.
195. Siegel JM, Rogawski MA. A function for REM sleep: regulation of noradrenergic receptor sensitivity. *Brain Res* 1988;**13**:213–33.

REVIEW QUESTIONS

1. Human sleep is unusual in comparison with that of other mammals because of:
   A. The high amount of REM sleep
   B. The high amount of REM sleep as a percentage of total sleep time
   C. The low amount of REM sleep
   D. None of the above
2. A good way to prove that a substance is involved in REM sleep generation is to:
   A. Inject it in the pons or other candidate area and note if REM sleep increases
   B. Measure its release in the pons or other region and see if it is selectively released in REM sleep
   C. Block it with an antagonist and see if REM sleep is blocked
   D. Apply the manipulations in A to C to control areas adjacent to the areas of interest
   E. All of the above
3. Transection and lesion studies have localized the critical REM sleep generation mechanisms to:
   A. The pons and adjacent midbrain
   B. The medulla
   C. The hypothalamus
   E. The cerebral cortex
4. *Choose all that apply:* Which of the following statements regarding GABA is/are correct?
   A. GABA is the sleep chemical, promoting sleep throughout the brain.
   B. Profound arousal results when it is injected into certain pontine areas.
   C. GABA has actions outside of the brain and nervous system.
   D. GABA is unrelated to sleep onset or maintenance.
5. Narcolepsy is due to:
   A. Generalized degeneration of the hypothalamus.
   B. Localized cell-specific cell losses within the hypothalamus
   C. Amygdala dysfunction
   D. Frontal cortex damage
   E. All of the above
6. In the egg-laying mammals echidna and platypus, REM sleep is:
   A. Nonexistent
   B. Largely restricted to brainstem structures
   C. Largely restricted to forebrain structures
   D. Much greater in hours/day than in other mammals
   E. B and D
7. *True or false:* Studies in animals have shown that the isolated forebrain can generate a REM sleep-like state.
8. *True or false:* Narcolepsy is due to generalized degeneration of the hypothalamus.
9. *True or false:* The cessation of activity in monoaminergic neurons, including the histamine and noradrenergic neurons of the tuberomammillary nucleus and the locus coeruleus, is linked to GABA release.

ANSWERS

1. **D.** Amounts of total sleep time, total non-REM sleep time, REM sleep, and REM sleep time as a percentage of total sleep time are not unusual in humans.
2. **E.** Because REM sleep generation mechanisms are located in the pons, excitatory substances may trigger or increase REM sleep even if they have no substantial normal role in the control of this state. It is important to show the normal release of the substance in question and that blocking the effects of normal release blocks REM sleep. The same strategy applies to NREM sleep–related substances injected in forebrain regions.
3. **A.** However, the "REM sleep" aspects that appear in the isolated pons or pons connected to medulla but disconnected from rostral structures are not normal in physiologic form. This finding and other data point to a two-way interaction between forebrain, medullary, and pontine structures in normal REM sleep.
4. **B** and **C.** GABA is released in some regions selectively during sleep, but in others selectively during waking or just in REM sleep or just in NREM sleep. Any pharmacologic manipulation that activates GABA receptors throughout the brain (as benzodiazepines do) would be expected to produce an abnormal mixture of aspects of sleep-waking states. GABA also acts on receptors in the heart, kidney, T cells, and many other nonneural tissues.
5. **B.** Current evidence suggests that damage is limited to hypocretin (orexin) neurons, with adjacent melanin concentrating neurons intact. At this point it appears that only hypocretin neurons are lost in human narcolepsy, making the damage the most restricted of any neurologic disease. This conclusion is subject to future modification as other systems are examined more closely! Of course, even localized cell loss will produce changes throughout the brain, including up- and downregulation of receptors, sprouting of new connections, and so on.
6. **E.**
7. **False.** The caudal midbrain and pons are required for REM sleep. If the midbrain and pons are attached to the forebrain, a REM sleep—like state with PGO waves and EEG aspects of REM sleep is seen, even though structures caudal to the pons are disconnected from the forebrain.
8. **False.** Current evidence suggest that damage is limited to hypocretin (orexin) neurons, with adjacent melanin-concentrating neurons intact. At this point it appears that only hypocretin neurons are lost in human narcolepsy, making the damage the most restricted of any neurologic disease. This conclusion is subject to future modification as other systems are examined more closely!
9. **True.** Microdialysis studies have shown increased GABA release onto these neuronal groups during REM sleep.