Chronic Sleep Deprivation

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Chapter 6

Abstract

Chronic sleep deprivation, also referred to as chronic sleep restriction, is common, with a wide range of causes including shift work and other occupational and economic demands, medical conditions and sleep disorders, and social and domestic responsibilities. Sleep dose–response experiments have found that chronic sleep restriction to less than 7 hours per night resulted in cognitive deficits that (1) accumulate (i.e., become progressively worse over time as sleep restriction persists), (2) are sleep-dose sensitive (i.e., the less sleep that is obtained, the faster the rate at which deficits develop), and (3) do not result in profound subjective sleepiness or full self-awareness of the cumulative deficits from sleep restriction. The mechanisms underlying the sleep dose–response cumulative neurobehavioral and physiologic alterations during chronic sleep restriction remain unknown. Individual variability in neurobehavioral responses to sleep restriction appear to be as large as those in response to total sleep deprivation and as stable over time, suggesting a traitlike (possibly genetic) differential vulnerability to the effects of chronic sleep restriction or differences in the nature of compensatory brain responses to the growing sleep loss.

Chronic sleep restriction occurs frequently and results from a number of factors, including medical conditions (e.g., pain), sleep disorders, work demands (including extended work hours and shift work), and social and domestic responsibilities. Adverse effects on neurobehavioral functioning accumulate as the magnitude of sleep loss escalates, and the result is an increased risk of on-the-job errors, injuries, traffic accidents, personal conflicts, health complaints, and drug use.

Chronic sleep restriction, or partial sleep deprivation, has been thought to occur when one fails to obtain a usual amount of sleep.[1,2] Half a century ago, Kleitman first used the phrase *sleep debt* to describe the circumstances of delaying sleep onset time while holding sleep termination time constant.[3] He described the increased sleepiness and decreased alertness in individuals on such a sleep–wake pattern, and proposed that those subjects who were able to reverse these effects by extending their sleep on weekends were able to "liquidate the debt."[3, p. 317]

The term *sleep debt* is usually synonymous with chronic sleep restriction because it refers to the increased pressure for sleep that results from an inadequate amount of physiologically normal sleep.[4] To determine the effects of chronic sleep loss on a range of neurobehavioral and physiologic variables, a variety of paradigms have been used, including controlled, restricted time in bed for sleep opportunities in both continuous and distributed schedules,[5] gradual reductions in sleep duration over time,[6] selective deprivation of specific sleep stages,[7] and limiting the time in bed to a percentage of the individual's habitual time in bed.[8] These studies have ranged from 24 hours[9] to 8 months[6] in length.

Many reports published before 1997 concluded that chronic sleep restriction in the range commonly experienced by the general population (i.e., sleep durations of less than 7 hours per night but greater than 4 hours per night) resulted in some increased subjective sleepiness but had little or no effect on cognitive performance capabilities. Consequently, there was a widely held belief that individuals could "adapt" to chronic reductions in sleep duration, down to 4 to 5 hours per day. However, nearly all of these reports of adaptation to sleep loss were limited by problems in experimental design.[8] Since 1997, experiments that have corrected for these methodological weaknesses have found markedly different results from those earlier studies, and have documented cumulative objective changes in neurobehavioral outcomes as sleep restriction progressed.[10] This chapter reviews the cognitive and neurobehavioral consequences of chronic sleep restriction in healthy individuals.

INCIDENCE OF CHRONIC SLEEP RESTRICTION

Human sleep need, or, more precisely, the duration of sleep needed to prevent daytime sleepiness, elevated sleep propensity, and cognitive deficits, has been a long-standing controversy central to whether chronic sleep restriction may compromise health and behavioral functions. Self-reported sleep durations are frequently less than 8 hours per night. For example, approximately 20% of more than 1.1 million Americans indicated that they slept 6.5 hours or less each night.[11] Similarly, in polls of 1000 American adults by the National Sleep Foundation, 15% of subjects (aged 18 to 84 years) reported sleeping less than 6 hours on weekdays, and 10% reported sleeping less than 6 hours on weekends over the past year.[12] Scientific perspectives on the duration of sleep that defines chronic sleep restriction have come from a number of theories.

THEORETICAL PERSPECTIVES ON SLEEP NEED AND SLEEP DEBT

Basal Sleep Need

The amount of sleep habitually obtained by an individual is determined by a variety of factors. Epidemiologic and experimental studies point to a high between-subjects variance in sleep duration, influenced by environmental, genetic, and societal factors. Although not clearly defined in the literature, the concept of basal sleep need has been described as habitual sleep duration in the absence of preexisting sleep debt.[13] Sleep restriction has been defined as the fundamental duration of sleep below which waking deficits begin to accumulate.[14] Given these definitions, the

basal need for sleep appears to be between 7.5 and 8.5 hours per day in healthy adult humans. This number was based on a study in which prior sleep debt was completely eliminated through repeated nights of long-duration sleep that stabilized at a mean of 8.17 hours.[15] A similar value was obtained from a large-scale dose–response experiment on chronic sleep restriction that statistically estimated daily sleep need to average 8.16 hours per night to avoid detrimental effects on waking functions.[4]

Core Sleep versus Optional Sleep

In the 1980s, it was proposed that a normal nocturnal sleep period was composed of two types of sleep relative to functional adaptation: core and optional sleep.[16,17] The initial duration of sleep in the sleep period was referred to as core, or "obligatory," sleep, which was posited to "repair the effects of waking wear and tear on the cerebrum."[16, p. 57] Initially, the duration of required core sleep was defined as 4 to 5 hours of sleep per night, depending on the duration of the sleep restriction.[16] The duration of core sleep has subsequently been redefined as 6 hours of (good-quality, uninterrupted) sleep for most adults.[14] Additional sleep obtained beyond the period of core sleep was considered to be optional, or luxury, sleep, which "fills the tedious hours of darkness until sunrise."[16, p. 57] This core versus optional theory of sleep need is often presented as analogous to the concept of appetite: Hunger drives one to eat until satiated, but additional food can still be consumed beyond what the body requires. It is unknown whether the so-called optional sleep serves any function.

According to the core sleep theory, only the core portion of sleep—which is dominated by slow-wave sleep (SWS) and slow-wave activity (SWA) on an electroencephalogram (EEG)—is required to maintain adequate levels of daytime alertness and cognitive functioning.[16] The optional sleep does not contribute to this recovery or maintenance of neurobehavioral capability. This theory was strengthened by results from a mathematical model of sleep and waking functions (the three-process model) that predicted that waking neurobehavioral functions were primarily restored during SWS,[18] which makes up only a portion of total sleep time. However, if only the core portion of sleep is required, it would be reasonable to predict that there would be no waking neurobehavioral consequences of chronically restricting sleep to 6 hours per night, and that cognitive deficits would be evident only when sleep durations were reduced below this amount. Experimental data have not supported this prediction.[10] For example, findings from the largest sleep dose–response study to date, which examined the effects of sleep chronically restricted to 4, 6, or 8 hours of time in bed per night,[4] found that cognitive performance measures were stable across 14 days of sleep restriction to 8 hours time in bed, but when sleep was reduced to either 6 or 4 hours per night, significant cumulative (dose-dependent) decreases in cognitive performance functions and increases in sleepiness were observed.[4]

It appears, therefore, that the "core" sleep needed to maintain stable waking neurobehavioral functions in healthy adults aged 22 to 45 years is in the range of 7 to 8 hours on average.[18] Moreover, because extended sleep is thought to dissipate sleep debt caused by chronic sleep restriction,[3] it is not clear that there is such a thing as "optional" sleep. There is, instead, recovery sleep, which may or may not be optional, although there have very few studies of the sleep needed to recover from varying degrees of chronic sleep restriction.

Adaptation to Sleep Restriction

One popular belief is that subjects may be acutely affected after initial restriction of sleep length and may then be able to adapt to the reduced sleep amount, with waking neurocognitive functions unaffected further or returned to baseline levels. Although several studies have suggested that this is the case when sleep duration is restricted to approximately 4 to 6 hours per night for up to 8 months,[6,9] there is also evidence indicating that the adaptation is largely confined to subjective reports of sleepiness but not objective cognitive performance parameters.[4] This suggests that the presumed adaptation effect is actually a misperception on the part of chronically sleep-restricted people regarding how sleep restriction has affected their cognitive capability.

One factor thought to be important in adaptation to chronic sleep restriction is the abruptness of the sleep curtailment. One study examined the relationship between rate of accumulation of sleep loss, to a total of 8 hours, and neurobehavioral performance levels.[19] After 1 night of total sleep deprivation (i.e., a rapid accumulation of 8 hours of sleep loss), neurobehavioral capabilities were significantly reduced. When the accumulation of sleep loss was slower, achieved by chronically restricting sleep to 4 hours per night for 2 nights or 6 hours per night for 4 nights, neurobehavioral performance deficits were evident, but they were of a smaller magnitude than those following the night of total sleep loss. A greater degree of neurobehavioral impairment was evident in those subjects restricted to 4 hours for 2 nights than in those subjects allowed 6 hours per night, leading to the conclusion that during the slowest accumulation of sleep debt (i.e., 6 hours per night for 4 nights), there was evidence of a compensatory adaptive mechanism.[19]

It is possible but not scientifically resolved that different objective neurobehavioral measures may show different degrees of sensitivity and adaptation to chronic sleep restriction. For example, in the largest controlled study to date with statistical modeling of adaptation curves, cognitive performance measures showed little adaptation across 14 days of sleep restriction to 4 or 6 hours per night, compared with 8 hours per night,[4] whereas waking EEG measures of alpha and theta frequencies showed no systematic sleep dose–dependent changes over days.[14] Consequently, different neurobehavioral outcomes showed markedly different responses to chronic sleep restriction, with neurocognitive functions showing the least adaptation, subjective sleepiness measures showing more adaptation, and waking EEG measures as well as non–rapid eye movement (non-REM), SWS measures showing little or no response.[4,14] The reliability of the latter findings may depend on the dose of restricted sleep and other factors.

Two-Process Model Predictions of Sleep Restriction

Biomathematical models of sleep–wake regulation have been used to make predictions about recovery in response

to various sleep durations. The basis of almost all current biomathematical models of sleep–wake regulation is the two-process model of sleep regulation.[20] This model proposes that two primary components regulate sleep: (1) a homeostatic process that builds up exponentially during wakefulness and declines exponentially during sleep (as measured by slow-wave energy or delta power in the non-REM sleep EEG), and (2) a circadian process, with near–24-hour periodicity.

Since its inception, the two-process model has gained widespread acceptance for its explanation of the timing and structure of sleep. Its use has extended to predictions of waking alertness and neurobehavioral functions in response to different sleep–wake scenarios.[21] This extension of the two-process model was based on observations that as sleep pressure accumulated with increasing time awake, so did waking neurobehavioral or neurocognitive impairment, and as sleep pressure dissipated with time asleep, performance capability improved during the following period of wakefulness. In addition, forced-desynchrony experiments revealed that the sleep homeostatic and circadian processes interacted to create periods of stable wakefulness and consolidated sleep during normal 24-hour days.[22] Hence, it was postulated that waking cognitive function (alertness variable A) could be mathematically modeled as the difference between the quantitative state for the homeostatic process (S) and the quantitative state for the circadian process (C), and thus A = S – C. Accordingly, predictions for changes in the neurobehavioral recovery afforded by chronically restricted sleep of varying durations could be made on the basis of sleep–wake times and circadian phase estimates, using the quantitative version of the two-process model.

The validity of the various biomathematical models based on the two-process model, and their ability to predict actual experimental results of the neurobehavioral effects of chronic sleep restriction have been evaluated in a blind test.[23] Because all current models are based on the same underlying principles as the two-process model, all yielded comparable predictions for neurobehavioral functioning in scenarios involving total sleep deprivation or chronic partial sleep restriction. All models accurately predicted waking neurobehavioral responses to total sleep deprivation. However, they all failed to adequately predict sleepiness and cognitive performance responses during chronic sleep restriction.[4,23] Hence, it appears that the extension of the two-process model to prediction of waking alertness[21] does not account for the results of chronic sleep restriction. Because the two-process model has had a profound theoretical influence on predictions of sleepiness based on total sleep deprivation data, its failure to capture the dynamic changes in neurobehavioral measures during chronic sleep restriction suggests that additional biological factors are relevant to the brain's response to chronic sleep restriction.

EFFECTS OF CHRONIC SLEEP RESTRICTION

The effects of sleep loss may be quantified in a number of different ways, using a wide range of physiologic, neurocognitive, behavioral, and subjective tools. Many early studies examining the effects of chronic sleep restriction on cognitive performance were conducted outside a controlled laboratory setting, with little or no control over potentially contaminating factors, such as the level of napping, extension of sleep periods, diet, stimulant use (e.g., caffeine, nicotine), activity, or exposure to zeitgebers (environmental time cues). The majority of these studies concluded that there were few or no detrimental effects on waking neurobehavioral capabilities, or subjective effects of the sleep restriction. For example, restriction of nocturnal sleep periods to between approximately 4 and 6 hours per night for up to 8 months produced no significant effects on a range of cognitive outcomes, including vigilance performance,[9] psychomotor performance,[6] logical reasoning, addition, or working memory.[9] In addition, few effects on subjective assessments of sleepiness or mood were reported.[9]

Later studies, however, with far greater experimental control and appropriate control groups, have demonstrated significant cumulative sleep dose–response effects on a wide range of physiologic and neurobehavioral functions, which we summarize here.

Sleep Architecture

Sleep restriction alters sleep architecture, but it does not affect all sleep stages equally. Depending on the timing and duration of sleep, and the number of days it is reduced, some aspects of sleep are conserved, occur sooner, or intensify, and other aspects of sleep time are diminished. For example, studies examining sleep architecture during chronic periods of sleep restriction have demonstrated a consistent conservation of SWS at the expense of other non-REM and REM sleep stages.[4,24,25] In addition, elevations in SWA, derived from spectral analysis of the sleep EEG in the range of 0.5 to 4.5 Hz, during non-REM sleep have also been reported during and after chronic sleep restriction.[4,25]

Because of the conservation of the amount of SWS and SWA during restricted sleep protocols, independent of sleep duration (e.g., 8 hours of time in bed or 4 hours of time in bed), it has been proposed that, with regard to behavioral and physiologic outcomes, these phenomena provide the recovery aspects of sleep. It remains to be determined whether the lack of SWS and SWA response to chronic restriction of sleep to 4 hours a night, relative to steady increases in physiologic and neurobehavioral measures of sleepiness,[4] can account for the latter deficits. Consequently, although SWS and non-REM SWA may be conserved in chronic sleep restriction (to 4 to 7 hours per night), they do not appear to reflect the severity of daytime cognitive deficits or to protect against these deficits, raising serious doubts about SWS and non-REM SWA being the only aspects of sleep critical to waking functions in chronic sleep restriction.[4]

Sleep Propensity

With the development and validation of sleep latency measures as sensitive indices of sleep propensity,[26] the effects of chronic sleep restriction could be evaluated physiologically. Objective EEG measures of sleep propensity, such as the multiple sleep latency test[26] (MSLT) and the

maintenance of wakefulness test[27] (MWT), are frequently used to evaluate sleepiness (see Chapters 4 and 143).

The daytime MSLT[26] has been shown to vary linearly after 1 night of sleep restricted to between 1 and 5 hours of time in bed.[26] Progressive decreases in daytime sleep latency have been documented (i.e., increases in sleep propensity) across 7 days of sleep restricted to 5 hours per night in healthy young adults,[28] a finding confirmed in a later study using the psychomotor vigilance test (PVT).[8]

Dose–response effects of chronic sleep restriction on daytime MSLT values have been reported in a controlled laboratory study in commercial truck drivers.[24] A significant increase in sleep propensity across 7 days of sleep restricted to either 3 or 5 hours per night was observed, with no increase in sleep propensity found when sleep was restricted to 7 or 9 hours per night.[24] Similarly, sleep propensity (as measured by the MWT[29]) during 7 days of sleep restriction to 4 hours per night was reported to increase, especially in subjects whose sleep was restricted by advancing sleep offset.[30]

An epidemiologic study of predictors of objective sleep tendency in the general population[31] also found a dose–response relationship between self-reported nighttime sleep duration and objective sleep tendency as measured by the MSLT. Persons reporting more than 7.5 hours of sleep had significantly less probability of falling asleep on the MSLT than those reporting between 6.75 and 7.5 hours per night (27% risk of falling asleep), and than those reporting sleep durations less than 6.75 hours per night (73% risk of falling asleep).[31] Although the MWT has been used less in experimental settings than the MSLT, it has also been found to increase in experiments in which adults were restricted to 4 hours for sleep for 7 nights,[30] and for 5 nights.[32] All of these studies suggest that chronic curtailment of nocturnal sleep increases daytime sleep propensity.

Oculomotor responses have also been reported to be sensitive to sleep restriction.[33] Eyelid closure and slow rolling eye movements are part of the initial transition from wakefulness to drowsiness. Eye movements and eye closures have been studied during sleep-loss protocols, under the premise that changes in the number and rate of movements and eyelid closures are a reflection of increased sleep propensity and precursors of the eventual onset of sleep.[34] It has been demonstrated experimentally that slow eyelid closures during performance are associated with vigilance lapses and are sensitive indices of sleep deprivation, and slow eyelid closures have been found to be a sign of drowsiness while driving.[33]

Increased slow eye movements attributed to attentional failures have been reported to be increased by reduced sleep time in medical residents.[35] Sleep restriction has also been found to decrease saccadic velocity and to increase the latency to pupil constriction in subjects allowed only 3 or 5 hours of time in bed for sleep over 7 nights.[36] These changes in ocular activity were positively correlated with sleep latency, subjective sleepiness measures, and accidents on a simulated driving task.[36]

Waking Electroencephalogram

Slowing in certain waking EEG frequencies has been thought to reflect the increased homeostatic pressure for sleep during sleep restriction. EEG frequencies in the slower range (0.5 to 14 Hz)—in sleep-deprived individuals in particular—may herald an increased tendency for microsleeps. Significant increases in power densities in the delta range (3.75 to 4.5 Hz) and decreases in the alpha range (9.25 to 10 Hz) have been reported in subjects exposed to 4 hours of sleep restriction for 4 nights.[25] In contrast, no effect on waking alpha power (8 to 12 Hz) across days of restriction was evident in subjects restricted to 4 or 6 hours of time in bed for sleep per night for 14 nights.[14] An increase in theta power (4 to 8 Hz) across days of the sleep restriction protocol was evident; however, there was no significant difference in theta power changes between restriction conditions. It has been suggested that these changes in waking EEG frequency during sleep loss reflect "spectral leakage" indicative of elevated sleep pressure manifesting in wakefulness.[37] Few studies have investigated the "leakage" of slower EEG activity in humans, but animal studies suggest that this process may enable the brain to discharge some of the accumulated homeostatic sleep drive without actually going to sleep.[37]

Cognitive Effects

Reduced sleep time can adversely affect different aspects of waking cognitive performance, especially behavioral alertness, which is fundamental to many cognitive tasks. Behavioral alertness can be measured with the PVT, which requires vigilant attention and has proved to be very sensitive to any reduction in habitual sleep time.[38,39] Studies have consistently shown that sleep restriction increases PVT response slowing[40] and lapses,[38] which are thought to reflect microsleeps.[3,41] As loss of sleep accumulates, relatively brief lapses of a half second can increase to well over 10 seconds and longer.[3,41,42] It is suggested that lapses produced by sleep loss involve shifts in neuronal activity in frontal, thalamic, and secondary sensory processing areas of the brain.[43] Lapses of attention occur unpredictably throughout cognitive performance in sleep-restricted subjects, and they increase in frequency and duration as a function of the severity of sleep restriction, which has led to the idea that they reflect underlying "wake state instability."[38,42,43] This instability appears to involve moment-to-moment fluctuations in the relationship between neurobiological systems mediating wake maintenance and sleep initiation.[43]

One early study of chronic sleep restriction effects on cognitive performance examined the effects of reducing habitual sleep time by 40% for 5 nights.[44] Decreases in performance on a vigilance and simple reaction time performance task were observed across the protocol with sleep restriction. Interestingly, however, there was no effect of sleep restriction on a choice reaction time task, suggesting that not all measures of performance are equally sensitive to chronic sleep restriction. This could result from any of a number of aspects of the psychometric properties of cognitive tests (e.g., learning curves) or from their neurobiological substrates; negative findings provide no insight into the reason for lack of sensitivity.

Two large-scale experimental studies published in 2003 described dose-related effects of chronic sleep restriction on neurobehavioral performance measures.[4,24] In one study, truck drivers were randomized to 7 nights of 3, 5,

7, or 9 hours of time in bed for sleep per night.[24] Cognitive performance was assessed using the PVT. Subjects in the 3- and 5-hour time-in-bed groups experienced a decrease in performance across days of the sleep restriction protocol, with increases in the mean reaction time, in the number of lapses, and in the speed of the fastest reaction on the PVT.[24] In the subjects who were allowed 7 hours of time in bed per night, a significant decrease in mean response speed was also evident, although no effect on lapses was evident. Performance in the group allowed 9 hours of time in bed was stable across the 7 days.

In an equally large experiment,[4] young adults had their sleep duration restricted to 4, 6, or 8 hours of time in bed per night for 14 nights, and daytime deficits in cognitive functions were observed for lapses on the PVT (Fig. 6-1), for a memory task, and for a cognitive throughput task. These performance deficits accumulated across the experimental protocol in those subjects allowed less than 8 hours of sleep per night.[4] Data from this study demonstrate that sleep restriction–induced deficits continued to accumulate beyond the 7 nights of restriction used in other experiments,[8,24] with performance deficits still increasing at day 14 of the restricted sleep schedule. By the end of the 14-day chronic partial-sleep restriction period, the level of cognitive impairments recorded in subjects in the 4-hour sleep restriction condition was equivalent to the level of impairment seen after 1 to 2 nights without any sleep (see Fig. 6-1). To understand the relationship between the different sleep-loss conditions and the equivalence in performance impairment observed, the amount of cumulative sleep loss for subjects in each condition was calculated.[4] The degree of sleep loss was greater in subjects allowed 4 hours of sleep each night for 14 nights (i.e., losing approximately 55 hours of sleep) than in subjects who remained awake for 88 hours (i.e., losing approximately 25 hours of sleep) (Fig. 6-2A), suggesting that impairments in waking performance should have been much worse in the 4-hour condition. However, this was not the case (see Fig. 6-1).

To reconcile this paradox, wake time was defined as the difference between the duration of each continuous wake period and the duration of habitual wake time. Accordingly, cumulative wake-time extension was calculated as the sum of all consecutive hours of wakefulness extending beyond the habitual duration of wakefulness that each subject was accustomed to at home. In the 4-, 6-, and 8-hour sleep restriction conditions, this yielded the same results as for cumulative sleep loss, because the definitions of cumulative wake extension and cumulative sleep loss were arithmetically equivalent. However, for the 0-hour total sleep deprivation condition, each day without sleep added 24 hours to the cumulative wake extension. Thus, over 3 days with 0 hours of sleep, cumulative wake extension was equal to 72 hours for each subject (see Fig. 6-2B), whereas cumulative sleep loss was only 23 hours (see Fig. 6-2A). These results illustrate that cumulative sleep loss and cumulative wake extension are different constructs that can have different quantitative values, depending on the manner in which sleep loss occurs. They also suggest that sleep debt can also be understood as resulting in additional wakefulness beyond an average of approximately 16 hours a day, which has a neurobiological cost that accumulates over time.[4]

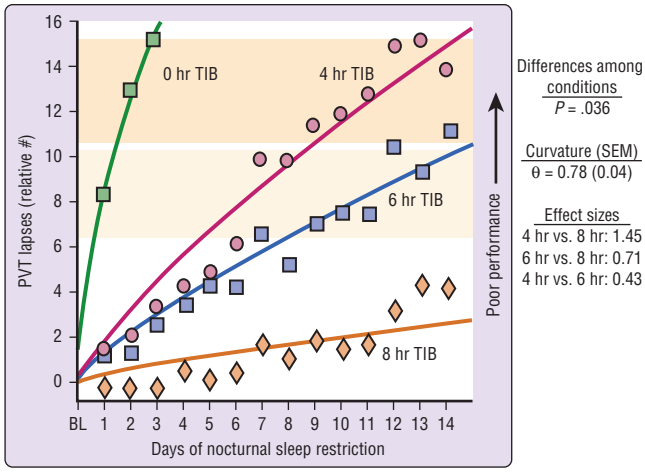

Figure 6-1 Psychomotor vigilance task (PVT) performance lapses under varying dosages of daily sleep. Displayed are group averages for subjects in the 8-hour *(diamond),* 6-hour *(light blue square),* and 4-hour *(circle)* chronic sleep period time in bed (TIB) across 14 days, and in the 0-hour *(green square)* sleep condition across 3 days. Subjects were tested every 2 hours each day; data points represent the daily average (07:30 to 23:30) expressed relative to baseline (BL). The curves through the data points represent statistical nonlinear model-based best-fitting profiles of the response to sleep deprivation for subjects in each of the four experimental conditions. The ranges (mean ± SE) of neurobehavioral functions for 1 and 2 days of 0 hours of sleep (total sleep deprivation) are shown as *light and dark bands,* respectively, allowing comparison of the 3-day total sleep deprivation condition and the 14-day chronic sleep restriction conditions. (Redrawn from Van Dongen HP, Maislin G, Mullington JM, et al. The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. Sleep 2003;26:117-126.)

It appears that the neurocognitive effects of restricting nocturnal sleep to 6 or 4 hours per night on a chronic basis are fundamentally the same as when sleep is chronically restricted but split each day between a nighttime sleep and a daytime nap.[45] Cognitive performance deficits also accumulate across consecutive days in which the restricted sleep occurs during the daytime and wakefulness occurs at night.[46] The primary difference between the nocturnally[4] and diurnally[46] placed restricted sleep periods is that the magnitude of neurobehavioral impairment is significantly greater with daytime sleep compared with nighttime sleep, reflecting a combined influence of the homeostatic and circadian systems. In another experiment, when recovery periods after total sleep time were restricted to 6 hours versus 9 hours time in bed for sleep per night, neither PVT performance nor sleepiness recovered to baseline levels, suggesting that restricting sleep can also reduce its recovery potential.[47]

All these studies suggest that when time in bed for sleep is chronically restricted to less than 7 hours per night in healthy adults (aged 21 to 64 years), cumulative deficits in a variety of cognitive performance functions become evident. These deficits can accumulate to levels of impairment equivalent to those observed after 1 or even 2 nights of total sleep deprivation.

These cognitive performance findings are consistent with those on the effects of sleep restriction on physiologic

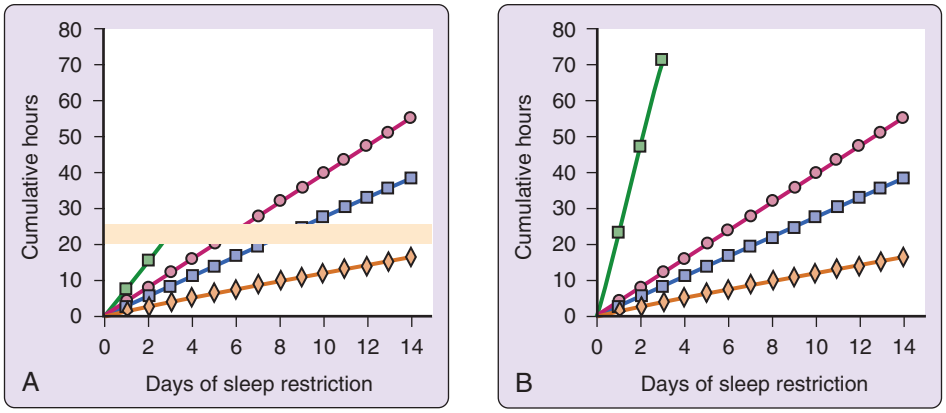

Figure 6-2 Cumulative buildup of sleep loss and wake time extension across days of sleep restriction and total sleep loss. **A,** Cumulative sleep loss relative to habitual sleep duration—that is, all hours of sleep habitually obtained (as measured at home during the 5 days prior to the experiment) but not received in the experiment because of sleep restriction. **B,** Cumulative wake extension relative to habitual wake duration—that is, all consecutive hours of wakefulness in excess of the habitual duration of a wakefulness period. Daily means are shown for subjects in the 8-hour *(diamond),* 6-hour *(light blue square),* 4-hour *(circle),* and 0-hour *(green square)* sleep period conditions. **A** also shows the range *(orange band)* of cumulative sleep loss (relative to habitual sleep duration) after 3 days in the 0-hour sleep condition, which was 23.1 ± 2.6 hours (mean ± SD). This was significantly less than the cumulative sleep loss after 14 days in the 4-hour sleep period condition ($t_{20} = 10.58$, $P < .001$). (Redrawn from Van Dongen HP, Maislin G, Mullington JM, et al. The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. Sleep 2003;26:117-126.)

sleep propensity measures (MSLT, MWT).[24,28,30,32] Collectively, they suggest that there is a neurobiological integrator that accumulates either homeostatic sleep drive or the neurobiological consequences of excess wakefulness.[4,24] There has as yet been no definitive evidence of what causes this destabilization of cognitive functions, but one intriguing line of evidence suggests that it may involve extracellular adenosine in the basal forebrain.[48]

Driving Performance

There is an increased incidence of sleep-related motor vehicle crashes in drivers reporting less than 7 hours of sleep per night on average.[49] Additional contributing factors to these sleep- or sleepiness-related crashes included poor sleep quality, dissatisfaction with sleep duration (i.e., undersleeping), daytime sleepiness, previous instances of driving drowsy, and time driving and time of day (driving late at night). It has been found that after 1 night of restricted sleep (5 hours), a decrease in performance on a driving simulator, with a concurrent increase in subjectively reported sleepiness, was found.[50] In addition, during chronic sleep restriction in a controlled laboratory, with sleep durations reduced to between 4 and 6 hours per 24 hours, placed either nocturnally or diurnally, significant increases in the rate of accidents on a driving simulator occurred with decreased sleep durations, independent of the timing of the sleep period.[51]

Subjective Sleepiness and Mood

In contrast to the continuing accumulation of cognitive performance deficits associated with nightly restriction of sleep to below 8 hours, subjective ratings of sleepiness, fatigue, and related factors repeatedly made by subjects on standardized sleepiness scales did not parallel performance deficits.[4] As a consequence, after 2 weeks of sleep restricted to 4 or 6 hours per night, subjects were markedly impaired and behaviorally less alert, but they thought themselves only moderately sleepy. This suggests that individuals frequently underestimate the actual cognitive impact of sleep restriction and believe themselves fit to perform. Experiments using driving simulators have found similar results, with drivers unable to accurately perceive their level of fatigue and cognitive impairment.[50]

Individual Differences in Responses to Chronic Sleep Restriction

Although the majority of healthy adults develop cumulative cognitive deficits and sleepiness with chronic sleep restriction, interindividual variability in the neurobehavioral and physiologic responses to sleep restriction is substantial.[3,38,39,42] Sleep restriction increases neurobehavioral performance variability in subjects,[38,39,41] and it also reveals clear neurobehavioral differences between subjects. This interindividual variability is quite apparent in sleep restriction studies. For example, not everyone was affected to the same degree when sleep duration was limited to less than 7 hours per day in the studies described earlier.[4,24] Some people experience very severe impairments even with modest sleep restriction, whereas others show little effect until sleep restriction is severe. However, this difference is not always apparent to the individual. It has been postulated that these individual differences are a result of state (basal level of sleepiness/alertness and basal differences in circadian phase) and trait differences (optimal circadian phase for sleep/wakefulness, sensitivity/responsiveness of sleep homeostat, and compensatory mechanisms),[52] but these factors have not been widely researched. For studies of the possible genetic contributors to differential vulnerability to sleep loss, it is significant that the neurobehavioral responses to sleep deprivation have been found to be stable and consistent within subjects,[23] suggesting they are traitlike.[23]

PHYSIOLOGIC EFFECTS

There is increasing evidence of physiologic and health-related consequences of chronic sleep restriction. Alterations in other physiologic parameters, such as endocrine (see Chapter 125) and immune function (see Chapters 25 and 26), have been recognized and have implications for health status and risk.

Several anecdotal and longitudinal studies have reported an increased incidence and risk of medical disorders and health dysfunction related to shift work schedules, which have been attributed to both circadian disruption and sleep disturbance. Further links between sleep disturbance and health effects have been reported in studies examining insomniac patients and patients with other sleep disorders and medical disorders that disturb sleep.[53] In addition, an elevated mortality risk in those individuals who reported sleeping less than 6.5 hours per night has been found.[11] One provocative discovery from this study was the finding that individuals sleeping more than 7.4 hours per night were also at an elevated risk of all-cause mortality. This finding is similar to that reported from the Nurses' Health Study,[54] where subjects reporting greater than 9 hours of sleep per night on average were at a higher risk for coronary events than those sleeping 8 hours per night. In addition, an increased risk of coronary events in women obtaining 7 hours of sleep or less per night was observed.[55] Also, increasing epidemiologic, cross-sectional, and longitudinal data suggest that reduced sleep duration is associated with larger body mass index (BMI). A meta-analysis study found a consistent, increased risk of obesity among short sleepers—both children (sleeping less than 10 hours per night) and adults (sleeping less than 5 hours per night)—but, as the authors pointed out, causal inference was difficult because of the lack of control of confounders and inconsistency in the methodologies used.[56]

Endocrine and Metabolic Effects

A number of studies have examined the effects of sleep loss on a range of neuroendocrine factors.[57,57a] Comparison of sleep restriction (4 hours per night for 6 nights) with sleep extension (12 hours per night for 6 nights) revealed an elevation in evening cortisol, increased sympathetic activation, decreased thyrotropin activity, and decreased glucose tolerance in the restricted as opposed to the extended sleep condition.[55] Similarly, an elevation in evening cortisol levels and an advance in the timing of the morning peak in cortisol, so that the relationship between sleep termination and cortisol acrophase was maintained, were found after 10 nights of sleep restricted to 4.2 hours of time in bed for sleep each night compared with baseline measures and a control group allowed 8.2 hours of time in bed for sleep for 10 nights.[58] Changes in the timing of the growth hormone secretory profile associated with sleep restriction to 4 hours per night for 6 nights, with a bimodal secretory pattern evolving, have also been reported.[59]

Immune and Inflammatory Effects

The majority of studies examining sleep loss and immune function have concentrated on total sleep deprivation or 1 night of sleep restriction. Changes in natural killer cell activity,[60,61] lymphokine-activated killer cell activity,[60] interleukin-6,[62] and soluble tumor necrosis factor–alpha receptor 1[61] have all been reported with total sleep deprivation and sleep restriction.

One study reported that antibody titers were decreased by more than 50% after 10 days in subjects who were vaccinated for influenza immediately after 6 nights of sleep restricted to 4 hours per night, compared with those who were vaccinated after habitual sleep duration.[63] But by 3 to 4 weeks after the vaccination, there was no difference in antibody level between the two subject groups. Therefore, sleep loss appeared to alter the acute immune response to vaccination.

Cardiovascular Effects

Increased cardiovascular events and cardiovascular morbidity have been reported with reduced sleep durations.[11,54] Additionally, this relationship has been found in a case-control study examining insufficient sleep resulting from work demands.[64] In the Nurses' Health Study, Ayas and colleagues[54] reported that coronary events were increased in female subjects obtaining 7 hours of sleep or less per night compared with those averaging 8 hours per night, and Liu and associates[64] reported a twofold to threefold increase in risk of cardiovascular events with an average sleep duration of 5 hours or less per night. Shift workers who typically experience chronic reductions in sleep time as well as circadian disruption have been found to have reduced cardiovascular health.[65]

The mechanism that links chronic sleep restriction and increased cardiovascular risk is unknown, but one potential pathway may be via activation of inflammatory processes during sleep loss. C-reactive protein (CRP) is a predictive inflammatory marker of increased risk for cardiovascular disease. Increased CRP levels have been found in patients with obstructive sleep apnea, who commonly experience reduced sleep time as well as hypoxia,[66] and an increase in CRP levels was reported after both total sleep deprivation and sleep restriction (4 hours in bed for sleep per night) in healthy subjects.[67]

❖ Clinical Pearl

Chronic sleep deprivation can be caused by sleep disorders, work schedules, and modern lifestyles. Regardless of its cause, chronic sleep deprivation results in cumulative adverse effects in daytime awake functions, including sleep propensity, cognitive performance, driving safety, mood, and physiologic conditions.

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Sleep Mechanisms and Phylogeny

Section 2

Jerome M. Siegel

Neural Control of Sleep in Mammals

Chapter 7

Dennis McGinty and Ronald Szymusiak

Abstract

Mammalian sleep and wake states are facilitated by multiple brain regions. The lower brainstem is sufficient to generate wake, non–rapid eye movement (NREM)-like, and REM sleep states, but lesions in several brain regions, including sites in the medulla, mesencephalon, preoptic area (POA) of the hypothalamus, thalamus, and neocortex, all markedly reduce the amounts of NREM and REM sleep. Similarly, arousal and waking are facilitated by several chemically distinct neuronal groups localized in the midbrain, the posterior and lateral hypothalamus, and the basal forebrain. These include histaminergic, orexinergic, serotonergic, cholinergic, dopaminergic, and noradrenergic neurons. These arousal systems share the property of having long axons and extensive projections to widespread brain regions, including the diencephalon, limbic system, and neocortex. These widespread projections can account for the global aspect of arousal from sleep, characterized by concurrent changes in electroencephalographic (EEG), motor, sensory, autonomic, and integrative functions. Arousal systems also control the neuronal membrane potentials in thalamocortical neurons; these potentials, in turn, regulate the oscillatory mechanisms intrinsic to thalamocortical networks that underlie NREM or "synchronized" EEG patterns. Inhibition of arousal systems permits the emergence of synchronized EEG patterns.

Although several brain regions modulate sleep, the POA has a critical role in the control of NREM sleep. Groups of neurons in the POA exhibit increased activity during NREM and REM sleep and respond to physiologic signals, such as warming, that increase sleep. Several putative neurochemical sleep factors promote sleep through actions in the POA or adjacent basal forebrain. Signals from the circadian clock originating in the hypothalamic suprachiasmatic nucleus regulate the circadian timing of sleep through connections in this brain region. POA sleep-active neurons send inhibitory gamma-aminobutyric acid (GABA)ergic projections to the histaminergic, orexinergic, serotonergic, and noradrenergic arousal systems. Through coordinated inhibition of these arousal systems, the key elements of NREM sleep onset are enabled, including EEG synchronization and suppression of motor activity.

We take it for granted that the brain controls sleep and waking, and this has been confirmed. Research spanning the past 80 years has identified specific groups of neurons and neurochemical mechanisms that carry out the control of sleep and waking and generate core aspects of the phenomena of sleep and waking, such as the electroencephalographic (EEG) patterns that define these states. Many methods have been used. The story is complex, but a surprisingly coherent picture of sleep–wake control has emerged.

DIVERSE BRAIN REGIONS MODULATE WAKING AND NREM SLEEP

Isolated Forebrain

The capacity of various brain regions to generate sleep and wake states was first studied by isolating or removing major regions. The physiology of the chronically maintained isolated forebrain or chronic *cerveau isolé* preparation can be examined in dogs and cats.[1] Acutely after complete midbrain transections, the isolated forebrain exhibits continuous EEG slow waves and spindles. *Thus, structures below the midbrain must normally facilitate awake-like EEG states.* However, if a brainstem transection is made lower, at the mid-pontine level, an activated or wakelike forebrain EEG state becomes predominant immediately after the transection, but with some residual episodes of EEG slow-wave activity.[2] In this preparation, the forebrain exhibits evidence of conditioning, and other signs of an integrated waking state. These studies argue that neuronal groups localized between the midpons and upper midbrain are important for generating a waking-like state. After 5 to 9 days of recovery from surgery, the chronic *cerveau isolé* rat preparation exhibits a circadian pattern of EEG activation and synchronization.[3] In this preparation, preoptic area (POA) lesions are followed by a continuously activated EEG, abolishing the circadian facilitation of synchronization. *Thus, the isolated forebrain can generate a sustained wake-*

like state, and the POA must play a critical role in initiating the sleeplike EEG state of the isolated forebrain (see later). Wakelike and sleeplike EEG states appear to depend on a balance between wake-promoting and sleep-promoting systems.

Diencephalon

Chronic diencephalic cats, whose neocortex and striatum have been removed, exhibit behavioral waking with persistent locomotion and orientation to auditory stimuli, a quiet sleeplike or non–rapid eye movement (NREM)-like state with typical cat sleeping postures, and a REM-like state including antigravity muscle atonia, rapid eye movements, muscle twitches, and pontine EEG spikes.[4] EEG patterns recorded in the thalamus showed increased amplitude in conjunction with the NREM-sleep–like state, although true spindles and slow waves are absent. The thalamic EEG exhibits desynchronization during the REM-like state. *In summary, at least in the cat, the neocortex and striatum are not required for any behaviorally defined sleep–wake states, and an NREM-like state occurs in the absence of sleep spindles and slow waves.*

Thalamus

Cats subjected to complete thalamectomy (athalamic cats) continue to exhibit episodes of EEG and behavioral sleep and waking, although there is an absence of spindles in the NREM sleep EEG,[4] and they exhibit chronic insomnia, with reductions in both NREM and REM sleep. Fatal familial insomnia,[5] a neurodegenerative disease characterized by progressive autonomic hyperactivation, motor disturbances, loss of sleep spindles, and severe NREM sleep insomnia, typically begins after age 40 years. Neuropathologic findings reveal initial severe cell loss and gliosis in the anterior medial thalamus, including the dorsomedial nucleus. However, patients with paramedian thalamic stroke, with magnetic resonance imaging (MRI)-verified damage to the dorsomedial and centromedial nuclei, present with either severe hypersomnolence or increased daytime sleepiness, not insomnia.[6] *In summary the thalamus plays a critical role in regulating cortical EEG patterns during waking and sleep, and portions of this structure appear to have hypnogenic functions.*

Lower Brainstem

After recovery from the acute effects of the complete midbrain transections (described earlier), the lower brainstem can generate rudimentary behavioral waking, a NREM-like state, and a REM-like state.[4] The behavior of the midbrain-transected cats could be characterized as having three states, including "waking" (identified by crouching, sitting, attempts to walk, dilated pupils, and head orientation to noises), and two sleeplike states. In the first sleeplike state, cats lay down in a random position, pupils exhibited reduced but variable miosis, and eyes exhibited slow and nonconjugate movements, and they could be aroused by auditory or other stimuli. If this stage is not disturbed, cats enter another stage, characterized by complete pupillary miosis, loss of neck muscle tone, and rapid eye movements, identifying a REM-like state. Additional studies support the hypothesis that the lower brainstem contains sleep-facilitating processes. Low-frequency electrical stimulation of the dorsal medullary reticular formation in the nucleus of the solitary track produced neocortical EEG synchronization.[7] Lesions or cooling of this site were followed by EEG activation.[8]

In summary, widespread structures in the mammalian nervous system, from the neocortex to the lower brainstem, have the capacity to facilitate both sleeplike and waking-like states and to modulate the amounts of sleep.

RETICULAR ACTIVATING SYSTEM AND DELINEATION OF AROUSAL SYSTEMS

The transection studies just reviewed support the concept of a pontomesencephalic wake-promoting or arousal system. No discovery was historically more significant than the description of the reticular activating system (RAS) by Moruzzi.[9] Large lesions of the core of the rostral pontine and mesencephalic tegmentum are followed by persistent somnolence and EEG synchronization, and electrical stimulation of this region induces arousal from sleep. Interruption of sensory pathways does not affect EEG activation. It was hypothesized that cells in the RAS generated forebrain activation and wakefulness.

The concept of the RAS has been superseded by the finding that arousal is facilitated not by a single system but instead by several discrete neuronal groups localized within and adjacent to the pontine and midbrain reticular formation and its extension into the hypothalamus (Fig. 7-1). These discrete neuronal groups are identified and differentiated by their expression of molecular machinery that synthesizes and releases specific neurotransmitters and neuromodulators. These include neuronal groups that synthesize serotonin, noradrenalin, histamine, acetylcholine, and orexin/hypocretin (herein called orexin). Each of these systems has been studied extensively in the context of the control of specific aspects of waking behaviors. Here we will give only a brief overview of each, focusing on their contribution to generalized brain arousal or activation. Before proceeding, we point out certain general properties of these neuronal systems.

1. Arousal is a global process, characterized by concurrent changes in several physiologic systems, including autonomic, motor, endocrine, and sensory systems, and in EEG tracings. Thus, it is intriguing that most arousal systems share one critical property: the neurons give rise to long, projecting axons with extensive terminal fields that impinge on multiple regions of the brainstem and forebrain. These diffuse projections enable the systems to have multiple actions, as might be expected of arousal systems. In this review, we emphasize the ascending projections—that is, projections from the brainstem and hypothalamus to the diencephalon, limbic system, and neocortex, as these are particularly germane to the generation of cortical arousal. Some arousal systems also give rise to descending projections, which are also likely to play a role in regulating certain properties of sleep–wake states, such as changes in muscle tone.
2. The release of neurotransmitters and neuromodulators at nerve terminals is initiated by the propagation of action potentials to the terminals. Thus, neurotransmitter release is correlated with the discharge rate of

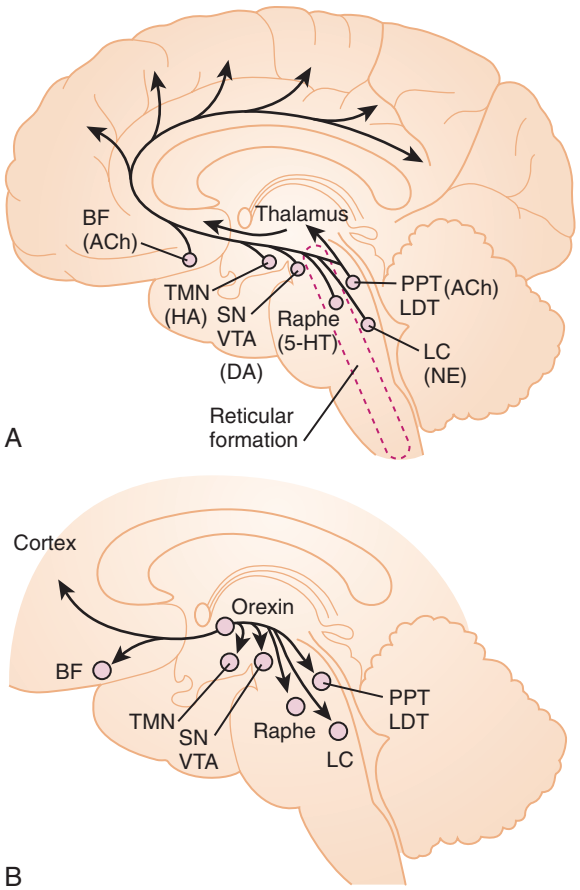

Figure 7-1 A, Sagittal view of a generic mammalian brain providing an overview of the wake-control networks described in the text. The upper brainstem, posterior and lateral hypothalamus, and basal forebrain contain several clusters of neuronal phenotypes, with arousal-inducing properties. These clusters include neurons expressing serotonin (5-HT), norepinephrine (NE), acetylcholine (ACh) in both pontomesencephalic and basal forebrain clusters, dopamine (DA), and histamine (HA). **B,** Sagittal view of brainstem and diencephalon showing localization of orexin-containing neurons and their projections to both forebrain and brainstem. All of these groups facilitate EEG arousal (waking and REM) and/or motor-behavioral arousal (waking). The arousal systems facilitate forebrain EEG activation both through the thalamus and the basal forebrain and through direct projections to neocortex. Arousal systems also facilitate motor-behavioral arousal through descending pathways.

neurons. Most arousal systems have been studied by recording the discharge patterns of neurons in "freely moving" animals, in relationship to spontaneously occurring wake and sleep states. Increased discharge during arousal or wake compared with sleep constitutes part of the evidence for an arousal system.

3. The actions of a neurotransmitter on a target system are determined primarily by the properties of the receptors in the target. The neurotransmitters and neuromodulators underlying arousal systems each act on several distinct receptor types, with diverse actions. In addition, postsynaptic effects are regulated by transmitter-specific "reuptake" molecules, which transport the neurotransmitter out of the synaptic space, terminating its action. Pharmacologic actions are usually mediated by actions on specific receptor types or transporters (see examples later).
4. Chronic lesions of individual arousal systems or genetic knockout (KO) of critical molecules have only small or sometimes no effect on sleep–wake patterns (with the exception of serotonin and orexin KOs; see later), even though acute manipulations of these same systems have strong effects on sleep–wake. The absence of chronic lesions or KO effects is probably explained by the redundancy of the arousal systems, such that, over time, deficiency in one system is compensated for by other systems or by changes in receptor sensitivity. Electrophysiologic studies show that the arousal systems are normally activated and deactivated within seconds or minutes. Thus, effects of acute experimental manipulations of particular arousal-related neurotransmitters, as with administration of a drug, may better mimic the normal physiologic pattern and be more informative as to their function.
5. REM sleep is, on one hand, a sleep state, but, on the other hand, it is associated with neocortical EEG characteristics of wake. In parallel with these two sides of REM, it has been shown that arousal systems can be classified into two types, ones that are "off" in REM, befitting the sleeplike property of REM, and others that are "on" in REM, befitting the wakelike properties of REM. Some arousal-promoting systems (summarized later) also play a role in REM control. Detailed analyses of the control of the role of these systems in REM sleep can be found in Chapters 8 and 9.

WAKE-ON, REM-OFF AROUSAL SYSTEMS

Serotonin

Neurons containing serotonin, or 5-hydroxytryptamine (5-HT), innervate the forebrain and are found in the dorsal raphe (DR) and median raphe (MR) nuclei of the midbrain. These neurons project to virtually all regions of the diencephalon, limbic system, and neocortex. Although it was initially hypothesized that serotonin might be a sleep-promoting substance,[10] much evidence shows that the immediate effect of release of serotonin is arousal (reviewed in reference 11). Although there is some heterogeneity, the discharge rates of most DR and MR neurons are highest during waking, lower during NREM, and there is minimal discharge in REM; release of serotonin in the forebrain is highest in waking. Because of the great diversity of serotonin receptors (there are at least 14 types), the effects of serotonin on target neurons are complex. Some receptor types are inhibitory, some are excitatory. At least one class of receptors (5-HT_{2A}) appears to facilitate NREM sleep; 5-HT_{2A} KO mice have less NREM.[12] Another type (5-HT_{1A}) is inhibitory to REM sleep, as 5-HT_{1A} KO mice have increased REM.[13] Selective serotonin reuptake inhibitors (SSRIs) and serotonin–norepinephrine reuptake inhibitors (SNRIs) are used to treat a variety of medical and psychiatric problems, and some drugs in this class have arousing or alerting properties. Serotonin has a wide range of functions in addition to the modulation of sleep–wake.

Norepinephrine

Norepinephrine (NE)-containing neuronal groups in mammals are found throughout the brainstem, but the primary nucleus giving rise to ascending projections is the locus coeruleus (LC). NE neurons in the LC project throughout the diencephalon, forebrain, and cerebellum. LC neurons exhibit regular discharge during waking, reduced discharge during NREM sleep, and near-complete cessation of discharge in REM sleep, a pattern congruent with a role in behavioral arousal.[14] Acute inactivation of the LC or a lesion in the ascending pathway from the LC increases slow-wave EEG activity during sleep.[15] Distinct roles for alpha-1, alpha-2, and beta NE receptor types are established. Direct application of alpha-1 and beta agonists in preoptic area and adjacent basal forebrain sites induces increased wakefulness (reviewed in reference 16). The arousal-producing effects of psychostimulant drugs such as amphetamines depend partly on induction of increased NE release and inhibition of NE reuptake, as well as on enhanced dopamine action (see later).

Histamine

Histamine (HA)-containing neurons in mammals are discretely localized in the tuberomamillary nucleus (TMN) and adjacent posterior hypothalamus (PH). HA neurons project throughout the hypothalamus and forebrain, including the neocortex, as well as to the brainstem and spinal cord. Perhaps the most familiar evidence for an arousal-promoting action of central HA is that administration of histamine (H_1)-receptor antagonists (antihistamines) that penetrate the blood–brain barrier cause sedation. Transient inactivation of the TMN region results in increased NREM sleep.[17] HA neurons exhibit regular discharge during waking, greatly reduced discharge during NREM sleep, and cessation of discharge in REM sleep.[18] HA neurons express the inhibitory H_3-type autoreceptors. Administration of an antagonist of this receptor causes disinhibition and increased waking. Blockade of the critical HA-synthesizing enzyme increases NREM sleep.[19]

Orexin

The loss of orexin neurons is known to underlie the human disease narcolepsy, whose major symptoms are cataplexy and excessive sleepiness.[19-20a] Orexin-containing neurons are localized in the midlateral hypothalamus, and like other arousal systems, they give rise to projections to all brain regions including the brainstem.[21] Among the targets of orexin terminals are other arousal-promoting neurons including HA, 5-HT, and NE neurons. Orexin-containing neurons are active in waking, and they are "off" in both NREM and REM sleep.[22,23] Local administration of orexin in several brain sites induces arousal.[24] (See also Chapters 8 and 16.)

WAKE-ON, REM-ON AROUSAL SYSTEMS

Acetylcholine

Groups of acetylcholine (ACH)-containing neurons are localized in two regions: in the dorsolateral pontomesencephalic reticular formation (RF) (the pedunculopontine tegmental [PPT] and laterodorsal tegmental [LDT] nuclei) and in the basal forebrain.[25] The pontomesencephalic ACH neuronal group projects to the thalamus, hypothalamus, and basal forebrain; the basal forebrain group projects to the limbic system and neocortex. Neurons in both groups exhibit higher rates of discharge in both waking and REM than in NREM sleep,[26] and release of ACH is also increased in these states.[27] Pharmacologic blockade of ACH receptors induces EEG synchrony and reduces vigilance, and inhibition of the ACH-degrading enzyme cholinesterase enhances arousal.[28]

Dopamine

Dopamine (DA)-containing neurons are primarily localized in the substantia nigra and the adjacent ventral tegmental area of the midbrain and the basal and medial hypothalamus.[29] Putative dopaminergic neurons exhibit highest activity in waking and REM sleep. Release of DA in the frontal cortex is higher during wakefulness than during sleep.[30] DA is inactivated primarily through reuptake by the dopamine transporter. Stimulant drugs such as amphetamines and modafinil act primarily through DA receptors, particularly by binding to and suppressing the dopamine transporter, reducing reuptake.[31] The degeneration of the nigrostriatal DA system is the primary neuropathologic basis of Parkinson's disease, and excessive daytime sleepiness is one manifestation of it.[32] DA agonists used to treat periodic limb movement in sleep and restless leg syndrome do not usually induce arousal, probably because they have effects on both presynaptic and postsynaptic DA receptors; presynaptic receptors inhibit transmitter release, counteracting postsynaptic stimulation.

Glutamate

Glutamate (Glu)-containing neurons are found throughout the brain, including in the core of the pontine and midbrain RF.[33] However, the projections of the brainstem Glu system have not been described. Glu is the primary excitatory neurotransmitter of the brain. Arousal is increased by application of Glu to many sites.[34] Glu actions are mediated by receptors controlling membrane ion flux, including the *N*-methyl-D-aspartate (NMDA) receptor, and "metabotrophic" receptors controlling intracellular processes. Humans may be exposed to systemic administration of NMDA receptor antagonists in the form of anesthetics (e.g., ketamine) or recreational drugs (PCP). The effects are dosage dependent: low dosages produce arousal, and high dosages produce sedation. In rats, exposure to NMDA antagonists induces a potent long-lasting enhancement of NREM slow-wave activity.[35]

SLEEP-PROMOTING MECHANISMS

We have reviewed evidence for multiple neurochemically specific arousal systems and noted that the activity of each of these neuronal groups is reduced during NREM sleep. In most groups, the reduction in neuronal discharge precedes EEG changes that herald sleep onset. How is the process of sleep onset orchestrated?

A POA Sleep-Promoting System

Von Economo postulated a POA sleep-promoting area more than 70 years ago on the basis of his observation that postmortem examinations of patients who had had encephalitis and severe insomnia showed inflammatory lesions in this area of the brain.[36] Patients with hypersomnia had lesions in the vicinity of the PH. To von Economo, this suggested the concept of opposing hypothalamic sleep-promoting and wake-promoting systems. In rats, symmetrical bilateral transections of the POA resulted in complete sleeplessness, and symmetrical bilateral transections of the PH caused continuous sleep.[37] Rats with both POA and PH transections exhibited continuous sleep, as with PH transections alone. This was interpreted as showing that the POA normally inhibits the PH wake-promoting region. The PH wake-promoting system can now be understood as the rostral extension of arousal-promoting systems and pathways summarized earlier.

The existence of a sleep-promoting mechanism in the POA has been confirmed by a variety of methods. Experimental lesions of this area result in insomnia. Bilateral lesions of the POA with diameters of 1 to 2 mm in rats and cats induce partial sleep loss. Larger bilateral lesions (3 to 5 mm in diameter) that extend into the adjacent basal forebrain yield more severe insomnia (reviewed in reference 38). After POA lesions that result in partial sleep loss, residual sleep is characterized by reduced slow-wave (delta) EEG activity.[39] Delta EEG activity in NREM sleep is augmented by POA warming (see later). Because delta activity is recognized as a marker of enhanced sleep drive, this finding suggests that POA output contributes to the regulation of sleep drive. Electrical or chemical stimulation of the POA evokes EEG synchronization and behavioral sleep.

c-Fos Mapping

Lesion and stimulation studies argue that the POA must contain sleep-active neurons. This has been confirmed in several species (reviewed in reference 38). The identification of sleep-active POA neurons was advanced by the application of the c-Fos immunostaining method.[40] Rapid expression of the protooncogene *c-fos* has been identified as a marker of neuronal activation in many brain sites.[41] Thus, c-Fos immunostaining permits functional mapping of neurons, identifying neurons that were activated in the preceding interval. After sustained sleep, but not waking, a discrete cluster of neurons exhibiting Fos immunoreactivity is found in the ventrolateral preoptic area (VLPO).[40] The VLPO is located at the base of the brain, lateral to the optic chiasm. Sleep-related Fos immunoreactive neurons are also localized in the rostral and caudal median preoptic nucleus (MnPN).[42] The MnPN is a midline cell group that widens to form a "cap" around the rostral end of the third ventricle. Examples of c-Fos immunostaining and the correlations between c-Fos counts and sleep amounts are shown in Figure 7-2. The number of sleep-related Fos immunoreactive neurons in the MnPN is increased in rats exposed to a warm ambient temperature, in association with increased NREM sleep.[42]

The VLPO and the MnPN contain a high density of neurons with sleep-related discharge.[43] Most sleep-active

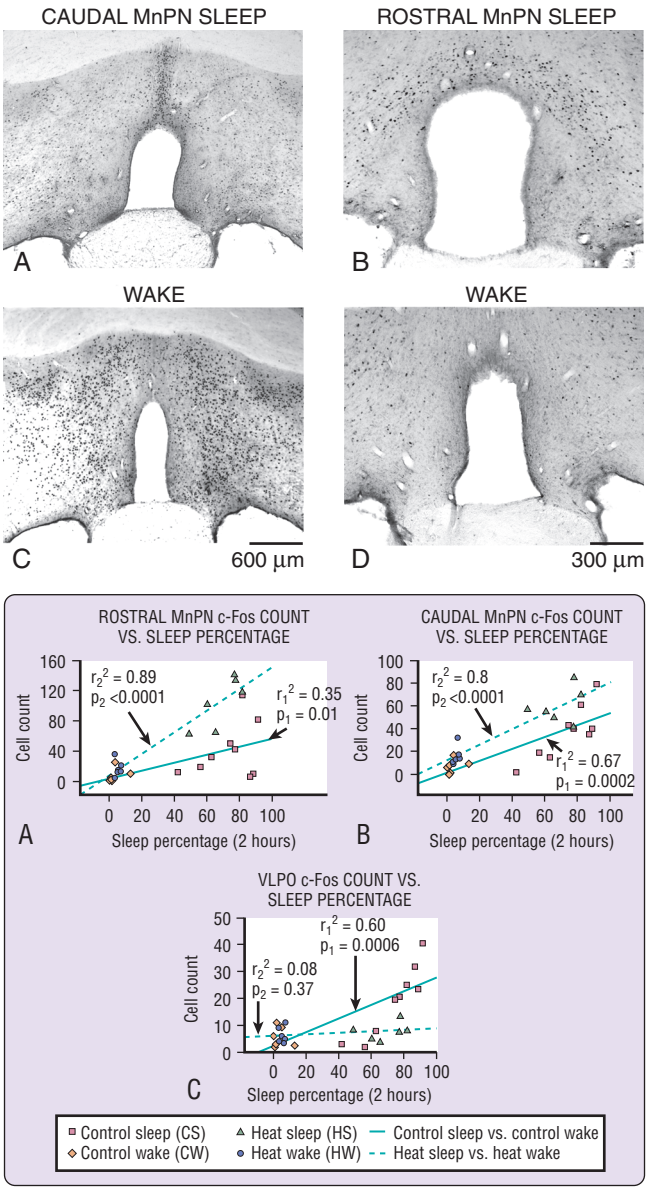

Figure 7-2 *Upper:* Examples of c-Fos immunostaining of preoptic area (POA) neuronal nuclei, identified by *dark spots* after either sustained spontaneous sleep or wake. c-Fos immunostaining is a marker of neuronal activation and is a method for mapping the localization of sleep-active neurons in the brain. After sleep, increased staining was seen in the midline (**A**) or around the top of the third ventricle (**B**) compared with the wake samples (**C** and **D**). These sites correspond to the caudal and rostral median preoptic nucleus (MnPN). Similar results were seen in the ventrolateral preoptic area (VLPO). In other POA sites, c-Fos immunostaining was seen following both waking and sleep.
Lower: Regression functions and correlations relating c-Fos counts and sleep amounts before sacrifice among individual animals. In all sites, high correlations were found between sleep amounts and c-Fos counts at a normal ambient temperature. Groups of animals were studied in both normal and warm ambient temperatures. In a warm ambient temperature, c-Fos counts after sleep and correlations between counts and sleep amounts were increased in MnPN sites (**A** and **B**), but they were suppressed in the VLPO (**C**). (From Gong H, Szymusiak R, King J, et al. Sleep-related c-Fos expression in the preoptic hypothalamus: effects of ambient warming. Am J Physiol Regul Integr Comp Physiol 2000;279:R2079-R2088.)

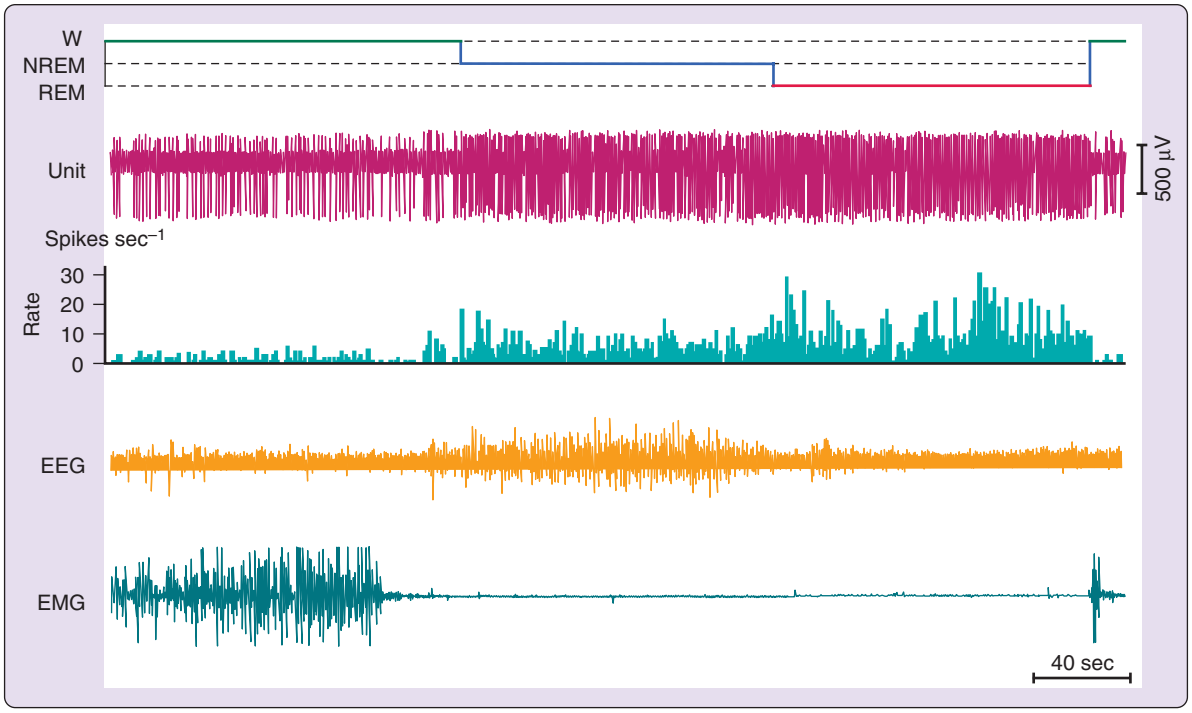

Figure 7-3 Example of sleep-active neurons in the median preoptic nucleus (MnPN). Shown is a continuous recording of discharge of an MnPN neuron during a wake-NREM-REM cycle *(top).* Discharge rate increased at the onset of sleep, as indicated by the increased amplitude of the EEG. Discharge rate increased further in association with REM sleep *(right).* Such sleep-active neurons were the majority of neurons encountered in the median preoptic nucleus (MnPN) and the ventrolateral preoptic area (VLPO). The presence of sleep-active neurons provides one critical piece of evidence for the importance of a brain region in the facilitation of sleep. (From Suntsova N, Szymusiak R, Alam MN, et al. Sleep-waking discharge patterns of median preoptic nucleus neurons in rats. J Physiol 2002;543:665-677.)

neurons in these nuclei are more activated during both NREM and REM sleep than in waking (Fig 7-3). A majority of VLPO neurons exhibit an increase in activity during the immediate transition periods between waking and sleep onset and display a progressive increase in discharge rate from light to deep NREM sleep. In response to 12 to 16 hours of sleep deprivation, VLPO neurons exhibit increased activation during sleep, but rates during waking remain the same as in control rats. Many MnPN neurons show a gradual increase in firing rates *before* sleep onset, elevated discharge rates during NREM sleep, and a small but significant additional increase in discharge rate during REM sleep.

Other sites in the POA also exhibit sleep-related c-Fos immunoreactivity. Electrophysiologic studies describe sleep-active neurons throughout the POA, and lesions that do include the VLPO or MnPN are known to suppress sleep. This suggests that much of the diffuse sleep-related c-Fos immunoreactivity in the POA also labels neurons that are functionally important for sleep regulation.

THE ORCHESTRATION OF SLEEP BY THE POA HYPNOGENIC SYSTEM

How do POA sleep-active neurons initiate and sustain sleep? VLPO neurons that exhibit c-Fos immunoreactivity following sleep express glutamic acid decarboxylase (GAD), the synthetic enzyme that produces the *inhibitory* neurotransmitter, gamma-aminobutyric acid (GABA). The majority of MnPN neurons that exhibit sleep-related Fos-immunoreactivity also express GAD.[44] VLPO neurons send anatomic projections to HA neurons in the TMN.[45] Additional projections of the VLPO include the midbrain DR and the LC.[46] The MnPN also projects to both the DR and LC.[47] Both MnPN and VLPO also project to the perifornical lateral hypothalamic area, the location of cell bodies of the orexin arousal system.[48] Thus, discharge of VLPO and MnPN GABAergic neurons during sleep is expected to release GABA at these sites. Indeed, GABA release is increased during NREM sleep and further increased in REM sleep in the PH, DR, and LC (reviewed in reference 38).[49] Sleep-active neurons in VLPO and MnPN exhibit discharge-rate-change profiles across the wake-NREM-REM cycle that are reciprocal to those of wake-promoting HA, 5-HT, NE, and orexin neurons (Fig 7-4). These findings support a hypothesis that POA sleep-active neurons, through release of GABA, inhibit multiple arousal systems.

The PH and LH areas also facilitate both motor and autonomic activation, and these processes are under GABAergic control.[50] Sleep-related GABAergic inhibition of PH and LH provides a basis for sleep-related deactivation of autonomic and motor functions. POA lesions suppress REM as well as NREM sleep. REM sleep loss after POA damage may be a secondary consequence of NREM sleep disturbance. Alternatively, POA mechanisms may facilitate REM sleep as well as NREM sleep.

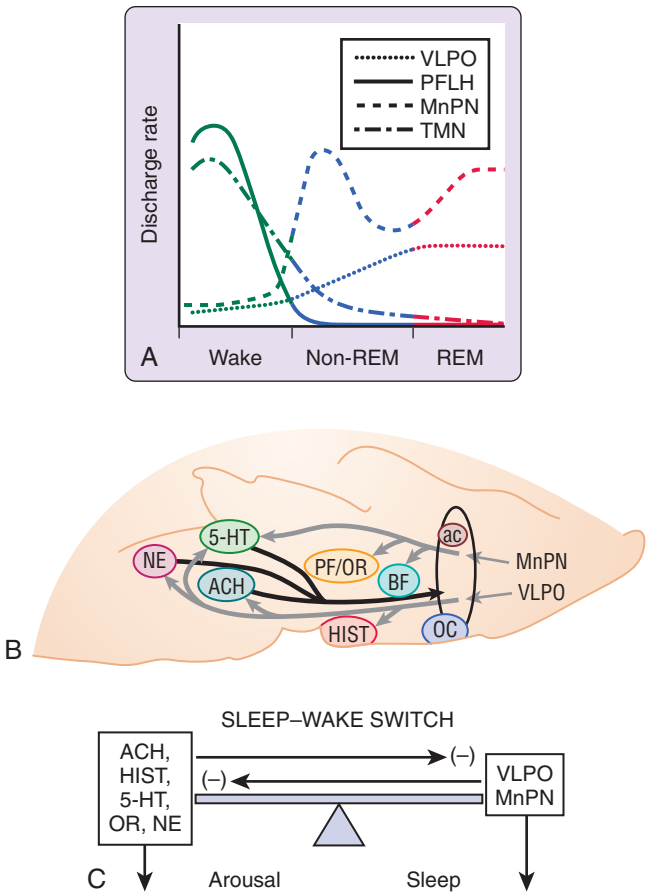

Figure 7-4 Interactions of the preoptic area (POA) sleep-promoting neuronal system with arousal systems that can account for the orchestration of the sleep process. **A,** The neuronal discharge rates across the wake-NREM-REM cycle of sleep-active neurons from the ventrolateral preoptic area (VLPO) and the median preoptic nucleus (MnPN), and from arousal-related (wake-active) neurons in the perifornical lateral hypothalamus (PFLH) and tuberomammillary nucleus (TMN). These neuronal groups have generally reciprocal discharge patterns, although MnPN and VLPO neurons have peak activity at different times during NREM episodes. The wake-active, NREM-diminished, REM-off discharge pattern shown for TMN and a subgroup of PFLH neurons is also characteristic of putative serotoninergic neurons of the dorsal raphe nucleus (DR) and putative noradrenergic neurons of the locus coeruleus (see also **C**). **B,** Sagittal section of the diencephalon and upper brainstem of the rat showing anatomic interconnections of MnPN and VLPO neurons with arousal-related neuronal groups. The MnPN and VLPO distribute projections to sites of arousal-related activity including the (1) basal forebrain, (2) PFLH, which includes orexin-containing neurons, (3) histamine (HIST)-containing neurons of the tuberomammillary nucleus, (4) pontomesencephalic acetylcholine (ACH)-containing neurons, (5) pontomesencephalic serotonin (5-HT)-containing neurons, and (6) noradrenergic (NE)-containing neurons of the pons, particularly the locus coeruleus (LC). 5-HT, NE, and ACH arousal-related neurons provide inhibitory feedback to sleep-active neurons. The arousal-related neuronal groups also have widespread additional ascending and descending projections that control state-related functions throughout the brain. **C,** The sleep–wake switch or "flip-flop" model characterized by mutually inhibitory sleep-promoting and arousal-promoting neuronal groups, depicted as a seesaw, which can promote stable sleep or waking states. Activity of either sleep-promoting or wake-promoting neurons inhibits the neurons generating the opposing state. This network provides a mechanism for the global control of brain activity in the sleep–wake cycle. (Modified from McGinty D, Szymusiak R. Hypothalamic regulation of sleep and arousal. Front Biosci 2003;8:1074-1083.)

GABAergic neurons in the VLPO region are inhibited by ACH, 5-HT, and NE, transmitters of the arousal systems.[51] MnPN neurons are inhibited by NE.[52] Thus, POA sleep-active neurons inhibit arousal systems, and arousal systems inhibit POA sleep-active neurons. These mutually inhibitory processes are hypothesized to underlie a sleep-wake switch or "flip-flop" model (see Fig 7-4).[53] Activation of arousal systems would inhibit sleep-active neurons, thereby removing inhibition from arousal systems, facilitating stable episodes of waking. On the other hand, activation of sleep-promoting neurons would inhibit arousal-related neurons, removing inhibition from sleep-promoting neurons and reinforcing consolidated sleep episodes. This model provides a mechanism for the stabilization of both sleep and waking states.

Abnormalities in one or more of the components of this "flip-flop" system could result in less stable sleep and wake states, a possible explanation for the fragmentation of sleep in sleep disorders in which insomnia is an element, and the fragmentation of waking in narcolepsy.

THALAMIC–CORTICAL INTERACTIONS AND THE GENERATION OF THE SLEEP EEG

Changes in cortical EEG patterns are usually considered to be the defining feature of NREM sleep in mammals. Here we briefly review current understanding of thalamocortical mechanisms underlying NREM sleep EEG patterns, and of how modulation of thalamocortical circuits by arousal and sleep regulatory neuronal systems has an impact on key features of the sleep EEG.

Thalamocortical circuits exhibit two fundamentally different modes of operation across the sleep–waking cycle: a state of tonic activation, or desynchrony, during waking and REM sleep, and a state of rhythmic, synchronized activity that is characteristic of NREM sleep.[54] The two functional modes of thalamocortical activity are evident at the level of single neurons. During waking and REM sleep, thalamocortical neurons exhibit tonic firing of single action potentials (Fig. 7-5A) that are modulated by the levels of excitatory input from thalamic afferents, including specific sensory afferents.[54,55] During NREM sleep, relay neurons discharge in high-frequency bursts of action potentials, followed by long pauses (see Fig. 7-5B).

These two modes of action potential generation reflect the expression of intrinsic properties of thalamocortical neurons, and a specialized, voltage-sensitive Ca^{2+} current plays a critical role.[56] This Ca^{2+} current, known as the low-threshold or transient Ca^{2+} current (I_t), is inactivated (nonfunctional) when the membrane potential of thalamic relay neurons is relatively depolarized (less negative than –65 mV). Thus, when depolarizing input is delivered to a relay neuron that is resting at this level of membrane polarization, the cell responds with tonic single-spike firing (see Fig. 7-5C). When relay neurons are hyperpolarized (membrane potential more negative than –65 mV), I_t becomes activated, and depolarizing input evokes a slow Ca^{2+}-mediated depolarization (100 to 200 msec in duration) that is crowned by a burst of three to eight fast Na^+ action potentials (see Fig. 7-5D). There is a pause in the generation of fast action potentials after the burst, because

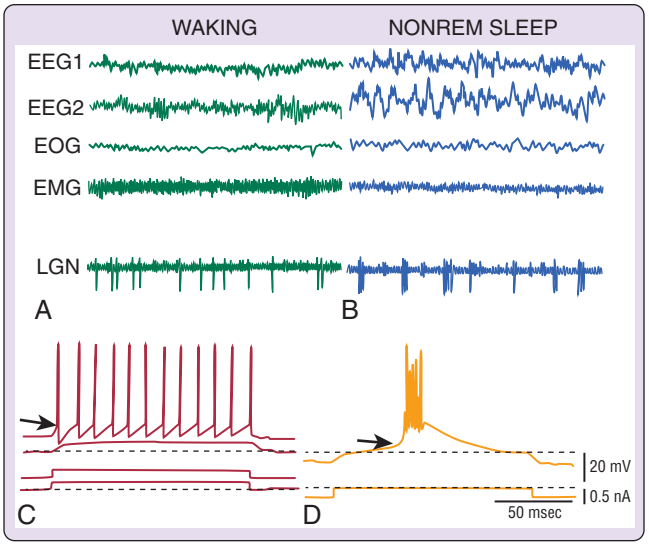

Figure 7-5 Thalamic neurons exhibit distinct patterns of action potential generation during waking/REM sleep and during NREM sleep. **A** and **B,** Typical extracellularly recorded discharge patterns of a neuron in the cat lateral geniculate nucleus during waking and NREM sleep. Note the change from tonic, single-spike firing during waking (**A**), to high-frequency-burst firing during NREM sleep (**B**). Tonic versus burst firing reflects intrinsic, voltage-dependent properties of thalamic neurons, and it can be recorded in neurons from isolated slices of thalamus. **C** and **D**, In vitro intracellular recordings of a relay neuron from guinea pig thalamus. In **C,** note direct current (DC) injections (*1* and *2*) and recordings of intracellular voltage (*3* and *4*). Spontaneous resting potential for the cell is indicated by the *dashed line.* A depolarizing current step (*1*) delivered at resting potential (> –65 mV) evokes a tonic depolarizing response in the neuron (*3*) that is subthreshold for action potential generation. When membrane potential is rendered more positive by DC injection, the same depolarizing step (*2*) evokes tonic generation of fast action potentials (*4*) that persist for the duration of the depolarizing pulse. In **D,** the neuron has been hyperpolarized below resting potential (< –65 mV) by negative DC injection, and the low threshold Ca^{2+} current (I_t) is activated. When a depolarizing pulse is applied on the background of hyperpolarization, a slow Ca^{2+} spike is evoked *(arrow),* and it is crowned by a high-frequency burst of fast Na^+ action potentials. I_t is inactivated in response to the Ca^{2+}-mediated depolarization, and membrane potential sags toward the hyperpolarized level despite the continuance of the depolarizing current step. EOG, electrooculogram; LGN, lateral geniculate nucleus. (**A** and **B** modified from McCarley RW, Benoit O, Barrionuevo G. Lateral geniculate nucleus unitary discharge during sleep and waking: state- and rate-specific effects. J Neurophysiol 1983;50:798-818; **C** and **D** modified from Jouvet M. Neurophysiology of the states of sleep. Physiol Rev 1967;47:117-177.)

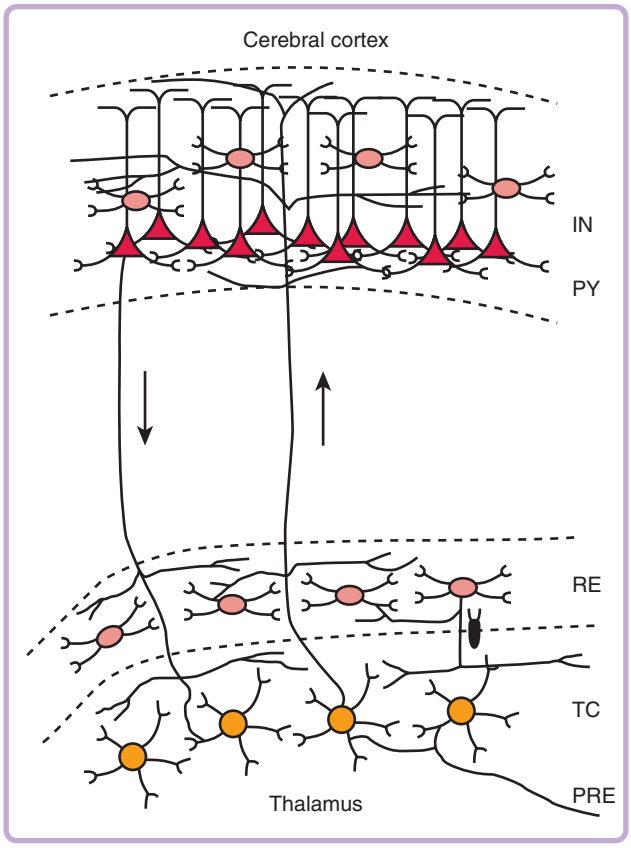

Figure 7-6 Schematic representation of thalamic and cortical cell types involved in the generation of sleep EEG rhythms, and of the synaptic connectivity among the cell types. Four cell types are shown: thalamocortical relay (TC) cells, thalamic reticular (RE) neurons, cortical pyramidal (PY) cells, and cortical interneurons (IN). TC cells receive excitatory inputs from prethalamic afferent fibers (PRE) arising from specific sensory systems and from cholinergic and monoaminergic arousal systems located in the brainstem and posterior hypothalamus. Activity in sensory systems is relayed to the appropriate cortical area by ascending thalamocortical axons *(up arrow).* TC neurons also send an axon collateral that makes synaptic contact with RE neurons. RE neurons are GABAergic, and they send an inhibitory projection back to TC neurons. Corticothalamic feedback is mediated by layer VI, PY neurons that project back to the same relay neurons from which they derive thalamic input, and they send an axon collateral to RE neurons. Corticothalamic projections are excitatory on both RE and TC cells, but cortical stimulation can evoke net inhibitory effects on TC neurons because of activation of GABAergic RE neurons. (From Destexhe A, Sejnowski TJ. Interactions between membrane conductances underlying thalamocortical slow-wave oscillations. Physiol Rev 2003;83:1401-1453.)

I_t is self-inactivated by the Ca^{2+}-mediated depolarization, and membrane potential falls below the threshold for action potential generation and back to the resting, hyperpolarized state (see Fig. 7-5D). Thus, the properties of I_t equip thalamocortical neurons with the ability to generate action potentials in two different modes: (1) tonic firing when stimulated from a relatively depolarized resting state, and (2) burst-pause firing from a hyperpolarized resting state.[54,55]

The major NREM sleep EEG rhythms, spindles, delta waves, and slow oscillations all arise through a combination of intrinsic neuronal properties (e.g., I_t) and the synaptic organization of cortical and thalamic circuits.[57] A schematic of the core circuitry responsible for the generation of sleep rhythms in the EEG is shown in Figure 7-6. Thalamocortical relay neurons receive excitatory input from sensory neurons and from several of the brainstem arousal systems that function to promote depolarization and tonic firing in relay cells during waking. During waking, this excitation is faithfully conveyed by ascending thalamocortical axons to the functionally relevant area of

cortex for processing and integration. Thalamic relay neurons also send a collateral projection to the adjacent portion of the thalamic reticular nucleus (RE), which is a thin band of neurons that surrounds most thalamic relay nuclei. RE neurons are GABAergic and they send an inhibitory projection back to the relay neurons. These reciprocal connections between relay and RE neurons are thought to be important for aspects of waking thalamic function.

The final critical piece of the basic circuitry for consideration is the feedback projection from layer VI pyramidal cells in cortex to both thalamic relay neurons and RE neurons. Corticothalamic projections are topographically organized, so that each cortical column has connectivity with the same relay neurons from which they derive thalamic inputs, and with the corresponding sector of the RE. In this anatomic/functional scheme, note the central location of RE neurons, which receive copies of thalamocortical and corticothalamic activity and sends an inhibitory projection back to the relay neurons. Although corticothalamic projections have excitatory effects on their postsynaptic targets in the thalamus, corticothalamic inputs to the RE are so powerful that the net response evoked in relay neurons by cortical stimulation is often inhibition.[57]

Sleep Spindles

In humans, EEG spindles are waxing and waning, nearly sinusoidal waves with a frequency profile of 10 to 15 Hz. Spindles are generated in the thalamus, as evidenced by the fact that thalamectomy eliminates spindles in the sleep EEG.[4] At the level of the thalamus, spindles are generated by an interplay between neurons in the RE and the relay nuclei.[54,55,57] RE neurons also possess I_t calcium channels and exhibit high-frequency-burst firing from a hyperpolarized background. A high-frequency burst in RE neurons will produce strong inhibitory postsynaptic potentials (IPSPs) in relay neurons that are followed by rebound slow Ca^{2+} spikes and a high-frequency burst. This burst of firing in the relay neuron is conveyed back to the RE, evoking an excitatory postsynaptic potential (EPSP) that triggers a calcium spike and a burst in RE neurons. In thalamic slices that preserve connectivity between the RE and the adjacent relay nuclei, this disynaptic circuit can generate spontaneous spindle-like oscillations that can propagate across the slice.[53] In the intact brain, the spindle oscillation in the thalamus is conveyed to the cortex by the pattern of burst firing in thalamic relay neurons.[54,57,58] However, relay of specific sensory information through the thalamus to the cortex is severely compromised during spindle oscillations (Fig. 7-7), because of the combination of (1) disfacilitation of relay neurons resulting from loss of excitatory input from arousal systems and (2) the rhythmic IPSPs evoked by RE input. This sensory deafferentation of the cortex is believed to play an important role in maintaining NREM sleep continuity.

Although the spindle oscillation originates in the thalamus, cortical feedback to the thalamus and cortico-cortical connections are important in synchronizing the occurrence of spindles over widespread thalamic and cortical areas.[57,58] Phasic activation of layer VI pyramidal neurons excites neurons in the RE and synchronizes IPSP–rebound burst sequences in thalamic relay neurons with cortical activity.

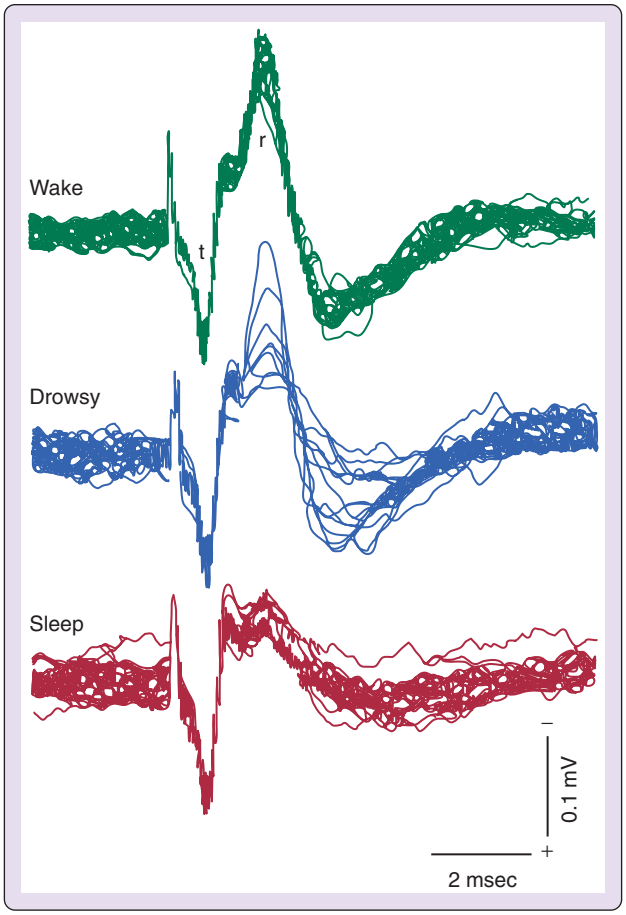

Figure 7-7 Blockade of synaptic transmission in the thalamus during drowsiness and sleep in the cat. Field potentials are evoked in the ventrolateral thalamus by stimulation of the cerebellothalamic pathway. The evoked response consists of a presynaptic volley (t) and the postsynaptic response (r). Note the variability of the postsynaptic response during drowsiness compared with during waking, and the complete absence of a postsynaptic response during sleep. (Modified from Steriade M, Llinás RR. The functional states of the thalamus and the associated neuronal interplay. Physiol Rev 1988;68:649-742.)

Delta Waves

The delta oscillation of NREM sleep appears to have both cortical and thalamic components. This is evidenced by the fact that cortical delta activity in the 1- to 4-Hz range persists after complete thalamectomy[4] and by the demonstration that isolated thalamic relay neurons can generate a spontaneous clocklike delta oscillation resulting from the interplay of I_t and a hyperpolarization-activated cation current, known as the h-current.[55] In the intact, sleeping brain, both sources of delta oscillation contribute to the frequency content of the cortical EEG. As discussed for spindles, corticothalamic and cortico-cortical connections function to synchronize delta oscillations over widespread cortical areas.

Slow Oscillations

Slow oscillations (less than 1 Hz) are a key aspect of the sleep EEG because they function to coordinate the occurrence of other synchronous EEG events (e.g., delta waves,

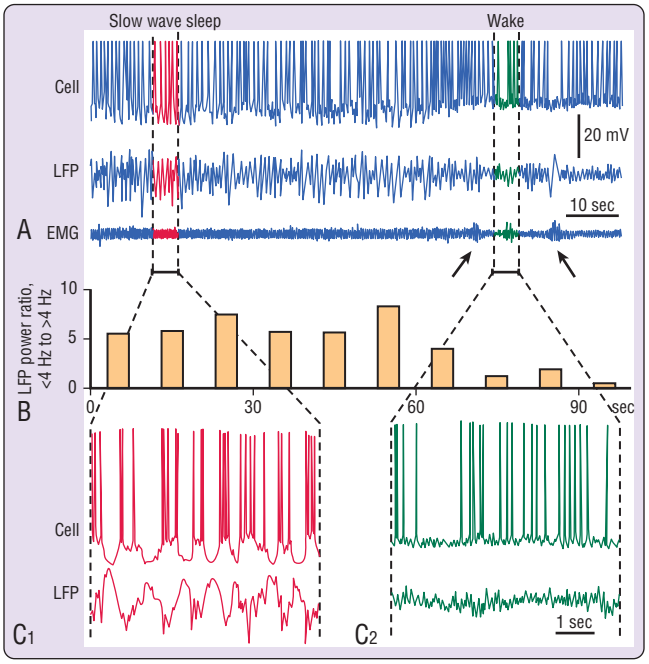

Figure 7-8 Slow oscillations in local cortical field potentials (LFP) and in the membrane potential of a cortical neuron during NREM sleep. **A,** Simultaneous intracellular, LFP, and EMG recording during sleep and wakefulness. the animal is in NREM sleep at the beginning of the recording with a transition to waking after about 70 seconds (*arrows* indicate EMG activation). Action potentials are truncated in the intracellular recording. **B,** Higher levels of delta power in the LFP are present during NREM sleep than during waking. Plotted are 10-second bins of the ratio of spectral power (<4 Hz/>4 Hz) recorded in the LFP. **C,** Intracellular activity and LFP recording from (**A**) shown at expanded timescale. Note clear fluctuations of the membrane potential between depolarized (up) and hyperpolarized (down) states during NREM sleep in association with the slow oscillation (<1 Hz) in the LFP. During wakefulness, cell is tonically depolarized and no sustained episodes of hyperpolarization are present. (From Mukovski M, Chauvette S, Timofeev I, Volgushev M. Detection of active and silent states in neocortical neurons from the field potential signal during slow-wave sleep. Cereb Cortex 2007;17:400-414.)

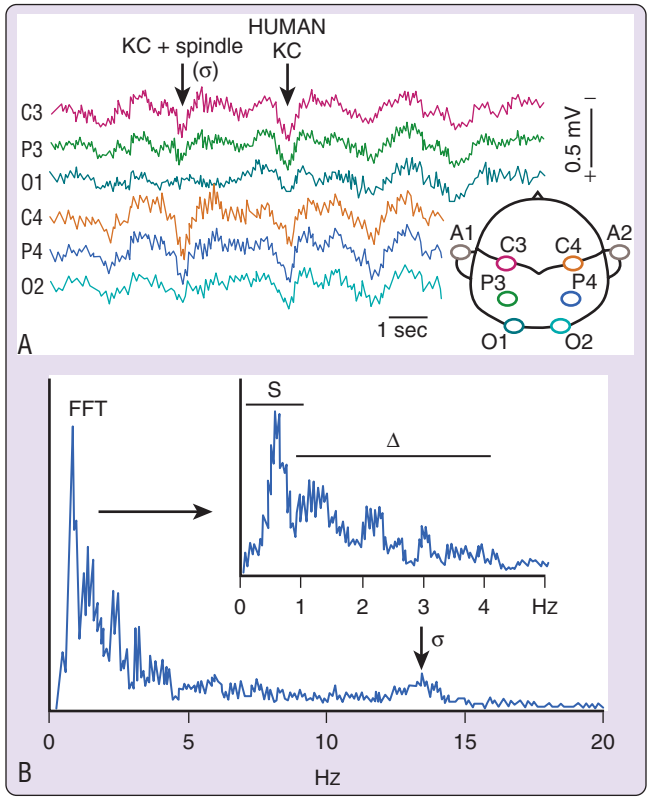

Figure 7-9 The cortical slow oscillation groups other sleep-related EEG events in humans. **A,** Recordings of human EEG during NREM sleep from three bilateral derivations. Note the synchronous occurrence of K-complexes (KC) and of spindles (σ) at multiple recording sites, and the regular recurrence of KC/delta waves with a periodicity of less than 1 Hz. **B,** Spectral decomposition from the C3 lead showing peaks in spectral power in the spindle frequency range (12 to 15 Hz), in the delta power range (1 to 4 Hz), and in the slow oscillation range (<1 Hz). (Modified from Sanchez-Vives MV, McCormick DA. Cellular and network mechanisms of rhythmic recurrent activity in neocortex. Nat Neurosci 2000;3:1027-1034.)

spindles, and K-complexes). Slow oscillations are entirely of cortical origin. They are absent from the thalamus in decorticate animals and are present in the cortex after thalamectomy as well as in isolated cortical slices.[57] Underneath the slow oscillations, fluctuations occur between two states of activity in nearly all cortical neurons (Figs. 7-8 and 7-9).[59,60] The "up" state is characterized by depolarization and generation of trains of action potentials. The up state occurs simultaneously in all cell types, including interneurons, and both fast IPSPs and EPSPs are characteristic of cortical neuronal activity during this state. Up states are followed by a prolonged period of hyperpolarization and quiescence, referred to as a "down" state (see Fig. 7-8).

Generation of up states occurs through recurrent excitation in local cortical circuits and depends on excitatory transmission through α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and NMDA receptors.[61] Transitions from up to down states involve a combination of activation of outward K^+ currents and disfacilitation resulting from depression of excitatory synapses.[61,62] Discharge of layer IV corticothalamic projection neurons during up phases can synchronize IPSPs in thalamic relay neurons through activation of RE neurons, causing the expression of EEG spindles and delta activity of thalamic origin on the background of the slow oscillation.[58] Slow oscillations organize the synchronization and propagation of cortical delta activity through cortico-cortical connections. Frequency spectra of the human NREM sleep EEG reflect this dynamic, with prominent spectral peaks in the delta (1 to 4 Hz) and slow oscillation (less than 1 Hz) frequency ranges (see Fig. 7-9).[63]

By orchestrating the temporal and spatial coherence of rhythmic oscillations in thalamocortical circuits, slow oscillations are thought to be important in promoting the functional sensory deafferentation of the cortex during sleep, which in turn enhances sleep continuity and sleep depth. The transitions between up and down states in cortical neurons have been hypothesized to underlie changes in synaptic plasticity, or synaptic strength, during sleep, and to contribute to sleep-dependent changes in learning and memory.[64]

Spindle and delta oscillations are blocked by stimulation of the rostral brainstem, which activates cholinergic, monoaminergic, orexinergic, and glutamatergic inputs to the thalamus. Activity of cholinergic and monoaminergic neurons facilitates thalamic *depolarization* through inhibition of potassium channels.[55] Thus, *inhibition* of these arousal systems facilitates hyperpolarization of thalamic neurons, permitting the activation of voltage-dependent membrane currents underlying spindles and slow waves. *The POA sleep-promoting system can control EEG patterns through inhibitory modulation of these arousal systems, as described earlier.*

INTEGRATION OF CIRCADIAN RHYTHMS AND SLEEP

The suprachiasmatic nucleus (SCN) of the POA generates the signals that bring about the circadian patterns of sleep–waking.[65] Direct projections from the SCN to areas of the POA implicated in sleep regulation are sparse, but multisynaptic pathways by which SCN signals can control of sleep-active neurons have been described. Lesions of a primary SCN projection target, the subparaventricular zone (SPVZ), also eliminate circadian rhythms of sleep–waking.[66] The SPVZ projects directly to the MnPN and indirectly to the VLPO, MnPN, and other POA regions through the dorsomedial hypothalamic nucleus (DMH).[67] Lesions of the DMH disrupt the circadian distribution of sleep–waking states in rats. In diurnal animals, activity of SCN neurons could inhibit sleep-promoting neurons during the light phase, or they may facilitate sleep-promoting neurons in the dark phase, with the reciprocal pattern occurring in nocturnal animals, or they may do both.

THE POA, THERMOREGULATION, AND CONTROL OF SLEEP

Several types of evidence support the hypothesis that sleep onset is coupled to body cooling. Depending on ambient conditions, sleep onset evokes heat loss–effector processes such as cutaneous vasodilation and sweating.[68] In humans, sleep onset occurs soon after vasodilation of the hands and feet, which increases heat loss. Sleep in humans and animals is modulated by ambient temperature. Mild to moderate ambient temperature elevation increases coincident sleep as well as subsequent sleep (reviewed in reference 38). An increase in heat production by selective activation of brown adipose tissue using a beta$_3$ adrenoreceptor agonist also increases NREM sleep.[69] Thus, it is reasonable to hypothesize that one function of sleep is body cooling, particularly after increased body warming during wake.

A circadian temperature rhythm is well documented. In humans, sleep normally occurs on the descending phase of the circadian temperature cycle, but sleep onset evokes a further decrease in temperature, even during continuous bed rest (reviewed in reference 38). Self-selected human bedtimes occur at the time of the maximal rate of decline of core body temperature.[70] The association of sleep onset and the circadian temperature rhythm could represent two independent outputs of the circadian oscillator. However, under certain experimental conditions, including short sleep–wake cycles, internal desynchronization, and forced desynchronization, the interactions of the temperature rhythm and sleep propensity can be studied and partially isolated from effects of prior waking (see Chapter 35). Although sleep may occur at any circadian phase, sleep propensity is increased on the late descending phase of the circadian temperature rhythm and is highest when temperature is low. Awakenings tend to occur as temperature increases, even if sleep time is short. This can be interpreted as evidence that the thermoregulatory mechanism that lowers body temperature also promotes sleep.

The coupling of sleep propensity and body cooling is controlled by the POA. The POA is recognized as a thermoregulatory control site on the basis of the effects of local warming and cooling, lesions, local chemical stimulation, and neuronal unit recording studies (reviewed in reference 71). In vivo and in vitro studies have confirmed that the POA contains populations of warm-sensitive and cold-sensitive neurons (WSNs and CSNs), which are identified by changes in neuronal discharge in response to locally applied mild thermal stimuli. Local POA warming promotes NREM sleep and EEG slow-wave activity in cats, rabbits, and rats (reviewed in reference 38). NREM sleep is increased for several hours during sustained POA warming.[72] Local POA warming also increases EEG delta activity in sustained NREM. Because delta EEG activity in sustained sleep is considered to be a measure of sleep drive, this finding supports a hypothesis that sleep drive is modulated by POA thermosensitive neurons. Augmentation of sleep by ambient warming is prevented by coincident local POA cooling.[72] Because POA cooling prevents activations of WSNs, this finding suggests that POA WSN activation mediates the sleep augmentation induced by ambient warming.

Most POA WSNs exhibit increased discharge during NREM sleep compared with waking; most CSNs are wake-active (reviewed in reference 38). Increases in WSN discharge and decreases in CSN discharge anticipate EEG changes at sleep onset by several seconds. Thus, activity of WSNs and CSNs is likely to modulate spontaneous sleep–wake as well as sleep induced by local POA warming. As summarized earlier, POA sleep-active neurons send afferents to putative arousal systems. Local POA warming suppresses the discharge of arousal-related neurons in the PH, DR (5-HT neurons), LH orexin neuronal field, and basal forebrain cholinergic field (Fig. 7-10) (reviewed in reference 38). *These studies demonstrate that functional inhibitory regulation of arousal-regulatory neurons originates in WSNs located in the POA, and they provide a mechanistic explanation for the coupling of sleep propensity to heat loss.*

HIERARCHICAL CONTROL MODEL

As reviewed in the beginning paragraphs of this chapter, there is evidence for the existence of sleep-promoting mechanisms at all levels of the neuraxis, including both forebrain and brainstem. Sleeplike behavior may be generated by an isolated lower brainstem, and sleep is facilitated by midbrain, thalamus, and neocortex. Lesions encompassing the *ventral* pontomesencephalic reticular formation and adjacent ventral structures, also produced severe sustained sleep reductions.[73] Both NREM and REM sleep

were reduced by about 50% a month after such lesions were created. However, a central role for the POA in the orchestration of sleep is supported by a variety of findings. To rationalize these diverse findings, it is useful to consider a hierarchical control concept, such as that applied previously to thermoregulation.[74] In this conceptualization, a fundamental behavior such as rest–activity or sleep–waking is organized at all levels of the neuraxis, just as rest–activity cycles are found in all orders of animals. The POA may act as a master controller, integrating sleep-promoting circuitry with sleep homeostatic processes, other behavioral and hormonal regulatory systems, and circadian signals. The limbic system and neocortex are likely sources of additional controls, related to more complex behavioral contingencies including learning processes, instinctive behaviors, and sensory stimuli. These controls may be conveyed to the hypothalamus through projections from neocortex and limbic system.

SLEEP-PROMOTING NEUROCHEMICAL AGENTS

Conceptions of sleep control based on neuronal circuitry, as outlined earlier, are deficient in that they do not provide explanations for quantitative features of sleep or sleep homeostasis. *The control of neuronal activity by neurochemical mechanisms, including the release of GABA, occurs in a time frame of seconds. Sleep regulation and homeostasis has a time frame of days, not seconds.* In addition, a complete description should account for biological variations in sleep such as the high daily sleep quotas in low-body-weight mammalian species and low quotas in very large species, higher sleep quotas in infants, sleep facilitation after body heating, and increased sleep propensity during acute infection. The investigation of biochemical mechanisms with sustained actions is needed to address these issues. Here we provide a brief overview of this approach, focusing on neurochemical agents with sleep-promoting properties. See Obal and Kreuger[75] for a detailed and complete review of biochemical mechanisms of sleep–wake regulation.

Adenosine

Adenosine is recognized as an inhibitory neuromodulator in the CNS, whose role in sleep is suggested by the potent arousal-producing effects of caffeine, an antagonist of adenosine A_1 receptors. Adenosine and its analogues were found to promote sleep after systemic injection, intracerebroventricular (ICV) administration, and intra-POA microinjection, and particularly after administration by microdialysis in the basal forebrain (reviewed in reference 76). In the basal forebrain and, to a lesser extent, in neocortex, adenosine recovered through microdialysis increased during sustained waking in cats. No increase was found in thalamus, POA, or brainstem sites. Application in the basal forebrain of an antisense oligonucleotide to the adenosine A_1 receptor mRNA, which blocks synthesis of receptor protein, slightly reduced spontaneous sleep, but it strongly reduced rebound after sleep deprivation.[77] Adenosine A_1 agonists delivered via microdialysis adjacent to basal forebrain neurons inhibited wake-active neurons during both waking and sleep.[78] A current hypothesis is that the effects of adenosine on sleep–waking are mediated by basal forebrain cholinergic neurons via A_1 receptors.[76] The proposed basal forebrain cholinergic neuronal site of action is problematic because destruction of these neurons has little effect on sleep.[79] However, adenosine could act at multiple sites. Adenosine A_{2A} receptors are also present in restricted brain regions and seem to mediate some sleep-promoting effects of adenosine.

Brain adenosine levels rise when ATP production is reduced; under these conditions, inhibition of neuronal activity is neuroprotective. It has been proposed that

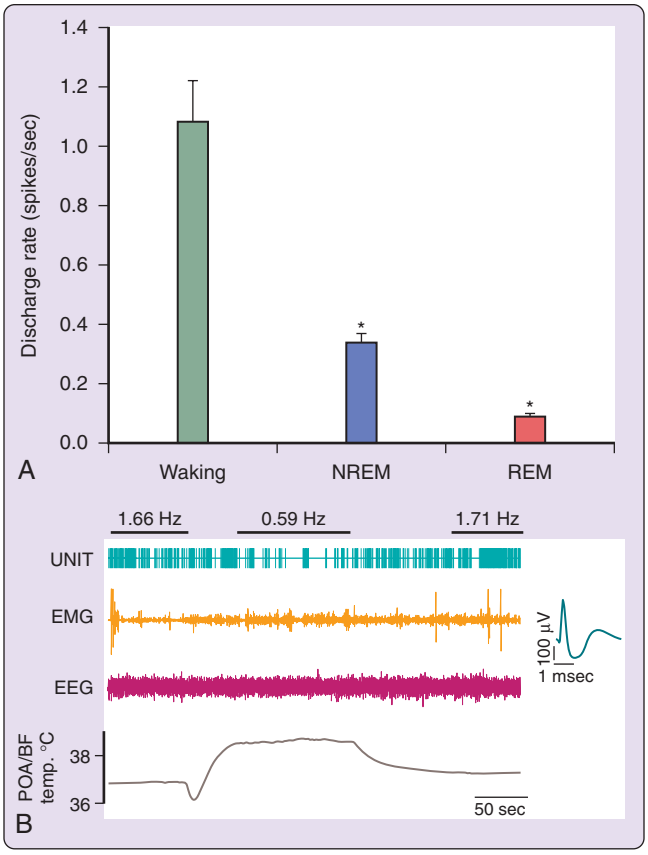

Figure 7-10 Effects of activation of preoptic area (POA) warm-sensitive neurons (WSNs) by local warming on discharge of a putative serotoninergic neuron of the dorsal raphe nucleus (DRN) in the rat. **A,** Pattern of activity of DRN neurons across the sleep–wake cycle. Putative DRN serotoninergic neurons exhibit reduced discharge during NREM compared with waking, and very low discharge in REM sleep. The identification of this pattern of discharge as representing serotoninergic neurons is supported by several types of indirect evidence. **B,** Effects of POA warming on the discharge of a putative serotoninergic REM-off neuron during waking. A POA warming pulse was applied for about 100 seconds *(bottom trace).* During POA warming, the discharge was reduced by 64%, to a typical NREM sleep rate. A waking state was maintained during warning, as shown by electroencephalographic and electromyographic recordings. Increased discharge of POA WSNs during spontaneous NREM sleep is thought to contribute to the concurrent reduction of DRN discharge. The central role of temperature-sensitive neurons in sleep control provides a mechanistic basis for the coupling of sleep and the circadian temperature rhythm (see text). (From Guzman-Marin R, Alam MN, Szymusiak R, et al. Discharge modulation of rat dorsal raphe neurons during sleep and waking: effects of preoptic/basal forebrain warming. Brain Res 2000;875:23-34.)

increased adenosine is a signal of reduced brain energy reserves that develop during waking, and that sleep is induced as an energy-restorative state.[80] With respect to the brain energy restorative concept, some, but not all, studies show reduced cerebral glycogen, an energy-supply substrate, after sleep deprivation.[81] There is no functional evidence that brain energy supply is compromised after sleep deprivation. This is a critical gap in the theory. Of course, adenosine could be a sleep-promoting signal based on functions other than energy supply limitation.

Proinflammatory Cytokines

Several proinflammatory cytokines have sleep-promoting properties. We summarize work on a well-studied and prototypic molecule, interleukin (IL)-1β. When administered intravenously, intraperitoneally, or into the lateral ventricles, IL-1β increases sleep, particularly NREM sleep, (reviewed in reference 82). Basic findings have been confirmed in several species. REM is usually inhibited by IL-1β. Much additional evidence supports a hypothesis that IL-1 modulates spontaneous sleep. Administration of agents that block IL-1 reduce sleep. In rats, IL-1 mRNA is increased in the brain during the light phase, when rat sleep is maximal. Sleep deprivation also increases IL-1 mRNA in brain. On the basis of these studies and others, it has been hypothesized that IL-1 plays a role in spontaneous sleep. Increased sleep associated with peripheral infection may also be mediated by responses to circulating IL-1, either through vagal afferents or via induction of central IL-1 or other hypnogenic signals. POA sleep-active neurons are activated by local application of IL-1, and wake-active neurons are inhibited.[83] IL-1 fulfills many criteria for a sleep-promoting signal, and it is likely to be particularly important in facilitating sleep during infection.

Prostaglandin D_2

Administration of prostaglandin D_2 (PGD_2) by ICV infusion or by microinjection into the POA increases sleep, but the most potent site for administration is the subarachnoid space ventral to the POA and basal forebrain (reviewed in reference 84). Sleep induced by PGD_2 administration is indistinguishable from normal sleep on the basis of EEG analysis. The synthetic enzyme lipocalin-PGD synthase (L-PGDS) is enriched in the arachnoid membrane and choroid plexus, but the receptor (the D-type prostanoid receptor) is localized more locally in the leptomeninges under the basal forebrain and the TMN. Knockout mice for L-PGDS had normal baseline sleep, but, unlike the controls, they had no rebound increase in NREM sleep after sleep deprivation. The PGD_2 concentration was higher in the cerebrospinal fluid during NREM sleep than in waking and was higher in the light phase in the rat, when sleep amounts are high. Sleep deprivation increased the PGD_2 concentration in the cerebrospinal fluid. On this basis, it was proposed that PGD_2 plays a central role in sleep homeostasis. The hypnogenic action of PGD2 is hypothesized to be mediated by adenosine release acting on an adenosine A_{2A} receptor. Administration of A_{2A} antagonists blocked the effects of PGD_2. A functional basis for the role of PGD_2 in sleep homeostasis or its primary localization in the meninges has not been identified.

Growth Hormone–Releasing Hormone

Growth hormone–releasing hormone (GHRH) is known primarily for its role in stimulating the release of growth hormone (GH). A surge in GH release occurs early in the major circadian sleep period in humans and in rats, specifically during the earliest stage 3/4 NREM sleep episodes in humans.[85] GHRH is a peptide with a restricted localization in neurons in the hypothalamic arcuate nucleus and in the adjacent ventromedial and periventricular nuclei. Neurons in the latter location are thought to be the source of projections to the POA and basal forebrain. GHRH promotes NREM sleep after intraventricular, intravenous, intranasal, or intraperitoneal administration, or after direct microinjection into the POA (reviewed in reference 75). Blockade of GHRH by administration of a competitive antagonist or by immunoneutralization reduces baseline sleep and rebound sleep after short-term sleep deprivation. Mutant mice with GHRH signaling abnormalities have lower amounts of NREM sleep. GHRH stimulates cultured GABAergic hypothalamic neurons; these may constitute the GHRH target in the sleep-promoting circuit.[86] It has been suggested that GHRH may elicit sleep onset in conjunction with release of GH as a coordinated process for augmenting protein synthesis and protecting proteins from degradation during the fasting associated with sleep. In brain, protein synthesis increases during sleep, and inhibition of protein synthesis augments sleep.[87] GHRH is proposed to be one element of a multiple-element sleep-promoting system.[75]

Sleep as Detoxification or Protection from Oxidative Stress

Oxidized glutathione (GSSR) was identified as one of four sleep-promoting substances in brain tissue extracted from sleep-deprived rats.[88] Infusion of GSSR or its reduced form (GSH) into the lateral ventricle of rats during the dark phase increases both NREM and REM sleep (reviewed in reference 89). The sleep-enhancing effects of GSSR and GSH could be based on their antioxidant functions (see later) or on their functions as inhibitory regulators of glutamatergic transmission. Glutamatergic stimulation, in excess, has known neurotoxic functions mediated by NMDA receptors. Thus, GSSR and GSH could function to protect the brain against excess glutamatergic stimulation and potential neurotoxicity, inducing sleep through NMDA receptor blockade.

Reactive oxygen species (ROS) include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), nitric oxide (NO), and peroxynitrite ($OONO^-$). ROS are generated during oxidation reactions or reactions between O_2^- and H_2O_2 or NO. ROS are normally reduced by constitutive antioxidants such as GSSR, GSH, and different forms of superoxide dismutase (SOD). GSH is lower in the hypothalamus of rats after 96 hours of sleep deprivation using the platform-over-water method.[90] Five to 11 days of deprivation using the disk-over-water sleep deprivation method reduces Cu/Zn SOD (cytosolic SOD) as well as glutathione peroxidase in the hippocampus and brainstem.[91] These studies suggest that, by reducing antioxidant availability, sleep deprivation can increase the risk of oxidative damage, and that sleep is protective against actions of

ROS. The possibility that sleep deprivation could cause neuronal damage was suggested by a finding that supraoptic nucleus neurons exhibited signs of subcellular damage after exposure to sleep deprivation.[92] Supraoptic nucleus neurons may be sensitive to sleep deprivation because of their high rate of protein synthesis.[92]

What are the signaling molecules related to oxidative stress that increase as a function of sustained wakefulness and can promote sleep? One possible signal is NO production, which can be a response to glutamatergic stimulation, to cytokines and inflammation, and to oxidative stress.[93] Activity of one NO synthetic enzyme, cytosolic nitric oxide synthase (NOS), is increased during the dark phase in rats, most strongly in the hypothalamus (reviewed in reference 94). Intravertebroventricular or IV administration of a NOS inhibitor strongly reduced sleep in rabbits and rats and suppressed NREM sleep response to sleep deprivation. Administration of NO donors increased sleep. NO inhibits oxidative phosphorylation and may stimulate the production of adenosine. NO production could, therefore, be a mediator of several sleep factors.

ROS may play a role in the pathology associated with patients with obstructive sleep apnea. These patients exhibit signs of increased oxidative stress, including increased O_2^- production by neutrophils, monocytes, and granulocytes derived from patients (see Chapter 20). They also exhibit elevated levels of proteins, such as vascular endothelial growth factor, that are normally induced by ROS. These changes were reversed by treatment with continuous positive airway pressure. On the basis of these and several additional findings, it has been proposed that the increased cardiovascular disease in patients with obstructive sleep apnea results from oxidative damage to vascular walls. Oxidative stress is hypothesized to play a central role in both vascular disease and neurodegenerative disease.

It is important to note that adenosine, ROS, glutamate, and NO have brief lives in the synaptic space—no more than a few seconds. If these molecules regulate sleep, they must have sustained release, or they may regulate the gene expression to generate sustained downstream effects. These mechanisms are the subject of current investigations.

SUMMARY

There has been rapid progress in the elucidation of the neural circuitry underlying the facilitation of sleep and the orchestration of NREM sleep as well as the facilitation of arousal. We have detailed models that can explain many of the phenomena of sleep–wake. Wake and arousal are facilitated by several chemically distinct neuronal groups, including groups synthesizing and releasing acetylcholine, serotonin, norepinephrine, dopamine, histamine, orexin/hypocretin, and glutamate. These neuronal groups distribute axons and terminal throughout the brain, providing a basis for concurrent changes in physiology associated with arousal. At the center of the sleep-promoting circuitry is the POA of the hypothalamus, confirming a hypothesis that is more than 70 years old. The POA sleep-promoting circuitry has reciprocal inhibitory connections with several arousal-promoting systems. A balance between the activities of sleep-promoting and arousal-promoting neuronal systems would determine sleep–waking state. The circadian clock in the suprachiasmatic nucleus has direct and multisynaptic connections, with POA sleep-promoting neurons providing a mechanistic basis for generation of the daily rhythm of sleep–waking. Both sleep-promoting and arousal-promoting neuronal groups are modulated by a host of processes, including sensory, autonomic, endocrine, metabolic, and behavioral influences, accounting for the sensitivity of sleep to a wide range of centrally acting drugs and behavioral manipulations.

The long-term regulation of sleep—sleep homeostasis—is the subject of competing and still incomplete hypotheses. Long-term sleep homeostasis may reflect the actions of several neurochemical processes that express "sleep factors." Some of these sleep factors, including adenosine, PGD_2, IL-1β, and GHRH, are known to act directly on the POA or adjacent basal forebrain neuronal targets. Sleep factors have been linked to distinct functional models of sleep homeostasis, including brain energy supply (adenosine), control of protein synthesis (GHRH), brain cell group "use" or temperature elevation (IL-1), or protection against oxidative or glutamatergic stress (NO, antioxidant enzymes). All of these factors may be involved in sleep homeostasis, but the relative importance of each factor for daily sleep is not established.

❖ Clinical Pearls

Sleep is reduced by a wide variety of localized brain lesions. This may account for the association of insomnia with a variety of neuropathologies. Degeneration of the hypothalamic wake-promoting cell type that expresses the neuropeptide orexin is known to underlie narcolepsy.

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REM Sleep*

Jerome M. Siegel

Chapter 8

Abstract

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

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

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

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

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

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

CHARACTERISTICS OF REM SLEEP

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

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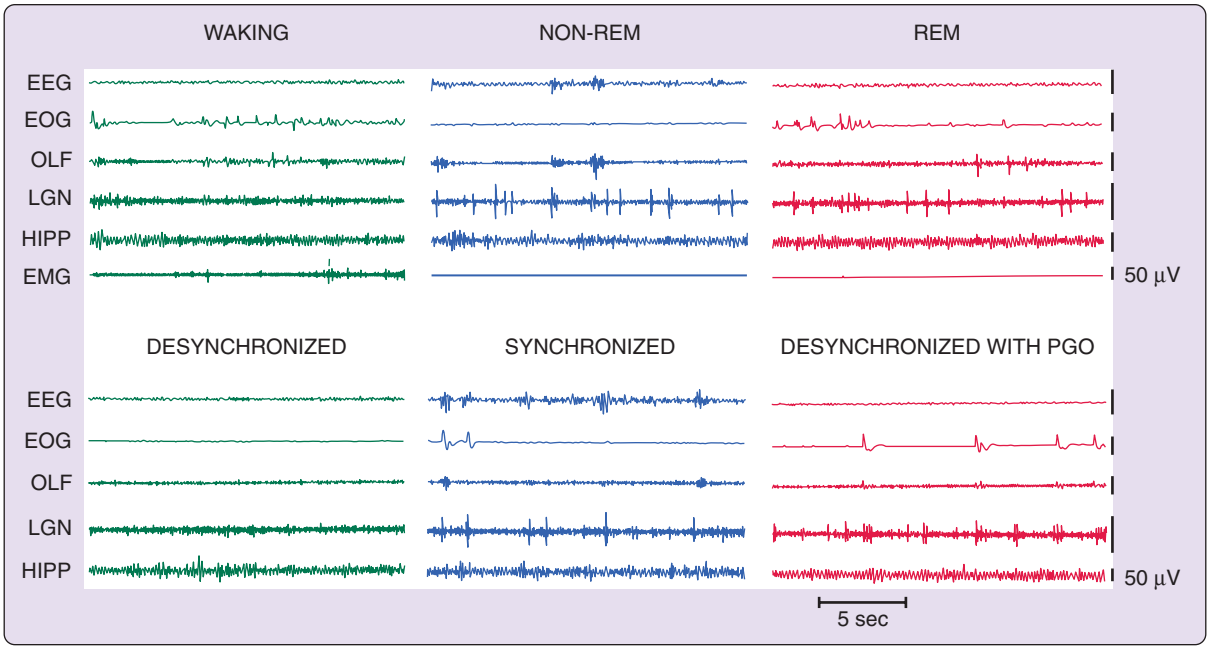

Figure 8-1 *Top,* Polygraph tracings of states seen in the intact cat. *Bottom,* States seen in the forebrain 4 days after transection at the pontomedullary junction. EEG, sensorimotor electroencephalogram; EMG, dorsal neck electromyogram; EOG, electrooculogram; HIPP, hippocampus; LGN, lateral geniculate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

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

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

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

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

REM GENERATION MECHANISMS

Technical Considerations

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Observation of Neuronal Activity

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

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

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

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

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

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

Summary

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

Transection Studies

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

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

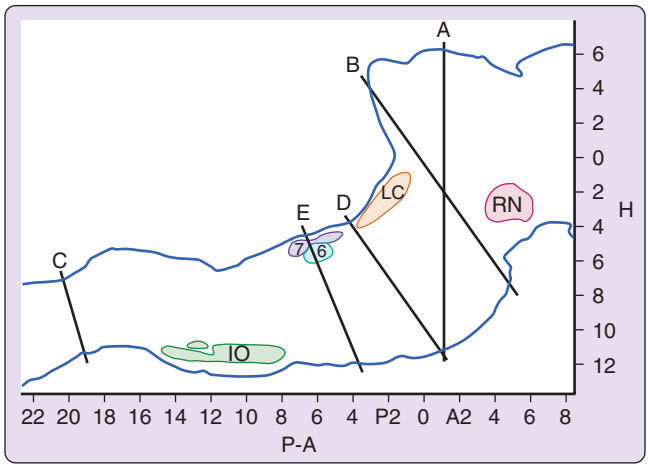

Figure 8-2 Outline of a sagittal section of the brainstem of the cat drawn from level L = 1.6 of the Berman atlas, indicating the level of key brainstem transection studies. H (horizontal) and P-A (anteroposterior) scales are drawn from the atlas. IO, inferior olive; LC, locus coeruleus; RN, red nucleus;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

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

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

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

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

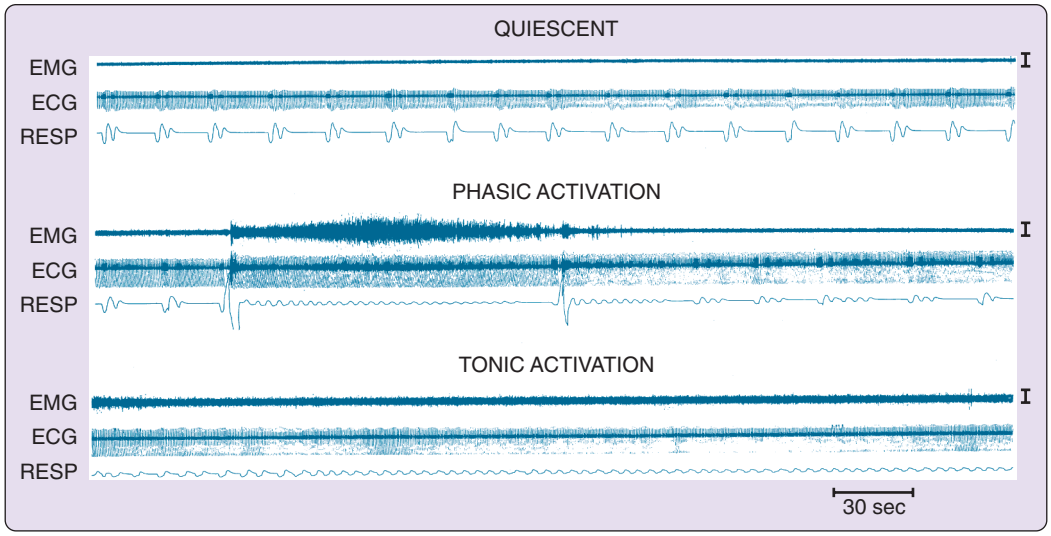

Figure 8-3 States seen in the chronic medullary cat. Note the absence of periods of atonia. Calibration, 50 μV. RESP, thoracic strain gauge measuring respiration. EMG, electromyogram, ECG, electrocardiogram. (From Siegel JM, Tomaszewski KS, Nienhuis R. Behavioral states in the chronic medullary and mid-pontine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

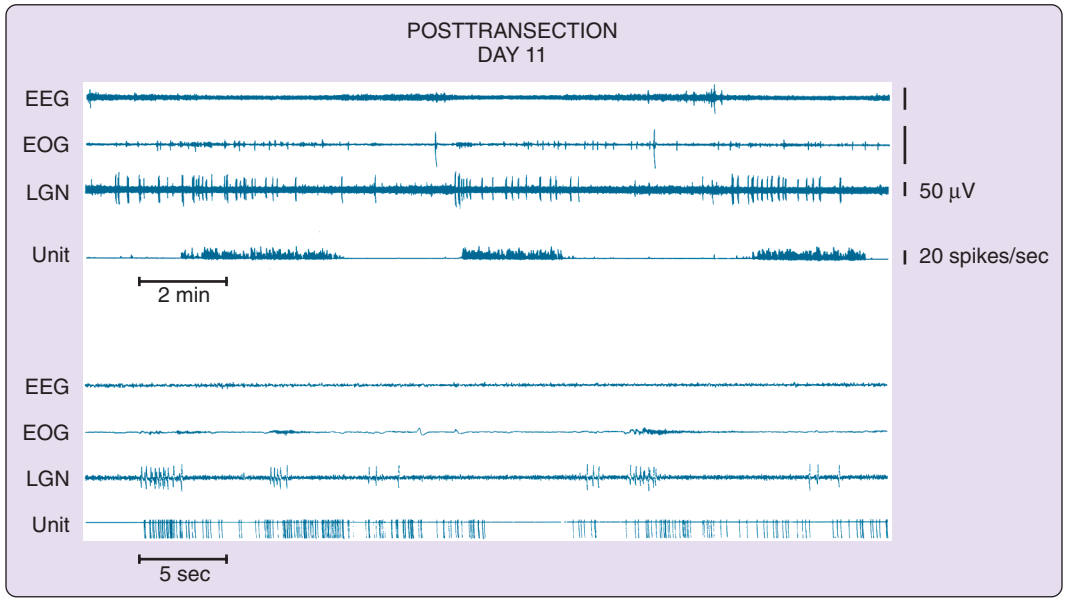

Figure 8-4 Midbrain unit: EEG, electrooculographic (EOG), and lateral geniculate nucleus (LGN) activity rostral to chronic transections at the pontomedullary junction. *Upper:* The unit channel displays the output of an integrating digital counter resetting at 1-second intervals. *Lower:* One pulse is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

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

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

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

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

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

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

Localized Lesion Studies

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

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

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

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

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

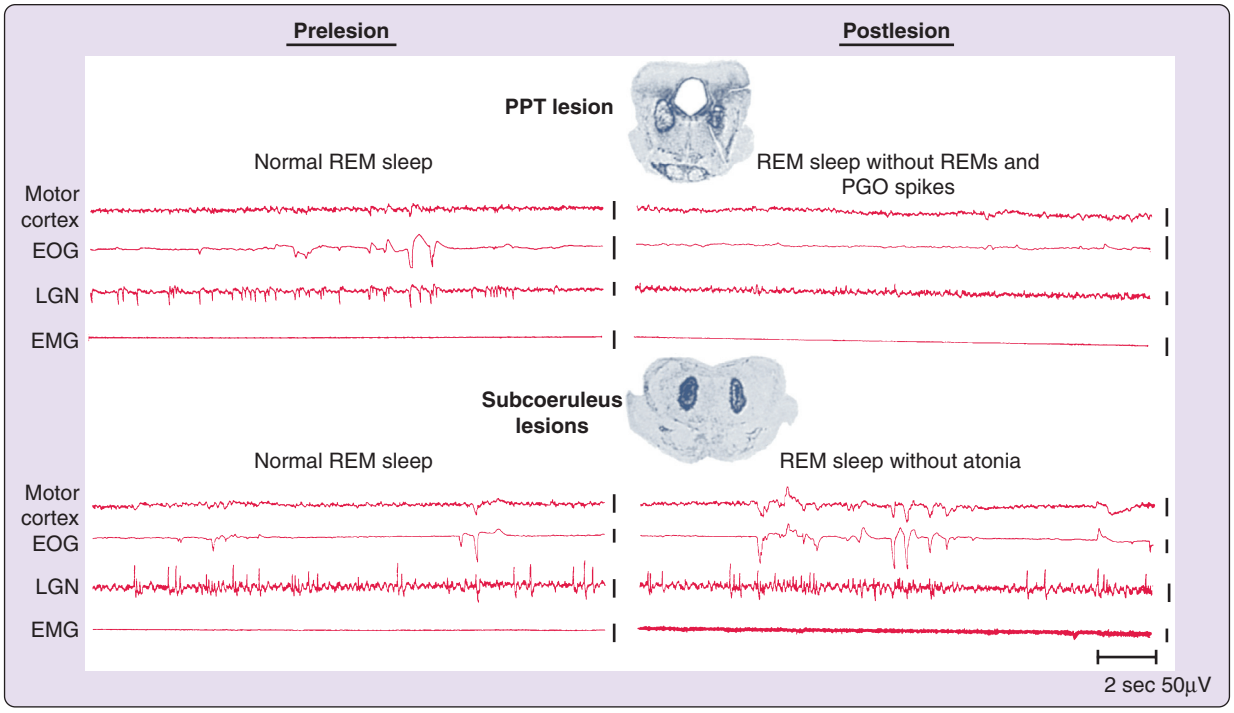

Figure 8-5 Twenty-second polygraph tracings of REM sleep before and after lesions, together with a coronal section through the center of the pontine lesions. EEG voltage reduction of REM sleep (recorded from motor cortex) was present after both lesions. *Top,* Radiofrequency lesions of the pedunculopontine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

Stimulation Studies

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

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

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

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

Neuronal Activity, Transmitter Release

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

Medial Brainstem Reticular Formation

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

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

Cholinergic Cell Groups

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

Cells with Activity Selective for REM Sleep

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

Monoamine-Containing Cells

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

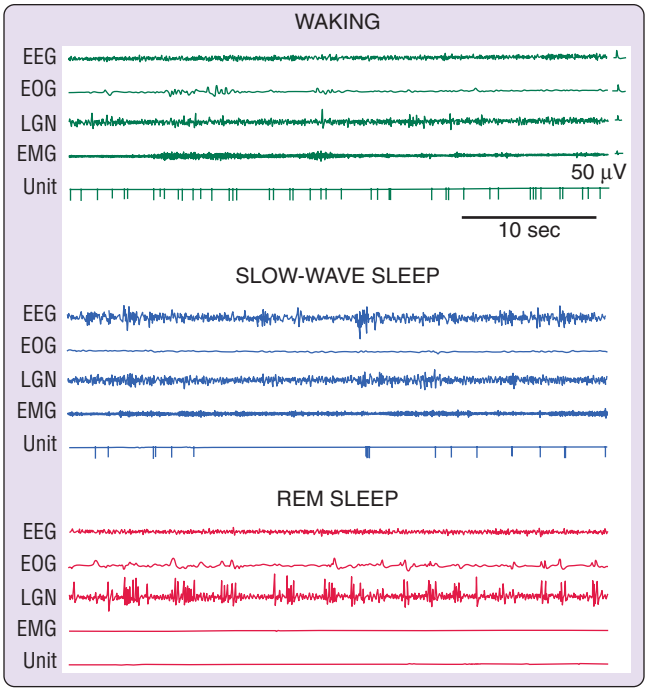

Figure 8-6 Activity of an REM sleep-off cell recorded in the locus coeruleus. EEG, sensorimotor electroencephalogram; EOG, electro-oculogram; LGN lateral geniculate activity; EMG, electromyogram; Unit, pulses triggered by locus coeruleus cell.

Other Cholinergic Cells in Lateral Pontine Regions

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

Fos Labeling

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

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

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

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

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

CONTROL OF MUSCLE TONE

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

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

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

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

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

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

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

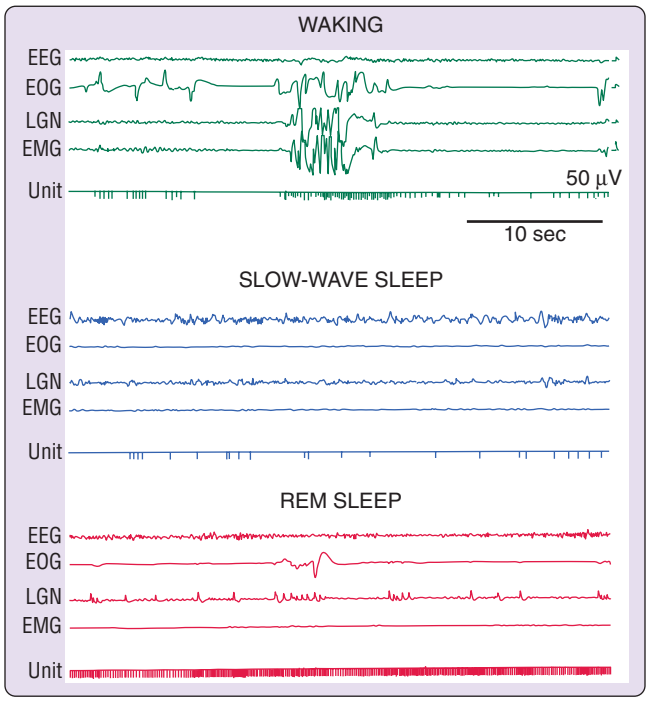

Figure 8-7 Activity of medullary REM sleep-on cell. Note the tonic activity during REM sleep. In waking, activity is generally absent even during vigorous movement. However, some activity is seen during movements involving head lowering and postural relaxation. EEG, electroencephalogram; EOG, electrooculogram;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

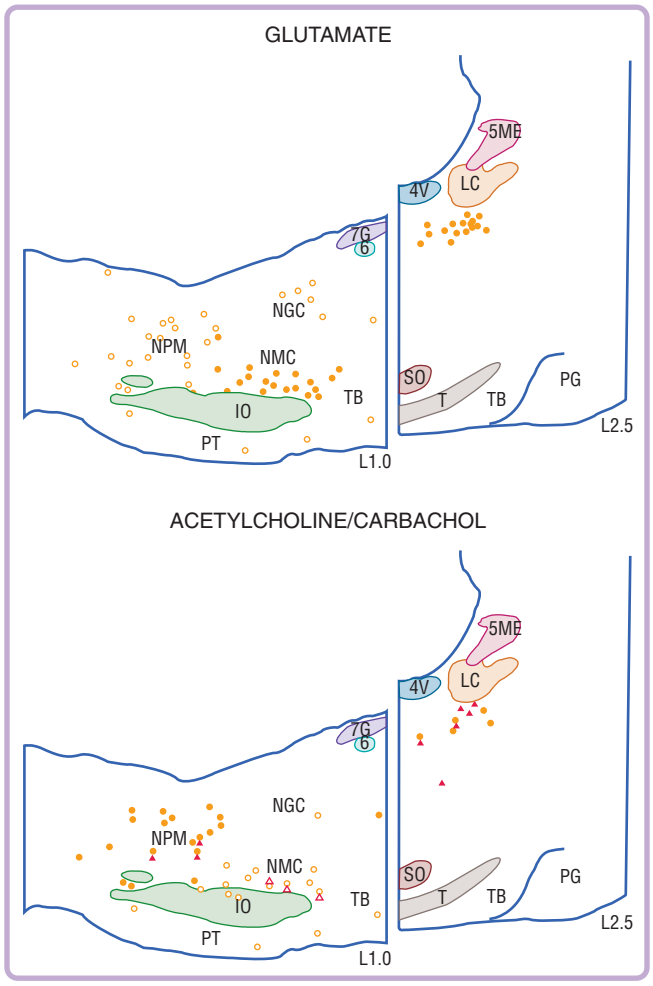

Figure 8-8 Sagittal map of pontomedullary inhibitory areas. Electrical stimulation produced atonia at all the points mapped. All electrically defined inhibitory sites were microinjected with glutamate or cholinergic agonists. *Filled symbols* represent points at which microinjections decreased muscle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

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

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

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

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

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

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

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

NARCOLEPSY AND HYPOCRETIN

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

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

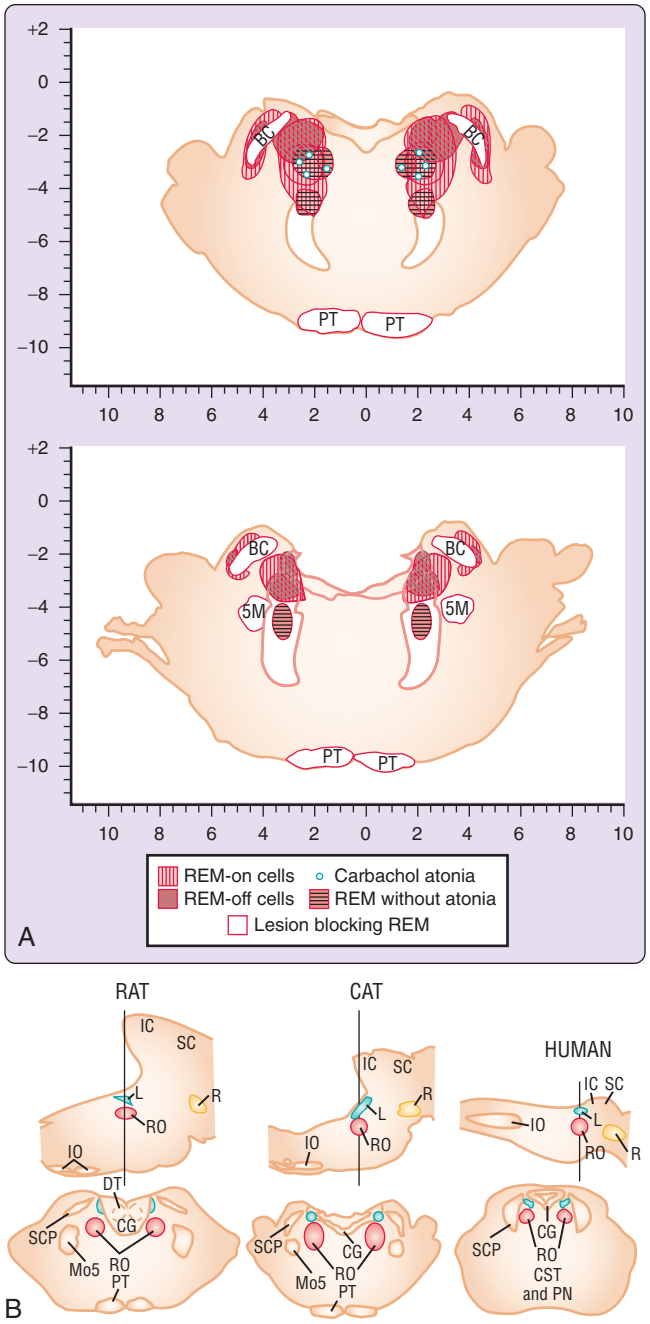

Figure 8-9 A, Anatomic relationship of REM sleep-on and sleep-off cells, carbachol-induced atonia sites, lesions blocking atonia but not preventing REM sleep, and lesions completely blocking REM sleep. **B,** Anatomic locations of REM on areas in cats, rat, and projected location in human, in sagittal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

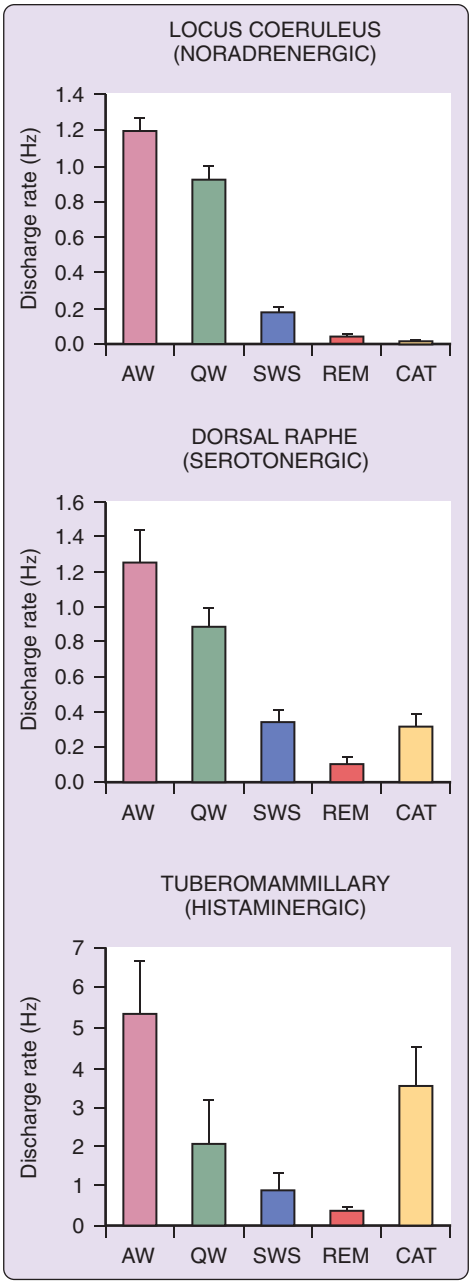

Figure 8-10 Comparison of mean discharge rates in sleep-waking states and cataplexy of REM-off cells recorded from three brain regions. Posterior hypothalamic histaminergic neurons remain active, whereas dorsal raphe serotonergic neurons reduced discharge, and locus coeruleus noradrenergic neurons cease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

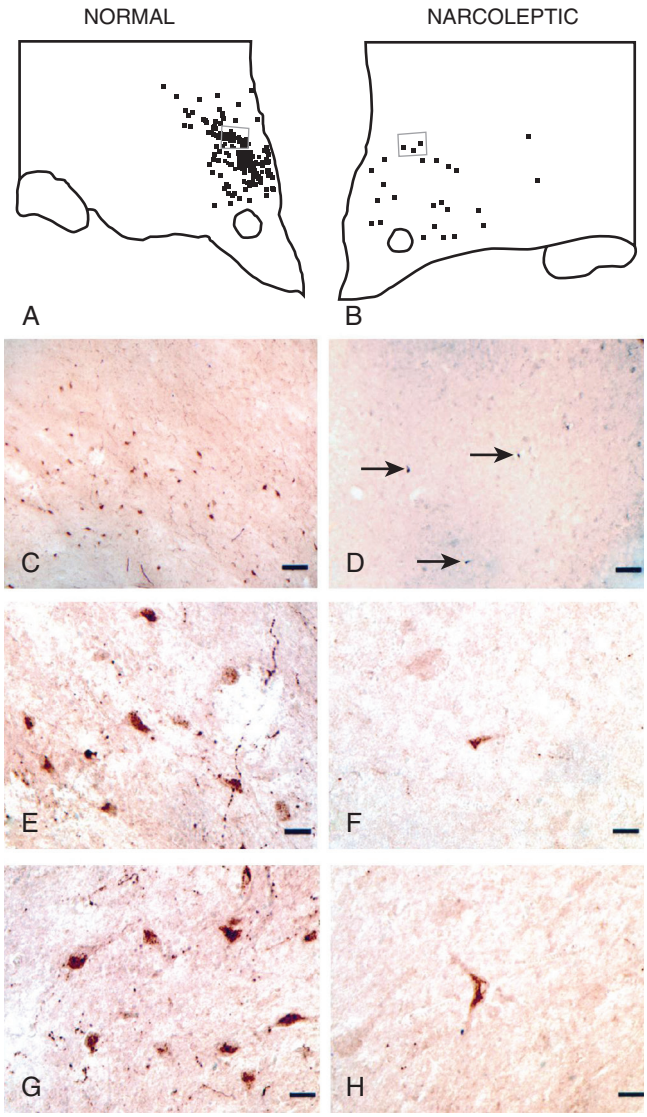

Figure 8-11 Loss of hypocretin cells in human narcolepsy. Distribution of cells in perifornical and dorsomedial hypothalamic regions of normal and narcoleptic humans. (From Thannickal TC, Moore RY, Nienhuis R, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000;27:469-474.)

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

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

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

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

THE FUNCTIONS OF REM SLEEP

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

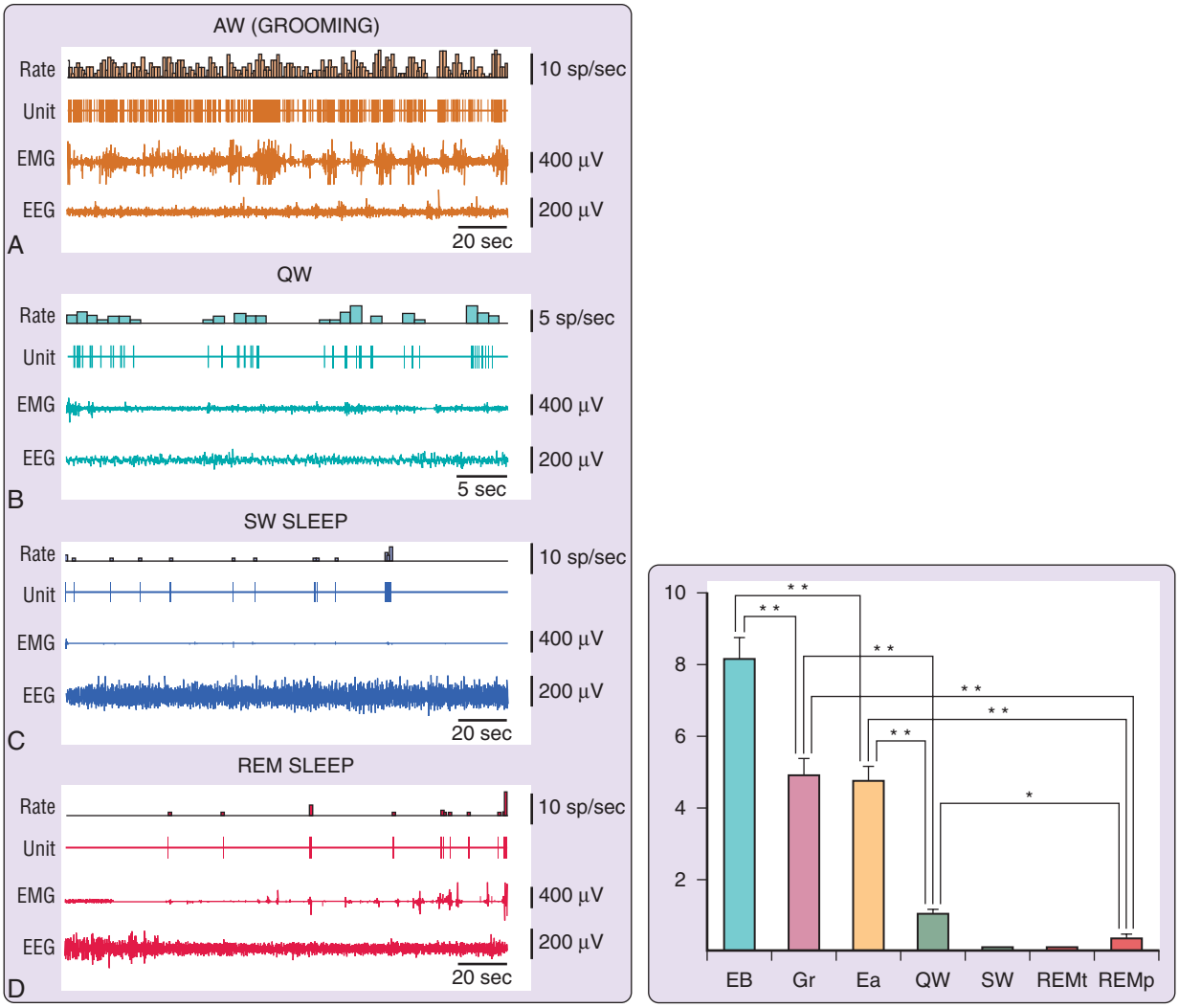

Figure 8-12 Firing rate of hypocretin cells in waking and sleep behaviors in freely moving rats. *Left:* The discharge pattern of a representative hypocretin neuron across the sleep-waking cycle in the freely moving rat. **A,** High firing rates are seen during AW (active waking—grooming). **B,** Reduced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

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

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

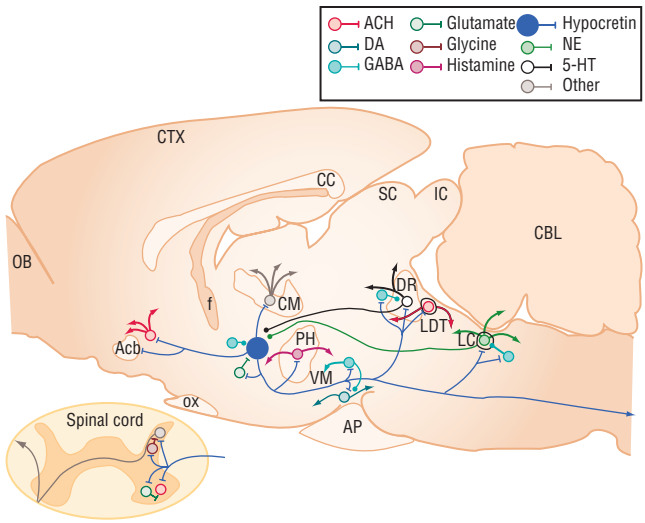

Figure 8-13 Major identified synaptic interactions of hypocretin neurons. *Lines terminated by perpendicular lines* denote excitation; *circular terminations* indicate inhibition. Acb, nucleus accumbens; ACH, acetylcholine; AP, anterior pituitary; CBL, cerebellum; CC, corpus callosum; CM, centromedian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

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

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

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

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

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

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

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

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

❖ Clinical Pearls

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

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Phylogeny of Sleep Regulation

Irene Tobler

Chapter 9

Abstract

It is remarkable that the function of sleep is still unknown, despite the general acceptance that sleep leads to some form of recuperation. Making use of the diversity of animals in the investigation of sleep or sleeplike behavior is likely to yield insights into its origins and functional significance. In particular, representatives of different evolutionary stages could reveal at which stage sleep evolved and became necessary for survival. Tracing the evolution of sleep to its origins is a powerful approach that can provide clues to its mechanisms and functions. Comparative studies of sleep at different levels of its evolution centered on the presence of sleep and its stages in the higher vertebrates (e.g., mammals and birds), and less attention was given to the possibility that sleeplike states are present also in the lower vertebrates, reptiles, amphibians, and fish. The limitations were set by the narrow electrophysiologic definition of vigilance states and the behavioral correlates derived from mammals and were applied to nonmammalian species. After sleep deprivation in mammals, there is typically a compensatory rebound. This widely occurring phenomenon led to the proposal that the homeostatic aspect of sleep regulation is an essential property of sleep and should be included in a broader definition of sleep.[1] This property sets it apart from the chronobiological definition of rest, with the important consequence that a relatively constant quota of sleep can be fulfilled by the interaction between sleep duration and intensity. This flexibility is especially important when animals are faced with situations in which the opportunity to sleep is limited.

Sleep in mammals is finely regulated: a constant daily quota is maintained through a balance of sleep duration and sleep intensity, a phenomenon that was termed *sleep homeostasis*. The main measure for sleep intensity is the amount of EEG slow wave activity (SWA; defined as EEG waves in the frequencies between 0.5 and 4 Hz, also referred to as delta activity) within non–rapid eye movement (NREM) sleep. Additional measures such as arousal threshold, sleep continuity, motor activity, and heart rate also are useful indicators of sleep homeostasis. The regulatory property of sleep becomes evident when sleep pressure is enhanced, such as by sleep deprivation, an intervention that is applicable to many diverse species. Loss of sleep is expected to activate compensatory mechanisms. Comparative data addressing the presence of regulatory mechanisms in evolution indicate that a need for sleep has evolved. This need is derived from the increase in compensatory variables. An important condition is that compensation occurs as a function of the duration of waking and its dissipation during sleep. The regulatory property of sleep distinguishes it from merely rest, because its intensity component surpasses the constraints of the ubiquitous circadian rest–activity rhythm. This feature may be the essence of an adaptive value for sleep.

Birds, perhaps because they are homeotherms, have NREM-REM sleep cycles very similar to those of mammals and also show compensatory mechanisms, including an increase in EEG sleep-intensity variables, when deprived of sleep. Species belonging to the lower vertebrates (reptiles and fish) and invertebrates (e.g., scorpions, cockroaches, and bees, with the exception of *Drosophila melanogaster*, where brain activity changes correlate with behavior) do not meet the electrophysiology criteria for the definition of sleep. Nevertheless, sleep deprivation in these species does elicit compensatory mechanisms. These evolutionarily simpler animals are useful for investigating basic mechanisms underlying compensatory activities at the molecular, neuroanatomic, and genetic level.

Extensive studies in fruit flies convincingly show that their sleep exhibits many of the properties of mammalian sleep, demonstrating that sleep is not a phenomenon unique to the most highly evolved vertebrates—the homoeothermic mammals and birds—but is present also, in simpler manifestations, in arthropods. *Drosophila* is being used as a model organism to investigate the molecular regulation of vigilance states. Genes regulating their sleep have been identified, and these genes might have a similar function in more evolved species. There are indications suggesting sleeplike features in crayfish, octopus, and *Caenorhabditis elegans*, but its existence in these species and yet in lower vertebrates needs to be further clarified.

SLEEP REGULATION IN MAMMALS

The Origins and Definition of Sleep Homeostasis

The aim of early studies on sleep was to establish the presence of NREM sleep and especially of REM sleep in a diversity of species at different evolutionary stages. Particular emphasis was given to determining the amount of each of these states. Most data stem from animals recorded in the laboratory or in captivity. It can be assumed that such values are not necessarily representative for the sleep need of a species. The amount of sleep reported for animals bred and raised in the laboratory rather represents excess sleep. A typical example is the finding that sloths recorded by telemetry while living in the wild slept 6 hours less than animals of the same species recorded in a laboratory setting.[2]

The implementation of EEG spectral analysis and other methods of signal analysis to complement the traditional vigilance-state scoring led to the recognition that a continuous process underlies the NREM-REM sleep cycle. This process is reflected in the time course of SWA in NREM sleep across a sleep period. The principle of homeostasis was defined by W.B. Cannon in 1939 as "the coordinated physiologic processes which maintain most of the steady states in the organism." Later, in 1980, the term *sleep homeostasis* was coined by Alexander Borbély,[3] who posited that sleep strives to maintain a constant level by

variation of its duration and intensity. A vast amount of experimental data, especially from humans and rodents, shows that homeostatic mechanisms counteract the deviations of sleep from an average reference level. When waking is prolonged, sleep duration, and more importantly, EEG SWA during NREM sleep, is enhanced. In contrast, when sleep is prolonged or human subjects take a nap during the day, SWA in the subsequent night reaches a predictably lower level compared with a night preceded by a normal wakefulness period. In animals, it is more difficult to prolong sleep experimentally. Still, blocking access to a running wheel in rats induced excess sleep, which in turn was followed by decreased SWA levels.[4]

The two-process model of sleep regulation postulates that a homeostatic Process S rises during waking, and declines during sleep, interacting with Process C, which represents the output of the endogenous circadian clock, the nucleus suprachiasmaticus (SCN). SWA best reflects the prior history of sleep and waking and thus is an ideal quantitative marker of the dynamics of the homeostatic Process S in humans (see Chapter 37). The time course of the homeostatic variable S was derived from EEG SWA. The model accounts for the timing of human sleep[5] and for the interaction of S and C, allowing predictions of the time course of daytime vigilance. In animals, sleep is polyphasic; thus, SWA is modulated by the alternation of sleep and waking bouts lasting from a few seconds up to several hours (their duration depends on the minimum criteria defining a sleep epoch). SWA turned out to be the best physiologic marker of sleep homeostasis in humans. Is this also true for animals? Strictly speaking, sleep homeostasis is fulfilled by a species when a (predictable) relationship is established between the degree of rebound and previous waking duration. It has been repeatedly shown for humans and in an increasing number of other mammals (squirrel monkey, rat, Syrian hamster, Djungarian hamster, cat, ground squirrel, and several mouse strains[6-14]) that SWA increases as a function of the duration of the sleep deprivation (Fig. 9-1; see Figs. 9-2 and 9-3).

Can the original two-process model predict sleep regulation also in rodents? Attempts at simulating SWA in rodents on the basis of the original two-process model of sleep regulation showed that its tenets can be used to predict experimental results in animals, despite their polyphasic sleep pattern. The original formulation was based on an extensive data set derived from experiments in the laboratory rat.[3] Experiments investigating the magnitude of the influence of Process C in animals are still scarce, and therefore Process C has yet to be incorporated in models of animal sleep. In rats, simulations of SWA closely resembled the data, demonstrating the validity of this simple model for a laboratory rodent.[15] Because light exposure can affect both C and S,[16-18] it was necessary to incorporate the SWA-reducing effect of light in order to optimize the correspondence between SWA data and its simulations. Simulations of SWA for inbred mouse strains enabled the investigation of mechanisms underlying sleep homeostasis at the genetic level.[7,19-21]

Strains differ considerably in the dynamics of the buildup of sleep pressure and subsequent SWA dissipation.[6,7] It still remains to be examined whether sleep homeostasis as conceptualized in the two-process model can be applied to mammals other than rodents. In addition, a more-refined modeling approach must be developed to simulate the short-term changes in SWA. In rodents, SWA in NREM sleep reaches high values in a matter of seconds, whereas in humans the manifestation of slow waves at the transition to NREM sleep episodes is progressive, lasting 20 to 30 minutes. The comparison of the dynamics of Process S in different species and strains is a powerful method to identify the mechanisms underlying sleep regulation.[6]

Using a quantitative simulation of NREM sleep homeostasis in the rat and two mouse strains, the biphasic time course of SWA during baseline conditions, during the initial increase after sleep deprivation, and during the subsequent prolonged negative rebound was reproduced (see Chapter 37, Fig. 37-5).[6,7,15]

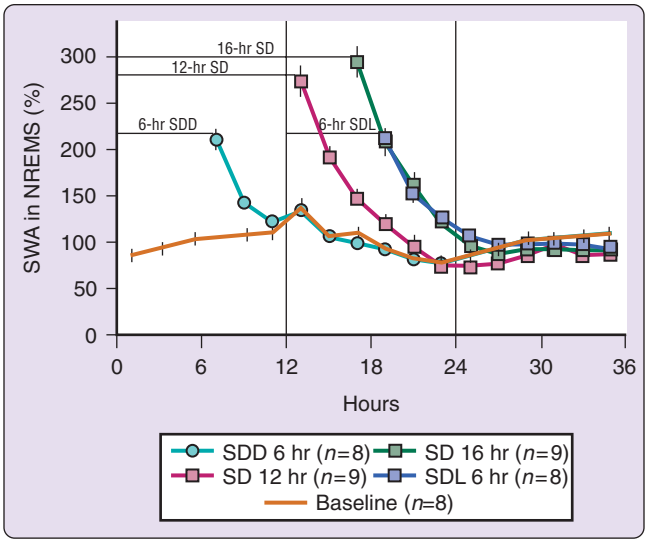

Figure 9-1 Effect of sleep deprivation (SD) by "gentle handling" on slow-wave activity (SWA) (electroencephalographic power density, 0.75 to 4.0 Hz) in non–rapid eye movement (NREM) sleep in the laboratory rat (Sprague Dawley). Mean values (n = 8 or 9 rats) of 2-hour intervals are expressed as a percentage of the corresponding 24-hour baseline value. SD duration was 6 hours beginning at either dark onset (6-hr SDD) or light onset (6-hr SDL), or 12 hours and 16 hours both beginning at dark onset (12-hr SD; 16-hr SD). SWA in NREM sleep during baseline is higher at light onset, after more wakefulness during the dark period than at dark onset, after the rats had slept considerable amounts. The experimental curves demonstrate the dose-response relationship of the duration of wakefulness and the magnitude of SWA increase during recovery. The levels of SWA are similar when recovery begins at dark onset and at light onset, when recovery is expressed relative to the 24-hour baseline. A difference between the two recovery curves would become apparent when each of the 2-hour intervals would be plotted relative to their corresponding 2-hour intervals. After SDD, recovery is biphasic: An additional SWA peak occurs at the beginning of the subsequent light period. After all manipulations, SWA attained recovery levels after approximately 12 hours, and in some cases, falls below the baseline; that is, there is a negative rebound.

Sleep Duration and Sleep Intensity

The hallmarks of sleep homeostasis in mammals are its duration, consolidation (i.e., brief awakenings and/or duration of consolidated NREM sleep and REM sleep epochs), and EEG SWA (also called *delta power)* in NREM sleep.

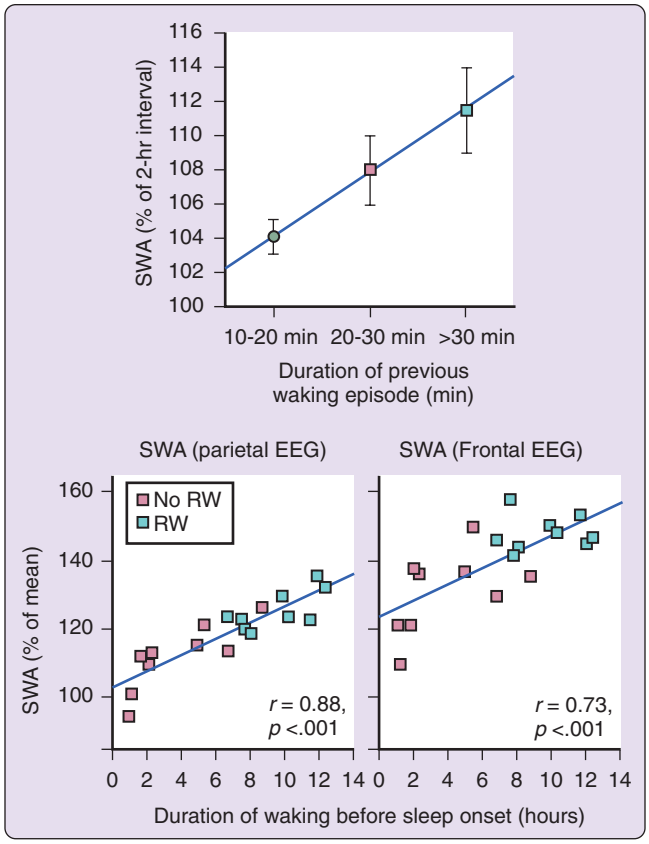

Figure 9-2 *Top panel,* slow-wave activity (SWA) (electroencephalographic [EEG] power density, 0.75 to 4.0 Hz) in non–rapid eye movement (NREM) sleep after spontaneous waking in the 12-hour baseline light period of an inbred mouse strain 129/Ola (n = 9). The waking episodes were subdivided into three categories according to their duration (10 to 20 minutes, 20 to 30 minutes, and longer than 30 minutes). The SWA increase in the 10-minute NREM sleep interval immediately following the waking episode is expressed relative to SWA in NREM sleep in the corresponding 2-hour baseline interval. Even after the short waking interval, SWA increases above baseline, increasing progressively as a function of the duration of the waking episode. (Modified from Huber R, Deboer T, Tobler I. Effects of sleep deprivation on sleep and sleep EEG in three mouse strains: empirical data and simulations. Brain Res 2000;857:8-19.) *Lower panels,* scatter plots of correlations between the duration of spontaneous wakefulness episodes and subsequent EEG SWA in NREM sleep increase in the first 2-hour interval after sleep onset (% of mean) in the frontal and parietal derivation. Two days are shown: the day when a running wheel (RW) was provided and when access to the wheel was prevented (no RW). Each individual (n = 9) contributed with 2 values (RW and no RW; different symbols). The line depicts the linear regression. (Modified from Vyazovskiy VV, Ruijgrok G, Deboer T, Tobler I. Running wheel accessibility affects the regional electroencephalogram during sleep in mice. Cereb Cortex 2006;16:328-336.)

A homeostatic balance between sleep duration and sleep intensity strives to maintain a constant daily quota of sleep. An increase of total sleep time is restricted by the constraints imposed by the circadian pacemaker, the SCN, providing the frame for the circadian rest–activity rhythm and the limitations ensuing from the environmental conditions. For most animals, it is maladaptive to engage in long periods of sleep during the wrong circadian phase.

Generally, compensation of a sleep deficit occurs by a minor increase in total sleep. In rodents, NREM and REM sleep are little increased after waking epochs in the range of 3 to 24 hours. Instead, the most striking feature manifested early during recovery sleep is the remarkable increase of slow waves in the NREM sleep EEG; this was first demonstrated in the rabbit,[22] extensively documented in the rat,[23,24] and shown in a diversity of different mouse strains.[6,7,19] The magnitude of the increase in sleep time or of SWA in NREM sleep depends on the species or strain, the duration of induced wakefulness, the efficacy of the deprivation, the quality of waking (behavior), and the circadian time point at which the deprivation ended. Apart from its clear relation to the duration of wakefulness, there is abundant evidence that the arousal threshold is consistently higher during NREM sleep with high amounts of slow waves, compared with more shallow NREM sleep with fewer slow waves.[25-27] Therefore, it has become generally accepted that SWA reflects NREM sleep intensity.[23,24]

It is evident that the main factor determining the degree of increase of SWA during recovery sleep is the duration of the waking state (i.e., the absence of sleep), but early studies questioned whether the quality of wakefulness also might affect SWA, a notion that would be consistent with a recovery function of sleep. However, using forced locomotion to achieve sleep deprivation and comparing the effects of different cylinder rotation rates[23,24] as well as voluntary running[28] failed to elicit major differences in the magnitude of increase of slow waves during recovery sleep. However, stressful waking experiences can contribute to an increase of SWA (e.g., in rats exposed to a social conflict).[29]

An important aspect that has received much attention in the interpretation of changes in SWA levels is the potential stressful effect of sleep deprivation per se. The gentle handling procedure introduced in Zurich consists primarily of keeping the animal active by introducing objects such as tissue and cardboard rolls, by tapping on the cage, and, when necessary, by tilting the cage (the animals are never touched, despite the use of the term "handling"[30,31]). Our procedure entails constant observation of the animals to ensure that they are awake. Great care is taken to avoid stressing the animals (that is why they are never touched) and to avoid interference with spontaneous feeding and drinking bouts.

A major influence of stress on the changes observed during recovery sleep appears unlikely because the forced locomotion procedure did not entail a significant increase in the level of plasma corticosterone,[32] and the expression patterns of genes were similar after 3 hours of spontaneous wakefulness or enforced sleep deprivation.[33] Moreover, in mice, corticosterone levels showed a negligible increase of this hormone after 6 hours of sleep deprivation compared to a massive increase after immobilization or spontaneous running activity.[34] A more refined study provided mice with a running wheel, which induces vigorous spontaneous running during the active phase; this study showed that indeed the longer waking epochs are followed by higher SWA levels (Fig. 9-2).[4] Studies in mice and ground squirrels found that SWA in NREM sleep also increased after *spontaneous*, *undisturbed* bouts of waking, and the magnitude of the increase depended on the duration of the

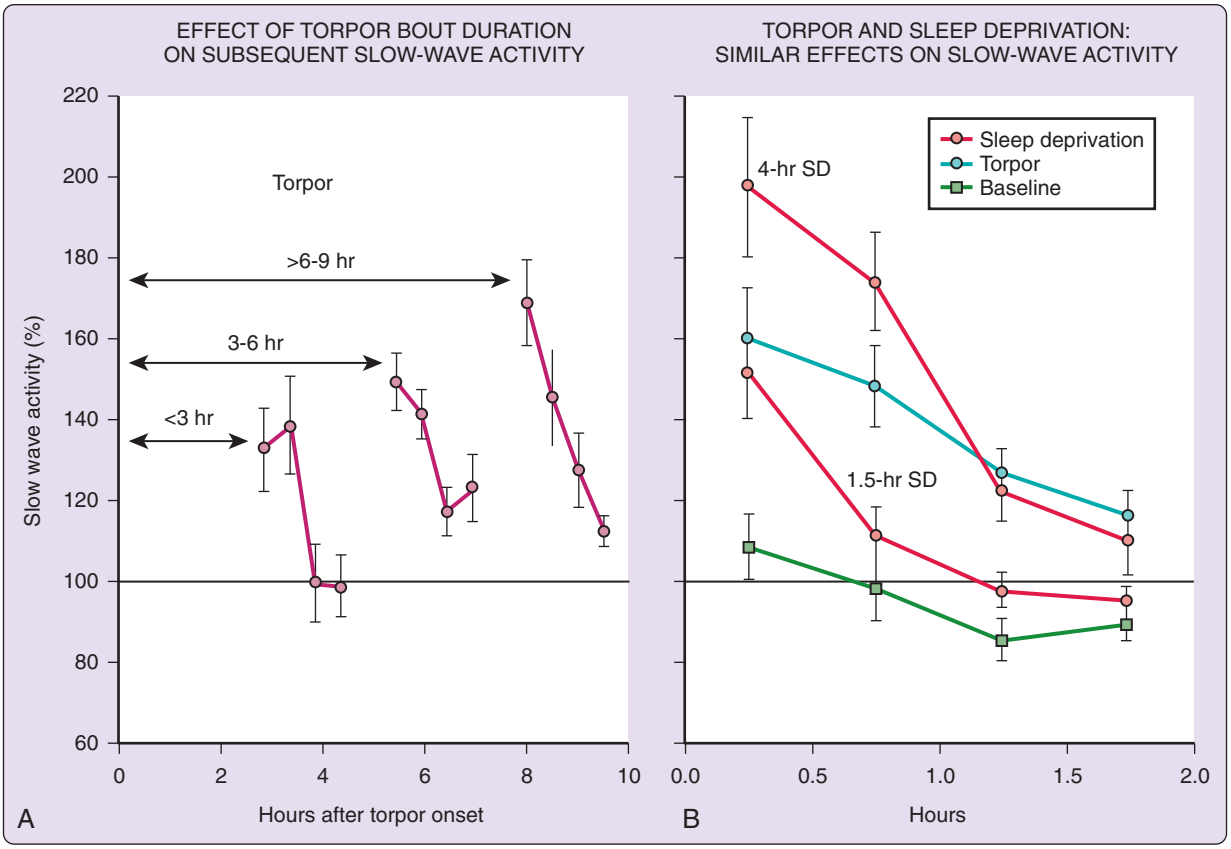

Figure 9-3 Time course of slow-wave activity (SWA) (mean electroencephalographic power density, 0.75 to 4.0 Hz) within non–rapid eye movement (NREM) sleep during sleep after torpor episodes of three different durations (less than 3 hours, 3 to 6 hours, and 6 to 9 hours) (**A**), and during baseline, recovery from 1.5 and 4-hours sleep deprivation (SD), and after torpor (**B**) (mean torpor duration = 4.8 ± 0.3 hours SEM) in the Djungarian hamster, *Phodopus sungorus*. **A,** Mean 30-minute values (n = 6, 15, and 7, for less than 3 hours, 3 to 6 hours, and 6 to 9 hours of torpor, respectively) during recovery after the spontaneous end of torpor. SWA is expressed relative to the individuals' 24-hour baseline day without torpor. A linear correlation between torpor bout duration and SWA increase was significant (n = 28, r = 0.42, P < .05). **B,** Time course of SWA after torpor and after 1.5 and 4-hour SD. Mean 30-minute values (n = 8, 8, and 7 for 1.5, 4-hour SD, and torpor, respectively). All recordings were performed in a short photoperiod, light–dark = 8:16, at 16° to 18°C. (Modified from Deboer T, Tobler I. Sleep regulation in the Djungarian hamster: Comparison of the dynamics leading to the slow-wave activity increase after sleep deprivation and daily torpor. Sleep 2003;26:567-572; and Deboer T, Tobler I. Slow waves in the sleep electroencephalogram after daily torpor are homeostatically regulated. Neuroreport 2000;11:881-885.)

previous waking bout (see Fig. 9-2).[6,7,10] These studies support the argument that an increase of EEG slow waves that follows a wakefulness interval is not due to stress.

It is unlikely that a global increase in metabolic rate during wakefulness is a critical variable contributing to the SWA increase after sleep deprivation. This possibility was rejected by the findings that changes induced by sleep deprivation performed either in a warm environment or at room temperature were similar, despite the higher levels of brain temperature in the warm condition.[35]

The fine regulatory property of sleep, especially the predictable changes in sleep intensity, and its so far ubiquitous manifestation, indicate that sleep must have an adaptive function, unless we postulate that sleep does not have a major function.[36,37] Under natural conditions, animals need to be vigilant, forage for food, reproduce, and avoid long, continuous bouts of deep sleep with high arousal thresholds. Evidently, compensating for sleep loss by balancing sleep duration and sleep intensity, depending on the timing and the situation, has added flexibility to sleep.

Daily Time Course of Slow-Wave Activity and Its Sleep-Wake Dependence

Having established that the main factor contributing to the initial level of EEG SWA in NREM sleep is the prior sleep and waking history, it follows that animals with a strong circadian amplitude of their rest–activity rhythm, which are awake preferably during the light or the dark period, therefore should exhibit high initial SWA values during the circadian preferred time for sleep. Moreover, as the need for sleep dissipates, SWA should decline progressively. This relationship is evident in nocturnal rodents (e.g. rat, hamster, and inbred mouse strains) and consistent in the diurnal chipmunk[38] and in humans. The global progressive SWA decline, despite the intermittent waking bouts typical for most mammals, reveals a stable, continuous process underlying the sleep–wake pattern. In contrast, in species with a minor or no preference for sleep in the light or dark period (rabbit, guinea pig, cat, blind mole rat), the decline of SWA in the course of sleep is negligible.[39-42]

Several experimental manipulations verified this relationship between the sleep-wake pattern and SWA. Changing the photoperiod from long to short days and vice versa in the rat and Djungarian hamster (*Phodopus sungorus*), or subjecting mice to short light–dark cycles over 24-hour intervals resulted in a dramatic redistribution of vigilance states, with no change in the total amount of sleep, but the time course of SWA followed the sleep–wake pattern as predicted.[16,17,43,44] Similarly, mice with an estrogen deficiency had a lower 24-hour sleep–wake amplitude than normal mice, and this sleep pattern was again predictably accompanied by a dampened SWA amplitude.[45]

There are large differences in the magnitude of a sleep deprivation–induced SWA increase between different mammals and even within mouse strains. Simulations show that the rate of the increase of SWA pressure (Process S), reflected by the time constants of its increase, is faster in the rat and mouse than in human beings (see Chapter 37, Fig. 37-5).[6,7,46] The remarkable difference in the increase of SWA elicited by 4 hours of sleep deprivation between two relatively small rodents, the Djungarian hamster and inbred mice[6,47-49] (Fig. 9-3, and see Fig. 9-1) might reflect different levels of Process S during their baseline conditions. Such an interpretation is supported by the different magnitude of the SWA response to sleep deprivation in human long and short sleepers (see Chapter 37). It is difficult to interpret the meaning of the differences in these fundamental dynamics within sleep between related species and within inbred mouse strains, whose genetics should be very similar.

Is REM Sleep Homeostatically Regulated?

Both total sleep deprivation and selective REM sleep deprivation elicit a subsequent compensatory increase in the amount of REM sleep (e.g., in cat, dog, rat, mouse, cow). Despite the considerable focus on the evolutionary origin of REM sleep, its rebound after sleep deprivation has not been addressed from an evolutionary perspective. There is as yet little indication for an intensity dimension of REM sleep, although in the rat[28,46] and rabbit[40] EEG theta activity in REM sleep was enhanced after 24 hours of sleep deprivation, and it declined progressively in the course of recovery sleep. Perhaps theta activity does reflect REM sleep intensity.

The Waking EEG Reflects Homeostatic Mechanisms

The marker for Process S, EEG SWA, can only be measured during sleep, leaving us with the difficulty of quantifying the buildup of sleep pressure as wakefulness progresses. In humans, theta activity in the waking EEG as well as the ratio of alpha to theta have been demonstrated as markers of increasing sleep pressure (see Chapter 37). In animals, in the course of sleep deprivation, it becomes increasingly difficult to keep them awake; more and more slow waves appear in the EEG despite the evident waking behaviors the animals engage in. This SWA during waking is a potential spillover of the slow waves typical for sleep intensity. It remains to be demonstrated that this is the case. Another remarkable feature of increasing sleep pressure during sleep deprivation in rodents is an increase in EEG theta activity during wakefulness[50] (EEG power between approximately 5 and 8 Hz). Such findings provide us with tools to quantify sleep pressure in animals under different conditions.

Circadian versus Homeostatic Aspects of Sleep Regulation

Several lines of evidence from animal experiments indicate that the homeostatic (Process S) and circadian (Process C) facets of sleep regulation are mediated by separate processes. Seminal experiments in the rat established that an intact circadian rhythm is not a necessary condition for sleep homeostasis. After the circadian facet of sleep regulation was disrupted by lesions of the SCN, and the rats became arrhythmic, a 24-hour sleep deprivation was followed by an intact ability to compensate for sleep loss.[51-53] Both SWA in NREM sleep and the amount of REM sleep increased. Therefore, the homeostatic response to sleep deprivation persists despite the abolished circadian rhythm (Fig. 9-4A). Similarly, arrhythmic fruit fly mutants subjected to sleep deprivation still retain the capacity to compensate for sleep loss (see Fig. 9-4B). Neither the phase nor the period of the free-running rest–activity rhythm of intact rats was affected by sleep deprivation,[54,55] but in the Syrian hamster it did lead to rapid resetting of the circadian clock.[56]

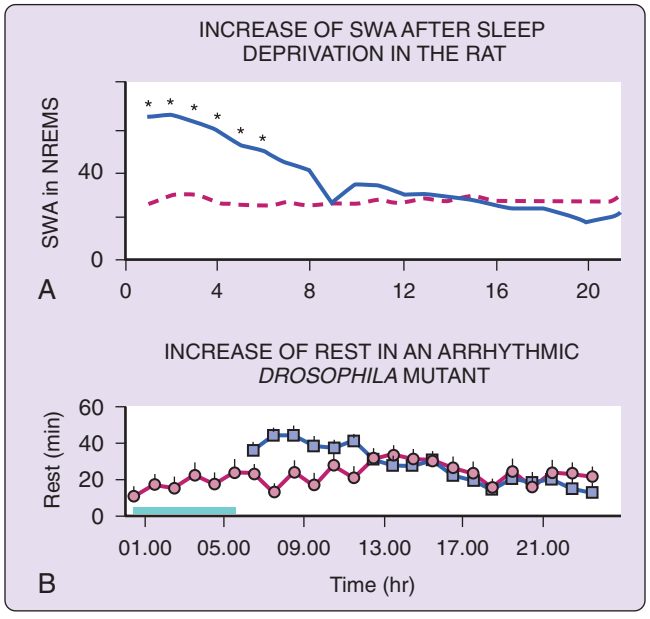

Figure 9-4 Independence of the homeostatic aspect of sleep regulation from the circadian component in the rat and the fruit fly *Drosophila melanogaster*. **A,** Increase of slow-wave activity (SWA) in non–rapid eye movement sleep (NREMS) in the rat after bilateral lesion of the nucleus suprachiasmaticus. Mean 3-hour values of sliding averages of 1-hour intervals for the baseline day preceding the 24-hour sleep deprivation (*dashed light blue line*) and the recovery light period ($n = 5$) (*dark blue curve*). (Modified from Tobler I, Borbély AA, Groos G. The effect of sleep deprivation on sleep in rats with suprachiasmatic lesions. Neurosci Lett 1983;42:49-54.) **B,** Increase in duration of rest (minutes) in an arrhythmic *Per*01 fly mutant recorded in constant darkness. *Blue bar,* 6 hours of deprivation of rest; *light circles,* amount of rest during baseline; *dark squares,* amount of rest during recovery. (From Greenspan RJ, Tononi G, Cirelli C, Shaw PJ. Sleep and the fruit-fly. Trends Neurosci 2001;24:142-145.)

Nevertheless, despite the predominance of the homeostasis aspect of sleep regulation, the vigilance states can affect the circadian process. Simultaneous recording of sleep stages and neuronal SCN activity of the rat demonstrated a feedback from sleep to the circadian pacemaker.[57] Also, conflict experiments examined the relationship between the two processes. In the rat, the conflict was induced by ending a 24-hour sleep-deprivation period either at the onset or in the middle of the dark period, the circadian period in which rats are predominantly awake.[23,58] SWA showed a two-stage rebound within the constraints of the circadian rhythm: an immediate increase followed by waking, and then a second, delayed increase at light onset. Because sleep in animals is largely polyphasic, short periods of sleep deprivation, such as 3 to 6 hours in rats or mice, theoretically can elicit considerable increases in sleep duration within the circadian constraint, but its magnitude depends on whether recovery is allowed within the light or the dark period.

Thus, it is not that the capacity to sleep attained an upper threshold, as has been argued. Instead, 3 hours of sleep deprivation leads to a small but significant increase in SWA with no change in NREM or REM sleep, whereas after 6 hours of sleep deprivation, NREM sleep (and, with a few hours' delay, also REM sleep) increases.[13,35] However, two bouts of 6 hours of sleep deprivation, once at the beginning of the light period and once at the beginning of the dark period, resulted in a major difference in the amount of sleep loss, and the time course and amount of increase in NREM sleep and SWA were different.[59] When the relationship between NREM sleep and SWA was analyzed by computing slow-wave energy (i.e., integrated SWA in NREM sleep), the compensation was identical under both sleep-deprivation conditions (Fig. 9-5). It is remarkable that even in cats, a species with a very small circadian sleep–wake modulation,[9,42] the effect of a short 4-hour sleep deprivation, performed at two different phases of the light–dark cycle, depended on the phase at which recovery was allowed.[9]

Generally, the circadian influence becomes evident when recovery from sleep deprivation begins in the circadian phase of predominant waking. The increase in total sleep time is invariably larger than when recovery occurs during the phase where sleep is predominant, but the SWA increase is less dependent on circadian factors.[58] A series of sleep-deprivation experiments in squirrel monkeys performed in constant light and initiated at the beginning of the subjective day, and therefore ending at different times of the circadian cycle,[8] led to the conclusion that the homeostatic component is weaker than circadian factors in determining the amount and intensity of sleep in the monkeys. However, a generalization to primates is not possible. In human subjects undergoing a forced desynchrony protocol, SWA showed little circadian modulation (see Chapter 37). Also the results obtained in species lacking a natural distinct preference for sleep in a particular circadian phase (guinea pig, rabbit, and some mouse strains,[40,60-62]) support the notion that sleep homeostasis is largely independent of the circadian rhythm.

The identification of clock genes in mice, humans, and fruit flies renewed the interest in the potential interaction between circadian and homeostatic aspects of sleep regulation. Studies using mouse mutants with deletions in circadian genes confirmed the presence of sleep homeostasis despite the absence of a circadian rhythm,[63-65] but for some mutants the homeostatic response to sleep deprivation was weak.[21,66] For state-of-the art knowledge on circadian genes affecting sleep homeostasis, see chapter 15).

In summary, caution is warranted in interpreting the effects of sleep deprivation on sleep, because sleep can vary its duration and its intensity. Moreover, the efficacy of the sleep-deprivation procedure can differ between species, and the spillover of slow waves into the waking EEG can confound the results. Simulations based on the two-

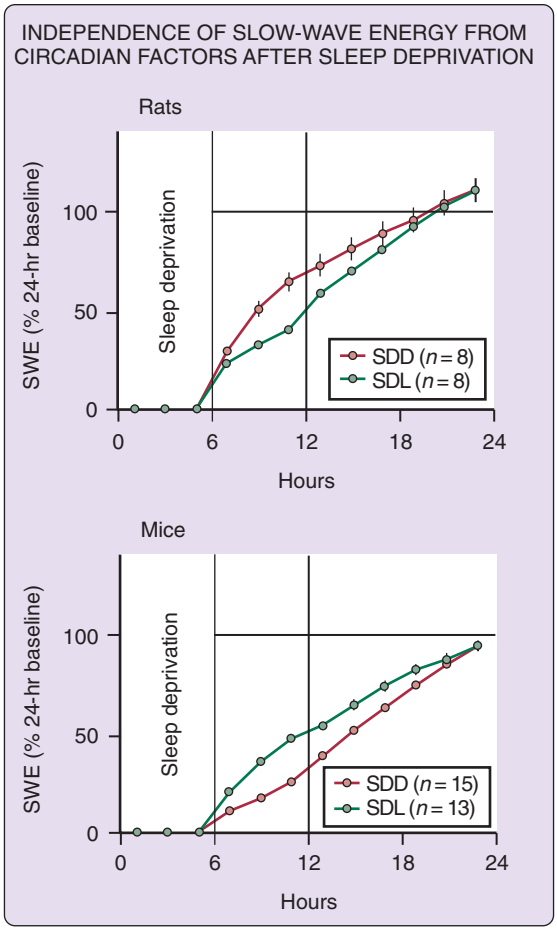

Figure 9-5 Time course of slow-wave energy (SWE) (cumulative electroencephalographic power density, 0.75 to 4.0 Hz in non–rapid eye movement [NREM] sleep) for the 18-hour recovery interval after 6 hours of sleep deprivation (SD) that began either at light onset (SDL) or dark onset (SDD). Curves connect 2-hour cumulative values of mean SWE (male Sprague-Dawley rats: SDL, $n = 8$; SDD, $n = 8$; male B1 mice [I/LnJ × C57BL/6]: SDL, $n = 13$; SDD, $n = 15$), expressed relative to mean SWE in NREM sleep in the corresponding individual 24-hour baseline. During recovery from 6 hours of sleep deprivation performed either at the beginning of the 12-hour light period (SDL) or at the beginning of the 12-hour dark period (SDD) in the rat ($n = 8$) and B1 mice ($n = 13$ to 15), NREM sleep and slow-wave activity in NREM sleep are significantly increased. The magnitude of the effect and its time course depend on the timing of the SD. The differences in time course become evident when SWE is computed. However, it is notable that in both species, SWE reaches identical values toward the end of the recovery period of both treatments. (Vyazovskiy V, Tobler I, unpublished data, 2007.)

process model, which accounts for the changes in duration of NREM sleep and its intensity, indicates that the considerable differences between strains are a consequence of different dynamics (i.e., of time constants) of the homeostatic process (see Chapter 37).[6]

Special Features

Herbivores: Cows and Horses, Ruminants and Nonruminants

The herbivores are a diverse group of animals encompassing ruminating and nonruminating species, some with considerably large body and brain weights; human-bred domesticated species; and animals living in the wild (giraffes, elephants, antelopes) (Videos 9-1 and 9-2). Therefore, the ungulates can help clarify whether sleep regulation is affected by these features. The impact of the specialization of nutrition, entailing many hours of rumination, on sleep is worth pursuing.

The effects of prolonged wakefulness have been investigated in three species: cows, donkeys, and ponies. Recumbence was prevented in cows for 14 to 22 hours per day for 2 to 4 weeks. This intervention, a major REM sleep deprivation (although NREM sleep and drowsiness were also reduced), was followed by both NREM and REM sleep enhancement during recovery.[67] Similarly, sleep time increased in a donkey that was sleep deprived for 48 hours.[68] In ponies, recorded in a stall and outdoors and subjected to visual and auditory sensory deprivation for a 4-day period in the stall, the amount of NREM sleep increased from day to day at the cost of *drowsiness*, a lighter form of NREM sleep, and REM sleep was unchanged. On exposure to the more disturbed conditions outdoors, there was a threefold increase of REM sleep during the first 2 days indicating a relationship with the increased environmental stimuli.[69,70]

Taken together, at least some aspects of sleep are regulated in large herbivores. It remains to be clarified whether their NREM sleep has a strictly regulated intensity component.

Sleep Regulation: Unihemispheric Sleep and Regional Aspects of Sleep

One of the most remarkable specializations of sleep evolved in aquatic mammals belonging to the orders Cetacea, Pinnipedia, and Sirenia. Some species of each of these orders engage in episodes of unihemispheric deep sleep (i.e., with a predominance of delta waves) that can last from minutes to hours while the other hemisphere exhibits an EEG resembling waking.[71] Although the invasive recordings with implanted electrodes, the restriction of the animals' movement by the recording cables, and the constraints of the recording environment might have influenced these findings, Ridgway[72] confirmed unihemispheric sleep in a bottle-nosed dolphin recorded by telemetry in a small pool. Refined recording equipment and conditions have been used to describe sleep in representatives of all orders of cetaceans.

Unihemispheric sleep provides a unique opportunity to investigate whether sleep regulation occurs simultaneously in both brain hemispheres. Maintaining six bottle-nosed dolphins awake for 35 to 72 hours resulted in an increase in delta sleep in three individuals and, in an early study, in a more regular alternation of deep sleep between the brain hemispheres,[71] providing evidence that slow waves in NREM sleep represent sleep intensity also in dolphins. Further experiments targeting the arousal threshold during unihemispheric sleep are lacking. The most revealing experiment was the attempt to restrict sleep deprivation to a single brain hemisphere by intervening only when the EEG of one hemisphere showed delta waves. Although some variability was seen between individuals in the magnitude of changes, the unilateral intervention resulted in a larger compensation of delta sleep during recovery in the deprived hemisphere in seven of the nine deprivations.[71]

These findings show that sleep does not necessarily encompass the entire brain and led to the notion that sleep might have local aspects in other species. Several lines of evidence supported the notion of topographic EEG SWA differences between and within hemispheres in humans and rodents.[31] Elaborate experiments manipulating the quality of waking revealed that specific aspects of the waking state can indeed induce SWA changes in the brain regions that were more active during waking.[30,73] Such enhanced neuronal activity was attained experimentally in rats and mice by stimulation of whiskers or spontaneously by use of a preferred paw during feeding[30,74] and unilateral sleep deprivation in dolphins.[71] A function of slow waves during sleep could be to enable recuperation of neurons in those regions that were most active during waking.[75]

Sleep and Hibernation

Hibernation and daily torpor, both characterized by a remarkable, physiologically regulated decrease in metabolism, as well as body and brain temperature, thereby showing similarity to the regulated decrease observed during sleep, have been used as models to understand sleep. Hibernation and daily torpor are specializations that reduce energy requirements most effectively in endothermic animals. In daily torpor, temperature is lowered to approximately 15° to 20°C for several hours during the circadian rest period.[76] In contrast, during hibernation, body temperature is lowered to 5°C or less for several months,[77] with short (less than 24-hour) euthermic interruptions.[77,78]

It is well established for several hibernators that they arouse from hibernation and, paradoxically, go to sleep. It is puzzling that animals arouse regularly from hibernation despite the high energy costs of the arousals.[78] Their SWA is high at the initiation of the sleep episode and subsequently decreases, exhibiting dynamics that are comparable to recovery after sleep deprivation. In three species of ground squirrels, the initial values of SWA during euthermia were higher after long hibernation bouts than after short ones, which again is a typical feature for recovery from sleep deprivation.[77,79,80] Thus hibernation, especially at low temperatures, and daily torpor seem to be incompatible with restorative processes that are expected to occur during sleep.[77,79]

The relationship of the torpid state, subsequent arousal, and sleep deprivation was extensively investigated in the Djungarian hamster *(Phodopus sungorus sungorus)*. They alternate days with torpor with days at euthermic levels, enabling comparisons within the same individuals (for a review, see reference 48). As in the hibernating ground squirrels, during euthermia, the initial value of SWA in

NREM sleep was high and was followed by a progressive decline closely resembling the pattern during recovery from sleep deprivation (see Fig. 9-3). Similarly, as after sleep deprivation, the initial level of SWA was higher after long torpor bouts than after short bouts (see Fig. 9-3),[81] and a better fit between the duration of torpor or sleep deprivation and the initial SWA values was attained by a saturating exponential function than by a linear function.[82] The kinetics of the increase in sleep pressure during torpor was 2.75 times slower than after sleep deprivation in the same species (see Fig. 9-3),[48] supporting the hypothesis that during hypothermia, the hamsters incur a sleep deficit that is recovered by returning to euthermia. Another similarity between recovery from torpor and sleep deprivation are the distinct regional differences between cortical areas in the degree of increase of EEG SWA, which invariably is higher above the frontal cortex compared to parietal or occipital regions. It still remains to be clarified whether the return to euthermia is triggered by a homeostatic need to recover.

Other Measures for Sleep Regulation: Sleep or Rest Consolidation

Abundant data document a compensatory increase of SWA and other EEG variables after prolonged wakefulness in species encompassing humans and rodents and many other mammals, including two nonhuman primates (the rhesus monkey *[Macaca mulatta]* and squirrel monkey),[8,83-85] three carnivores (the cat,[9,11,14,40,86] the dog,[87] and the ferret[88]), and rabbits and dolphins. However, variables such as sleep state consolidation, or alternatively, when sleep data are lacking, the number and consolidation of epochs with little or no motor activity, also change as a function of the duration of previous wakefulness. Sleep consolidation might not merely reflect higher SWA pressure, but might also provide an organism with an additional means to enhance the recovery value of sleep. Indeed, the higher SWA levels encountered in long NREM sleep episodes compared to shorter episodes support the notion of a higher recovery value of long, consolidated epochs.[59] Thus, the consolidation of sleep under increased sleep pressure by means of sleep-episode prolongation might be a powerful mechanism to achieve more-intense sleep.

Sleep continuity can be defined also by the frequency of short wake episodes (brief awakenings). In the rat, the frequency of wake episodes shorter than 32 seconds was reduced after 24 hours of sleep deprivation, leading to a consolidation of sleep during recovery. In further similar experiments in the rat and other rodents (guinea pigs and laboratory mice), the reduction in the number of brief awakenings correlated with the increase of SWA.[15,20,39,89] This inverse relationship indicates that brief awakenings might represent a behavioral correlate of sleep intensity.[15] The decrease of brief awakenings under enhanced sleep pressure likely contributes to the reduced variability of the NREM-REM sleep cycle shown in the rat,[89] bottle-nosed dolphin,[71] and cat[90] to the shortening of the sleep cycle, as well as to decrease in the duration of the single-cell activity discharge cycle in brainstem dorsal raphe nucleus.[90]

Motor activity is reduced during recovery from sleep deprivation in humans[91] and in several other mammals. In dogs subjected to sleep deprivation, motor activity assessed continuously by actigraphy was reduced by as much as 40% during recovery.[92] Similarly, in the rat, activity was reduced to 84% of baseline when recovery from 24 hours of sleep deprivation coincided with dark onset.[23] There are data showing that the amount of consecutive rest episodes as a marker for sleep intensity may be a useful measure to quantify sleep regulation in inbred mouse strains (e.g., 45). In summary, motor activity is a simple measure that can be recorded easily, allowing investigation of aspects of sleep regulation when invasive EEG interventions are not possible or in species where sleep cannot be defined by EEG criteria (see "Sleep Regulation in Invertebrates").

SLEEP REGULATION IN NONMAMMALIAN VERTEBRATES

Birds

Sleep in birds is similar to sleep in mammals (for a review, see reference 93), but the limited number of species investigated relative to the large diversity of avian species does not allow generalization. The similarity applies especially to the uncontested presence of the two stages NREM sleep and REM sleep, although the duration of REM sleep epochs in birds is typically much shorter. Three vigilance states can be distinguished by spectral analysis of the EEG concomitant with electromyogram (EMG) and electrooculogram (EOG) measures.

In birds, the differences between sleep stages are much smaller than in mammals,[94] in which NREM sleep power density values in the low-frequency range (0.25 to 6.0 Hz) exceed those of REM sleep and waking by approximately one order of magnitude. It was important to clarify whether sleep in birds is homeostatically regulated, because specific aspects of sleep regulation may be related to homeothermy. An early study in pigeons showed no effect of 12-hour sleep deprivation on SWA in NREM sleep.[94] Nevertheless, several other variables were affected by the sleep deprivation, which are consistent with a need for recovery. Sleep duration, the amount of REM sleep, and EOG activity in waking and NREM sleep were increased. In the meantime, a homeostatic SWA increase was documented in pigeons,[95] and sparrows.[96] It is still an open question whether SWA in birds affects the arousal threshold to stimuli, providing another measure to demonstrate the relation between their SWA and sleep intensity.

Many birds exhibit unilateral eye-blinking, which in some species was correlated to a wakelike EEG over the contralateral hemisphere.[97] In Barbary doves, frequency of eye blinking decreased substantially after 3- to 36-hour sleep deprivation (achieved by exposing the animals to a ferret on a leash).[98] The level of vigilance estimated by the blinking frequency depended on the duration of the sleep deprivation, suggesting a need to compensate for sleep loss. There is a trade-off between the benefits of frequent eye blinking (e.g., optimizing predator detection by increasing vigilance) and sleep with few interruptions.[98] The results imply that the birds benefited from sleep with closed eyes and that those benefits were reduced during sleep deprivation. This early finding was later complemented by EEG recordings in pigeons and mallard ducks. Both species had short epochs of EEG asymmetry during

sleep, resembling unihemispheric sleep in dolphins. However, in birds, these epochs were ultrashort, in the range of seconds.

In addition, mallards exposed to laboratory surroundings had higher amounts of such eye-openings and concomitant unilateral sleep than the same birds placed in the more protected space between conspecifics rather than at the periphery of the group.[97,99] This elegant experiment indicated that the lateralization might enhance survival, because it allows one hemisphere to scan the environment intermittently while the other remains in a sleeping state. The occurrence of such episodes may be the clue to "sleeping on the wing," which was determined in frigate birds wearing altimeters[100] and was postulated years ago on the basis of behavioral observations of swifts. The frigate birds were in constant motion day and night. Alternatively, birds might sleep while they are gliding[100a] or, as recordings in the laboratory in sparrows showing migratory behavior, drowsiness can be enhanced.[101]

Reptiles

The sleep EEG in reptiles differs in many ways from that in mammals. In particular, the vigilance state–related changes in EEG patterns are different. It is therefore remarkable that elements in their EEG did respond to sleep deprivation. Considerable diversity in the appearance of high-voltage slow waves (sharp waves and spikes) superimposed on the waking and sleeping EEG was found, suggesting that this type of EEG activity may be a precursor or a correlate of the slow waves associated with sleep in mammals.[102] A state-related change in firing rate of brainstem neurons (waking versus quiescence) was seen in box turtles.[103]

In the early 1970s, Flanigan[104] performed a unique set of sleep-deprivation experiments in reptiles belonging to the orders Crocodilia *(Caiman sclerops)*, Chelonia (box turtle *[Terrapene carolina]* and red-footed tortoise *[Geochelone carbonaria]*), and Squamata (iguanid lizards, green iguana *[Iguana iguana]*, and spiny-tailed lizard, also called black lizard *[Ctenosaura pectinata]*). The animals were subjected to 24 or 48 hours of stimulation by stroking, handling, or gently tugging the animal's leash when it showed signs of behavioral sleep. The increasing amount of interventions necessary to keep the turtles awake and the loss of muscle tone in the iguanas toward the last hours of sleep deprivation were clear signs of increasing sleepiness, indicating an accumulating need for sleep. Recovery consisted of a compensatory rebound: The latency to behavioral sleep shortened, the duration and amount of behavioral sleep increased, and EEG spikes and, importantly, arousal threshold (response to electrical stimulation of eye potentials or heart rate) were markedly enhanced. It is therefore possible that reptilian EEG spikes and sharp waves reflect sleep intensity. Their increase after prolonged wakefulness, similar to slow waves in mammals, suggests a functional similarity.

Amphibians and Fish

Data on sleep in amphibians and fish are scarce, although they show many behavioral aspects of sleep. Moreover, investigations of effects of sleep deprivation in amphibians are lacking. For example, a correlation between arousal threshold and behavioral sleeplike states is not established.

However, two early studies subjected two species of fish, carp and perch, to sleep deprivation, using behavioral measures to assess its effects.[105] Activation of carp for up to 96 hours by continuous illumination induced decreased latency to sleep behavior during the reinstatement of the dark period,[106] and in perch, the activation induced by exposure to 6 and 12 hours of light during the habitual rest period (i.e., the dark period) resulted in an increase of resting behavior during recovery.[105] The effect depended on the length of the light exposure, confirming the notion that homeostatic mechanisms might underlie the regulation of rest in fish.

The availability of the zebrafish *(Danio rerio)* genetic map makes this lower vertebrate an interesting candidate to screen for the genes involved in sleep regulation at the level of simpler vertebrates. Rest deprivation of larvae elicited behavioral sleep, or a rest rebound,[107] and deprivation of rest by electrical stimulation of adults elicited a sleep rebound, albeit only when recovery began at dark onset,[108] confirming the sleep-regulatory capacity in this species.

Taken together the findings in fish demonstrate that a sleep regulatory system might have evolved across the vertebrate classes. Fish may be powerful models to investigate the molecular networks and mechanisms underlying sleep regulation.

SLEEP REGULATION IN INVERTEBRATES

Simpler models are better suited than mammals and birds to dissect the molecular basis of sleep. Once it was established that in mammals and birds a series of additional variables other than the EEG could be used to define sleep and its homeostasis, the way was paved to investigate whether nonvertebrate organisms, such as arthropods, meet the behavioral criteria for sleep. The criteria have indeed been met, showing that arthropods evolved the capacity for a compensatory response after prolongation of the normal waking period. Initial evidence came from two species of cockroaches and the scorpion, the oldest living arthropod (marine scorpions can be traced back to the Silurian period of about 400 million years ago). The most compelling evidence for the existence of sleep in an invertebrate is available for the fruit fly, *Drosophila*, rendering this insect an ideal species to dissect the genetics of sleep and sleep regulation.[109-113]

Cockroaches (*Leucophea maderae*,[114] *Blaberus giganteus*,[115] and *Periplaneta australasiae*) (K. Sly and R. Brown, unpublished data, 1994) were prevented from resting for 3 hours at the end of the daily rest period; it was predicted that a compensatory increase of rest would best be manifested at the time of habitual activity. Rest exhibited a rebound after the intervention, whereas the control experiment, simply removing the insects from their home cages for 3 hours, elicited a smaller and shorter-lasting decrease of activity and an increase of immobile rest episodes. The data provided the first evidence for compensatory mechanisms in an invertebrate.[114] In a more-refined approach, behavior was scored as "activity" or "rest," but rest was subdivided on the basis of the position of the head, abdomen, and

antennae in order to clarify whether substates reflect different arousal thresholds. In one of the nine behavioral states, characterized by a horizontal body axis with the antennae touching the substrate, arousal was lower than in all other states. After disturbing the cockroaches for an entire 12-hour dark period, it was this behavioral state that showed a tendency to increase its duration and frequency during recovery; however, locomotion was also increased.[115]

The fruit fly (with its manifold mutants) has significantly contributed to our knowledge about the genetic basis of sleep regulatory mechanisms. Rest in this insect is not merely a state of inactivity but fulfills all the behavioral criteria for sleep.[110,111,113] Correlates of sleep and waking were established: The rest activity pattern was quantified and the arousal thresholds to several types of stimuli were investigated. Preventing the flies from attaining rest for different periods of time elicited a corresponding increase of rest during recovery (Fig. 9-6A) that was associated with increased sleep continuity and arousal thresholds (see Fig. 9-6B). Thus, both factors contributing to sleep homeostasis in mammals, duration and intensity, are firmly established for the fruit fly. Moreover, electrical field potentials in the fly brain correlated with behavioral state.[116] The definition of sleep and its regulatory capacity in *Drosophila* paved the way to screen thousands of fly mutants (see Fig. 9-6C), leading to the identification of the genetic components regulating their sleep. Several hundred fly mutants, including lines derived from single females collected in the wild, are being screened for short and long sleepers and for flies that lack a homeostatic response to the loss of sleep (reviewed by Cirelli[109]).

Already, there is large variability in sleep homeostasis in flies. *Drosophila* mutants lacking an enzyme involved in the catabolism of monoamines, dopamine acetyltransferase, as well as the loss-of-circadian-function fly, cyc01, showed enhanced rest rebound after sleep deprivation, whereas male flies with a null mutation for cycle (*BMAL1*) showed no rebound or very little rebound (reviewed in references 117 and 118). Homeostasis was apparently impaired in flies mutant for the clock gene *timeless*, but this result was based on the lack of a rebound after only 6 hours of sleep deprivation.[110] Longer periods of sleep deprivation should reveal whether a compensatory response can be induced in these mutants.

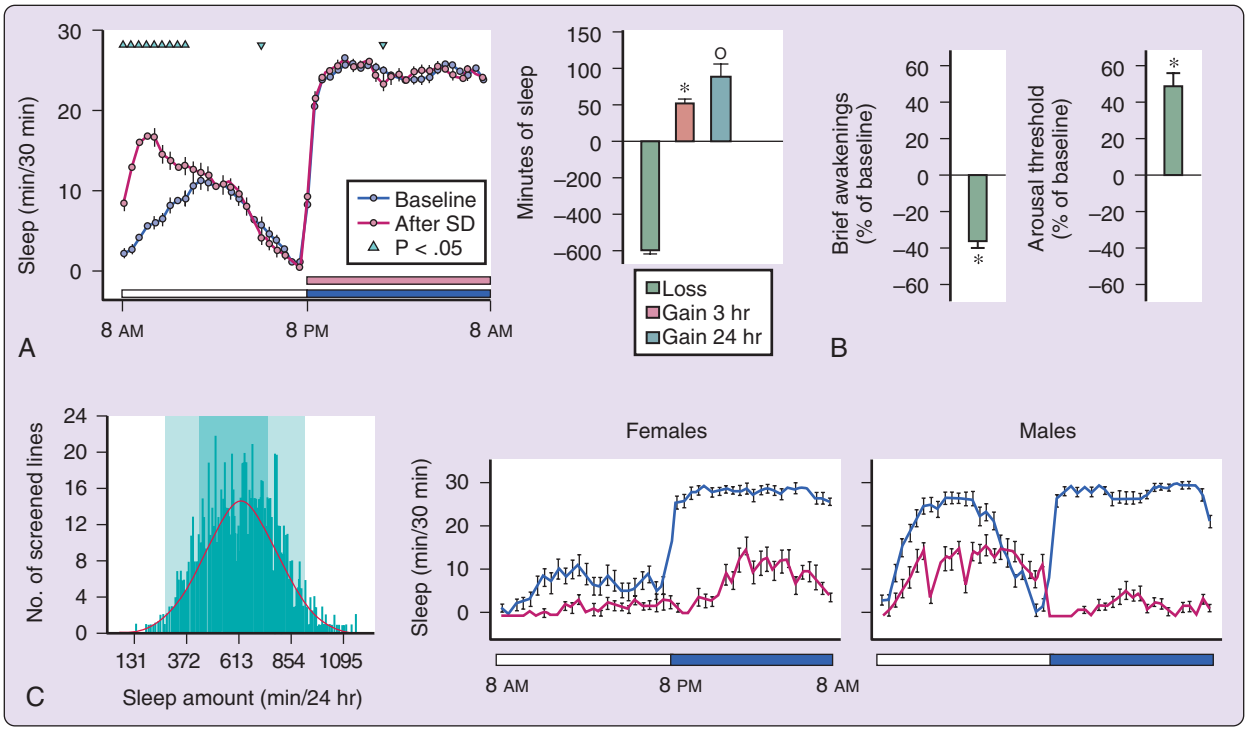

Figure 9-6 Sleep in the fruit fly. **A,** *Left, Solid circles* indicate the typical pattern of sleep in a population of about 100 female wild-type flies during baseline. Flies sleep mostly during the night. *Open circles* indicate sleep amount and distribution after 12 hours of sleep deprivation (SD). Time and duration of sleep deprivation are indicated by the *light blue bar* on the x-axis. The increase in sleep duration occurs mainly during the first 6 hours after sleep deprivation (paired *t* tests). Flies were maintained in a 12:12 light–dark cycle (light on at 8 AM). *Right,* Amount of sleep lost (during sleep deprivation) and of sleep recovered (after sleep deprivation) for the experiments shown at *left.* Sleep duration is significantly increased during the first 3 hours after sleep deprivation. *$P < .05$; O, $P < .1$, Duncan's multiple range test. In flies, as in mammals, the amount of sleep recovered after sleep deprivation represents only a fraction of the sleep lost. **B,** Sleep intensity is increased after sleep deprivation. *Left,* The number of brief awakenings is reduced during the first 3 hours after SD. *Right,* The arousal threshold is increased during the first 6 hours after sleep deprivation. Values refer to the experiment shown in **A**. *$P < .05$, Duncan's multiple range test. **C,** *Left,* Daily amount of sleep in 1547 mutant lines. Mean amount of sleep per 24 hours is 616 ± 169 (mean ± standard deviation; minimum, 131; maximum, 1155). *Shaded areas* show one *(dark blue)* and two *(light blue)* standard deviations from the mean. Only a very few mutant lines sleep less than 2 standard deviations from the mean (short sleepers). *Right,* Daily amount of sleep in female and male flies of a short-sleeper line *(light blue lines).* For comparison, the *dark blue line* in each panel represents the daily amount of sleep in wild-type flies. (From Cirelli C, Huber R, Tononi G. unpublished data, 2005.)

Sleep deprivation in bees led to compensatory changes including prolongation of rest and decreased antenna movements, complementing the early studies.[119,120]

Scorpions of the three species *Heterometrus longimanus*, *Heterometrus spinnifer*, and *Pandinus imperator* also show a clear daily rhythm of rest and activity, and on the basis of the reaction to a vibration stimulus, an intermediate alert state and a rest state could be defined. Deprivation of rest for 12 hours elicited an initial rise of activity and a significant increase in the resting state (Fig. 9-7).[94]

Among lower invertebrates, crayfish not only displayed most elements of behavioral sleep, including an enhanced arousal threshold to mechanical stimulation when in behavioral quiescence, but also a rest rebound after the quiescent state was prevented for 24 hours.[121] A series of experiments in cuttlefish *(octopus, Sephia pharaonis)* resulted in similar rebound mechanisms after 12 hours of vibratory stimulation,[122] thereby expanding the presence of a sleep-like state to mollusks. The evolutionary lowest organism in which the presence of sleep and sleep regulation was extensively investigated most recently is the nematode *Cenorhabditis elegans.*[123]

Many genes in *Drosophila* have a homologous counterpart in mice and humans. These genetic links mean that flies, zebrafish, mice, and perhaps also *C. elegans* can be used as models, which will eventually lead to our understanding of sleep in humans.

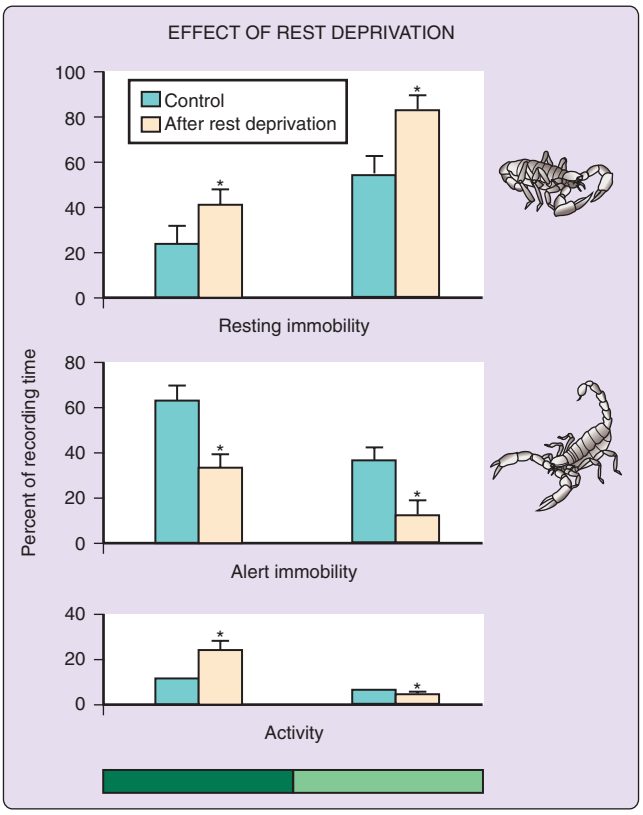

Figure 9-7 Effect of 12-hour rest deprivation on three vigilance states in the scorpion. Bars represent mean values ± SEM (standard error of the mean) ($n = 7$) for the 12-hour intervals of the control day and recovery after rest deprivation, ending at dark onset, separately for the 12-hour dark and 12-hour light period. *$P < .05$, Wilcoxon matched-pairs signed-rank test; differences between control and recovery. Dark and light conditions are indicated by the *dark green* and *light green bar* at the bottom of the figure. Note: The prolonged compensation of resting immobility at the cost of alert immobility, and note the initial activating effect of the intervention. (Modified from Tobler I, Stalder J: Rest in the scorpion—a sleep like state? J Comp Physiol [A] 1988;163:227-235.)

OUTLOOK

Compensation for the loss of sleep has been found in many mammals. Similar phenomena were described in birds, reptiles, fish, and some invertebrates. Most notably, *Drosophila* exhibits homeostatic compensation of rest after rest deprivation, leading to its use as a model to investigate the underlying mechanisms at the genetic level.

In natural environments, there are many disturbances that can prevent animals from obtaining their normal quota of sleep. A large variety of animals have developed mechanisms to compensate for the loss of sleep within the constraints of the circadian rest–activity rhythm by intensifying sleep. This is a powerful indication that there is a benefit to sleep that is reduced during sleep deprivation and is indispensable.

Rest behavior may be a state from which sleep evolved. A more-detailed characterization of rest in different classes of animals is helping to clarify the origin of sleep and to identify the unique properties of sleep in comparison to rest. Sleep, as it is defined in mammals and birds, has many properties that can be identified in lower vertebrates and invertebrates. An important common feature is that sleep is not merely a function of the circadian rest–activity rhythm but is determined by additional regulatory mechanisms. Sleep deprivation in vertebrates and invertebrates elicits compensatory responses. This regulatory property of sleep, which can be considered the essence of sleep, can be used to examine rest in a broad range of animals that, because of the absence of EEG criteria, are not considered to manifest sleep.

The effects of rest deprivation in invertebrates are, in some aspects, analogous to the compensation of sleep after sleep deprivation in mammals. Elementary properties of sleep may be found that will allow the investigation of the underlying mechanisms in less-complex organisms. Although these technologies allow the application of genetics to complex systems such as those encountered in mammals, the finding that *Drosophila* exhibits the criteria used to define sleep provides an interesting parallel avenue to clarify the involvement of genes in sleep regulation.[124]

❖ Clinical Pearl

Hibernation and daily torpor, which are the most effective ways to reduce energy requirements, result in reduction of body temperature and other physiologic changes that protect organs that have a high metabolic rate. Lessons learned from understanding spontaneous or artificially induced hibernation might lead to new treatments to protect the brain in patients with stroke and traumatic brain injury.

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Sleep in Animals: A State of Adaptive Inactivity

Jerome M. Siegel

Chapter 10

Abstract

In most adult animals sleep is incompatible with mating and feeding. Animals are believed to be vulnerable to predation during sleep. Humans frequently see sleep as an obstacle to a productive life. Many people use alarm clocks to truncate sleep and take stimulants, such as caffeine, to maintain alertness after insufficient sleep. Why do animals devote from 2 to 20 hours of the day to sleep, in what appears to be a nonproductive state? Why has evolution preserved this state? It is often said that sleep must have some unknown vital function to have persisted in all humans and throughout much, though not necessarily all,[1] of the animal kingdom. Without this so far undiscovered function, it has been said that sleep would be evolution's greatest mistake.

Sleep is not a maladaptive state that needs to be explained by undiscovered functions (which nevertheless undoubtedly exist). Rather, the major function of sleep is to increase behavioral efficiency. Greater waking activity does not necessarily lead to increased numbers of viable offspring and, hence, genetic success. Rather, genetic success is closely linked to the efficient use of resources and to the avoidance of risk. Thus, inactivity can reduce predation and injury. It also reduces brain and body energy consumption. It has often been stated that energy conservation is not a sufficient explanation for sleep because the energy saved in a night's sleep in humans is only equivalent to that contained in a slice of bread, although to the extent that sleep prevents locomotor activity the energy savings are much greater. In the wild, most animals are hungry and are seeking food most of the time they are awake. The ability of sleep to conserve energy when food is scarce will produce a major survival benefit. Conversely, if food is available but is time consuming to acquire, then it is highly advantageous for animals to be able to reduce sleep time without behavioral impairment.[2] Similarly, it is highly advantageous to reduce or eliminate sleep to allow migration and to respond to certain other needs.

Many have assumed that predation risk is increased during sleep, that is, that more animals are killed per hour during sleep than during waking. However there is scant evidence to support this contention.[1] Most animals seek safe sleeping sites, often underground, in trees, or in groups that provide communal protection. Those large herbivores that cannot find safe sleeping sites appear to have smaller amounts of sleep and sleep less deeply, making it difficult to see how they would get the unknown benefit that sleep supposedly confers. Large animals that are not at risk for predation, such as big cats and bears, can sleep for long periods, often in unprotected sites and appear to sleep deeply.[3]

ADAPTIVE INACTIVITY

Sleep should be viewed in the context of other forms of "adaptive inactivity." Most forms of life have evolved mechanisms that permit the reduction of metabolic activity for long periods of time when conditions are not optimal. In animals, this usually includes a reduction or cessation of movement and sensory response. The development of dormant states was an essential step in the evolution of life, and it continues to be essential for the preservation of many organisms. Many species have evolved seasonal dormancy patterns that allow them to anticipate periods that are not optimal for survival and propagation (predictive dormancy). In other species dormancy is triggered by environmental conditions (consequential dormancy). Many organisms spend most of their lifespan in dormancy. A continuum of states of adaptive inactivity can be seen in living organisms including plants, unicellular and multicellular animals, and animals with and without nervous systems.[3a]

In the plant kingdom, seeds are often dormant until the correct season, heat, moisture and pH conditions are present. One documented example of this was a lotus seed that produced a healthy tree after 1300 years of dormancy.[4] More recently it was found that a seed from a 2000-year-old palm tree seed produced a viable sapling. http://news.nationalgeographic.com/news/2005/11/1122_051122_old_seed.html. Some forms of vegetation can germinate only after fires that may come decades apart. These include the giant sequoias native to the southwest United States as well as many other species of trees and grass. Most deciduous trees and plants have seasonal periods of dormancy during which they cease photosynthesis, a process called abscission. These periods of dormancy enhance plant survival by synchronizing growth to optimal conditions. Clearly this mechanism has evolved to time germination to optimal conditions, that is, not in a dry clay pot or when growth conditions will not be optimal for survival. Energy savings is not the only reason for dormancy in plants or animals.

Many unicellular organisms (protozoans) have evolved to live in environments that can only sustain them for portions of the year because of changes in temperature, water availability or other factors. Their survival requires that they enter dormant states that can be reversed when optimal conditions reappear.

A tiny colony of yeast trapped inside a Lebanese weevil covered in ancient Burmese amber for up to 45 million years has been brought back to life and used to brew a modern beer (http://www.foodinthefort.com/tag/raul-cano/). Rotifers, a group of small multicellular organisms of microscopic or submicroscopic size (up to 0.5 mm long) have extended dormant periods lasting from days to months in response to environmental stresses, including lack of water or food.[5,6]

Parasites can become dormant within an animal's tissues for years, emerging during periods when the immune system is compromised.[7] Some invertebrate parasites also have extended dormant periods, defending themselves by forming a protective cyst.[8] In some cases the cyst can only be dissolved and the parasite activated by digestive juices. Many sponges have a similar dormant state which allows

them to survive suboptimal conditions by being encased in "gemmules."

Insect dormancy or diapause can be seasonal lasting several months, and anecdotal reports indicate that it, under some conditions, can last for several years to as long as a century.[9] This can occur in an embryological, larval, pupal, or adult stage. During diapause insects are potentially vulnerable to predation, as are some sleeping animals. Passive defense strategies are employed, such as entering dormancy underground or in hidden recesses, having hard shells and tenacious attachment to substrates. In a few cases insects have evolved a vibrational defensive response which is elicited when pupae are disturbed. Land snails and slugs can secrete a mucus membrane for protection and enter a dormant state when conditions are not optimal.[10]

Reptiles and amphibia that live in lakes that either freeze or dry seasonally and snakes that live in environments with periods of cold or extreme heat have the ability to enter dormant states (called brumination in reptiles). These dormant periods may occur just during the cool portion of the circadian cycle or may extend for months in winter.[11]

In the mammalian class, a continuum of states ranging from dormancy to continuous activity can be seen. Small animals that cannot migrate long distances and live in temperate or frigid environments often survive the winter by hibernating. Some bats, many species of rodents, marsupials and insectivores hibernate. This condition is entered from, and generally terminates in, sleep periods. During hibernation, body temperature can be reduced to below 10° C to as low as −3° C with greatly reduced energy consumption.[12,13] Animals are quite difficult to arouse during hibernation, with arousal taking many minutes. Consequently, hibernators are vulnerable to predation and survive hibernation by seeking protected sites. Torpor[12] is another form of dormancy which can be entered by mammals and birds daily. Torpor is entered and exited through sleep and can recur in a circadian rhythm or can last for weeks or months. Animals in shallow torpor are less difficult to arouse than hibernating animals but are still unable to respond quickly when stimulated. Some other mammals such as bears have extended periods of sleep in the winter during which their metabolic rate and body temperature are reduced by 4° to 5° C,[14] but they remain more responsive than animals in torpor.

Sleep can be seen as a form of adaptive inactivity lying on this continuum. What is most remarkable about sleep is not the unresponsiveness or vulnerability it creates, but rather its ability to reduce activity and body and brain metabolism, but still allow a high level of responsiveness relative to the states of dormancy described previously. The often cited example of a parent arousing at a baby's whimper but sleeping through a thunderstorm illustrates the ability of the sleeping human brain to continuously process sensory signals during the sleep period and trigger complete awakening to significant stimuli within a few hundred milliseconds. This capacity is retained despite the great reduction in brain energy consumption achieved in sleep relative to quiet waking.[14a,15,16]

Adolescent humans are less responsive than adults to stimuli presented during sleep, as anyone who has raised teenagers can attest. This may have been selected for by evolution, because protection from predators is provided by older members of the family group who also tend to the nocturnal needs of infants. The inactivity of children benefits the group by reducing their relatively large portion of the food needs of the family.

The continuum from adaptive inactivity to high levels of activity can be seen within the life cycle of some animals. Thus, some animals that live in climates with a pronounced seasonal reduction in food or light availability or a periodic increase in threat from predators may need to migrate to survive. Many species of birds do this as do certain species of marine mammals (discussed later). Although some may maintain circadian rhythms of activity during migration, others remain continuously active for weeks or months. Some vertebrate species do not ever appear to meet the behavioral criteria for sleep, remaining responsive, or responsive and active, throughout their lifetime.[1]

Humans with insomnia, who are typically not sleepy during the day despite reduced sleep at night, may be viewed as falling closer to migrating animals or short sleeping animals, in contrast to humans with sleep disturbed by sleep deprivation, sleep apnea or pain, who are sleepy during the day.[17] Individuals with restless legs syndrome are similarly unlikely to be sleepy during the day despite low levels of nightly sleep. Conversely, many individuals with hypersomnia appear to need more sleep and sleep more deeply, rather than being the victims of shallow or disrupted sleep that is compensated for by extended sleep time.

To summarize, evolution has produced a wide range of forms of diurnal or seasonal adaptive inactivity, some of which are accompanied by a virtual cessation of metabolism and responsiveness. Clearly, evolution rewards judicious activity, not continuous activity. Sleep is often viewed as a liability because of its reduced alertness compared to quiet waking. However, seen in the context of adaptive inactivity shown by most species, what is most notable about sleep in humans is its intermediate status, between the highly inactive unresponsive states seen in rotifers, insects, and hibernating mammals (which show little neuronal activity during hibernation), and the virtually continuous periods of activity and waking that have been seen in migrating birds and cetaceans.

QUANTITATIVE ANALYSES OF THE CORRELATES OF SLEEP DURATION

An increasing number of studies have attempted to correlate the data that has been collected on sleep duration in mammals with a number of physiological and behavioral variables in order to develop hypotheses as to the function of sleep. The data these studies are based on are not ideal. Only approximately 60 mammalian species have been studied with sufficient measurements to determine the amounts of rapid eye movement (REM) and non-REM (NREM) sleep over the 24-hour period. These species are by no means a random sample of the more than 5,000 mammalian species. Rather they are species that are viable and available for study in laboratories or in some instances for noninvasive (and less accurate) studies in zoos. In laboratories, animal subjects for sleep studies are typically fed *ad libitum* and are on artificial light cycles at thermoneutral temperatures. These environments differ greatly from

those in which they evolved. Digital recording and storage technologies now exist that will enable the collection of polygraphic data on animals in their natural environment[18] but they have not yet been widely used. Such observations are necessary to determine the variation in sleep times caused by hunger, response to temperature changes, predation and other variables that have driven evolution. Very few of these animals have been tested for arousal threshold, the nature and extent of sleep rebound, and other aspects of sleep whose variation across species might contribute to an understanding of sleep evolution and function. An important issue in comparing sleep times in animals is determining sleep depth. In humans we know that sleep depth, as assessed by either arousal threshold or electroencephalogram (EEG) amplitude, increases after sleep deprivation and is often greater during early stages of development when total sleep time is greatest. Can sleep time be profitably compared across animals without incorporating information on sleep depth? Should we assume that animals that sleep for longer periods also sleep more deeply as is true across human development, or should we assume the reverse, that short sleeping animals sleep more deeply as has been hypothesized?[19]

One of the earliest studies comparing REM and NREM sleep durations with physiological variables, found that sleep duration was inversely correlated with body mass.[20,21] A subsequent analysis found that this relationship applied only to herbivores, not to carnivores or omnivores.[3] This study also showed that, as a group, carnivores slept more than omnivores, who in turn slept more than herbivores (Fig. 10-1). Significant negative correlations were found between brain weight and REM sleep time (but not total sleep time). It should be emphasized that this latter correlation was extremely small, accounting for only 4% of the variance in REM sleep time between species (Fig. 10-2). The largest correlation emerging from these early studies was that between body or brain mass and the duration of the sleep cycle, that is, the time from the start of one REM sleep period to the start of the next, excluding interposed waking. This correlation accounted for as much as 80% of the variance in sleep-cycle time between animals and has held up in subsequent studies in mammals. Sleep cycle time is about 10 minutes in mice, 90 minutes in humans, and 2 hours in elephants. Another robust correlation in the Zepelin study was between adult REM sleep time and immaturity at birth, that is, animals that are born in a relatively helpless state have greater amounts of REM sleep as adults than animals born in a more mature state. Because sleep is linked to a reduction in body temperature[22] and a reduction in energy usage, it has been hypothesized that energy conservation may be a function of sleep.[23]

Several studies have reanalyzed the phylogenetic data set with the addition of the few more recently studied animals. These studies took a variety of strategies to extract relations from this data set. Lesku et al.[24] used a method of "independent contrasts" in an attempt to control for the relatedness of species being compared. They confirmed prior findings of a negative relationship between basal metabolic rate (which is correlated with body mass) and sleep time. In contrast to earlier and subsequent studies of the same data set, they reported a positive correlation between REM sleep and relative brain mass and a negative relationship between REM sleep time and predation risk.

Another recent study, confining its analysis to studies that met what they felt were more rigorous criteria, found that metabolic rate correlates negatively rather than positively with sleep quotas,[25] in contrast to earlier studies.[21] This result is not inconsistent with some prior work.[3] They also reported that neither adult nor neonatal brain mass correlates positively with adult REM or NREM sleep times, differing from earlier studies.[21,25] They find, in agreement with prior analyses, that animals with high predation risk sleep less.[3,26] In keeping with the concept that there is some fixed need for an unknown function preformed only during sleep, they propose that short-sleeping species sleep more intensely to achieve this function in less time, but they present no experimental evidence for this hypothesis.

A notable feature of the Lesku et al. study and the Capellini et al. studies is that both excluded animals that they decided had unusual sleep patterns. So the echidna, which combines REM and NREM features in its sleep[27] was eliminated from the analysis. The platypus, which has the largest amount of REM sleep of any animal yet studied[28] was also excluded from this analysis as it was from another study focusing on brain size relations.[29] The dolphin and three other cetacean species and two species of manatee were excluded from the Lesku et al. study because of their low levels of REM sleep and unihemispheric slow waves. Including these species in their analyses would undoubtedly negate or reverse the positive relationship they report between brain size and REM sleep, because the platypus has the largest amount of REM sleep time of any studied animal and one of the smallest brain sizes, and the dolphin, which appears to have little or no REM sleep, has a larger brain size than humans. As I will discuss hereafter, these "unusual" species that have been excluded from prior analyses may in fact hold the most important clues to the function of sleep across species.

In considering the possibility of universal functions of sleep across species, from humans to drosophila, it is important to appreciate the presence of REM and NREM sleep in birds. A recent correlational analysis of sleep parameters in birds, which paralleled the studies done in mammals, found no relationship between brain mass, metabolic rate, relative metabolic rate, maturity at birth and total sleep time or REM sleep time.[30] All values for these parameters were found to be "markedly nonsignificant." The only significant relation found was a negative correlation between predation risk and NREM sleep time (but not REM sleep time), in contrast to the relation reported previously in mammals between predation risk and REM sleep time (but not NREM sleep time). This lone significant relation explained only 27% of the variance in avian NREM sleep time. To summarize, a variety of correlation studies reach disparate and often opposite conclusions about the physiological and functional correlates of sleep time. It should be emphasized that with the exception of the strong relationship between sleep cycle length and brain and body mass, all of the "significant" correlations reported explain only a small portion of the variance in sleep parameters, throwing into question whether the correlational approach as currently used is getting at the core

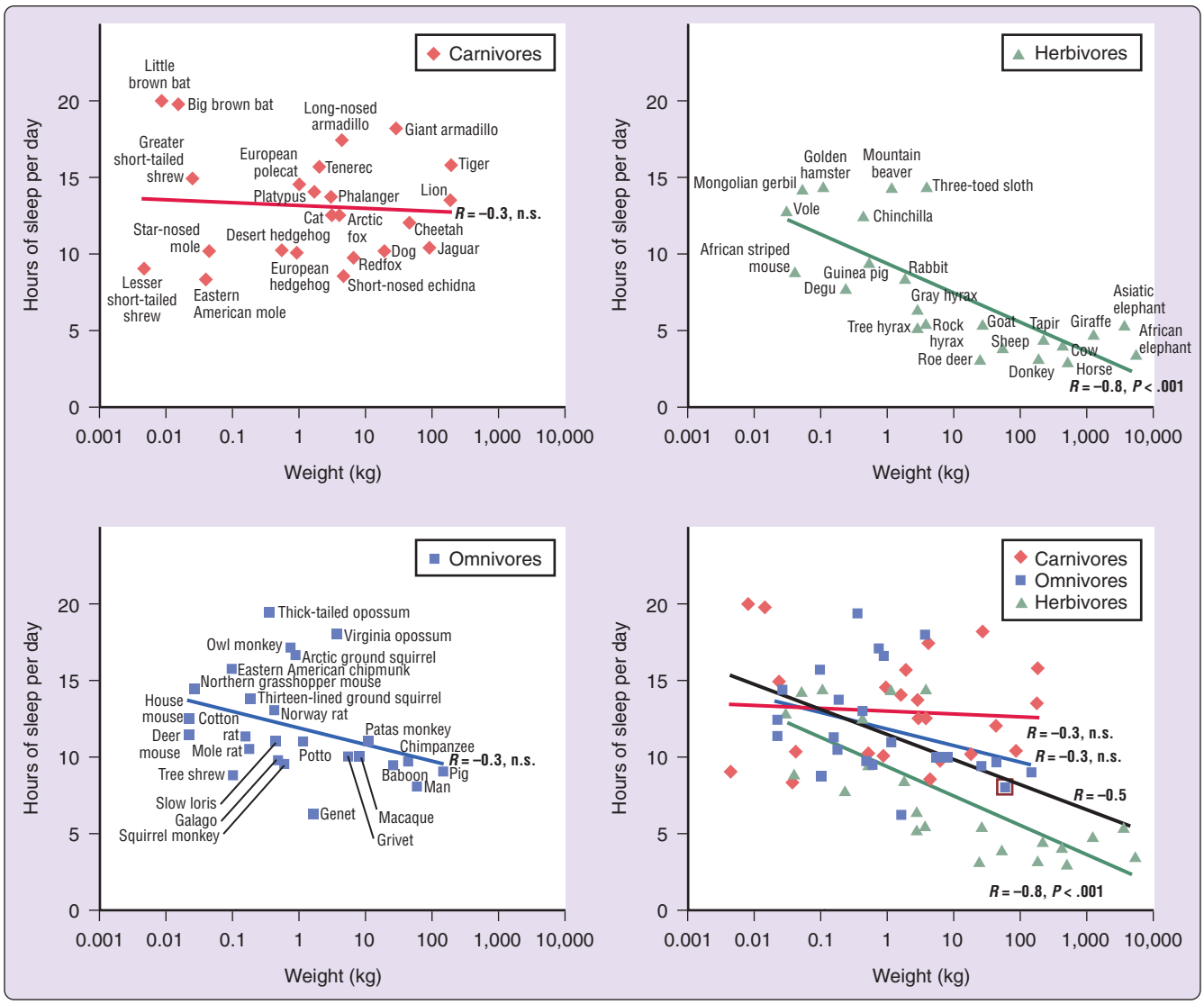
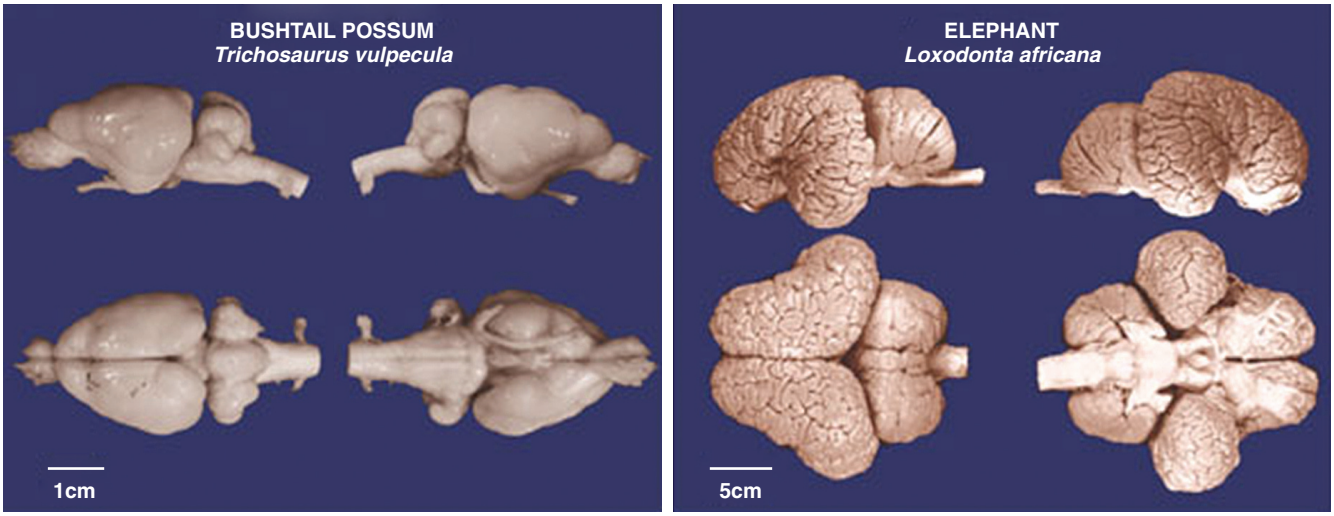


Figure 10-1 Sleep time in mammals. **A**, Carnivores are shown in dark red; **B**, herbivores are in green and **C**, omnivores in grey. Sleep times in carnivores, omnivores and herbivores differ significantly, with carnivore sleep amounts significantly greater than those of herbivores. Sleep amount is an inverse function of body mass over all terrestrial mammals (*black line*). This function accounts for approximately 25% of the interspecies variance (**D**) in reported sleep amounts. Herbivores are responsible for this relation because body mass and sleep time were significantly and inversely correlated in herbivores, but were not in carnivores or omnivores. Small red box in the combined figure (lower right) indicates human data point. (From Siegel JM. Clues to the function of mammalian sleep. Nature 2005;437:1264-1271.)

issues of sleep function. Despite similar genetics, anatomy, cognitive abilities and physiological functioning, closely related species can have very different sleep parameters and distantly related species can have very similar sleep parameters. Many such examples exist despite the relatively small number of species in which REM and NREM sleep time have been determined (Fig. 10-3).

THE DIVERSITY OF SLEEP

On the assumption that sleep satisfies an unknown, yet universal function in all animals, recent work has been carried out on animals whose genetics and neuroanatomy is better understood and more easily manipulated than that of mammals. Much of this work has focused on the fruit fly, *Drosophila melanogaster*. These animals appear to meet the behavioral definition of sleep. Their response threshold is elevated during periods of immobility but will rapidly "awaken" when sufficiently intense stimuli are applied. They make up for "sleep" deprivation with a partial rebound of inactivity when left undisturbed. However, major differences between the physiology and anatomy of these organisms and mammals make it difficult to transfer insights gleaned from studies of drosophila sleep to human sleep. The *Drosophila* brain does not resemble the vertebrate brain. Octopamine, a major sleep regulating transmitter in drosophila does not exist in mammals. Hypocretin, a major sleep regulating transmitter in mammals does not exist in *Drosophila*.[31] *Drosophila* are not homeotherms, whereas thermoregulation has been closely linked to fundamental aspects of mammalian sleep.[3,22,32] There is no evidence for the occurrence of a state resembling REM sleep in *Drosophila*. Thus the neurochemistry, neuroanatomy, and neurophysiology of sleep must necessarily differ



18 hours of sleep, 6.6 hours of REM

3.6 hours of sleep, 1.8 hours of REM

Figure 10-2 Sleep amount is not proportional to the relative size of the cerebral cortex or to the degree of encephalization, as illustrated by these two examples. (From Siegel JM. Clues to the function of mammalian sleep. Nature 2005;437:1264-1271.)

among *Drosophila*, man, and other mammals. Any commonality of sleep phenomena would have to be restricted to cellular level processes. Two recent studies have shown that drosophila sleep and sleep rebound is markedly impaired by genetic alteration of a potassium current that regulates neuronal membrane excitability.[33,34] Regulation of potassium currents may be a core function of sleep or it may instead affect the excitability of circuits regulating activity and quiescence, just as such currents affect seizure susceptibility.[35,36]

Caenorhabditis elegans, a roundworm with a nervous system much simpler than that of *Drosophila*, has also been investigated for sleeplike behavior.[37] *C. elegans* reaches adulthood in 60 hours and has periods of inactivity during this maturation called "lethargus" occurring before each of the four molts it undergoes prior to reaching maturity. Stimulation of *C. elegans* during the lethargus period produced a small but significant decrease in activity during the remainder of the lethargus period, but did not delay the subsequent period of activity or increase quiescence overall, phenomena that differ from the effects of sleep deprivation in mammals. It is not clear if adult *C. elegans* show any aspect of sleep behavior.[38]

Fundamental species differences in the physiology and neurochemistry of sleep have been identified even within the mammalian line. Although there are many similarities, the EEG aspects of sleep also differ considerably between humans, rats, and cats, the most studied species.[39-41] Human stage 4 NREM sleep is linked to growth hormone secretion. Disruption of stage 4 sleep in children is thought to cause short stature. However, in dogs, growth hormone secretion normally occurs in waking, not sleep.[42] Melatonin release is maximal during sleep in diurnal animals, but is maximal in waking in nocturnal animals.[43] Erections have been shown to be present during REM sleep in humans and rats,[44] however the armadillo has erections only in NREM sleep.[45] Blood flow and metabolism differ dramatically between neocortical regions in adult human REM sleep,[46] although most animal sleep deprivation and sleep metabolic studies treat the neocortex as a unit. Lesions of parietal cortex and certain other regions prevent dreaming in humans, even in individuals continuing to show normal REM sleep as judged by cortical EEG, rapid eye movements and suppression of muscle tone.[47] Humans before age 6 do not have dream mentation, perhaps because these cortical regions have not yet developed.[48] These findings make it questionable whether nonhuman mammals that have REM sleep, all of which have cortical regions whose structure differs from that of adult humans, have dream mentation.

SLEEP IN MONOTREMES

The mammalian class can be subdivided into three subclasses: placentals, marsupials, and monotremes. There are just three extant monotreme species, the short beaked and long beaked echidna and the platypus (Video 10-1). Fossil and genetic evidence indicates that the monotreme line diverged from the other mammalian lines about 150 million years ago and that both echidna species are derived from a platypus-like ancestor.[49-52] The monotremes have shown a remarkably conservative evolutionary course since their divergence from the two other mammalian lines. For example, fossil teeth from *Steropodon galmani* dated at 110 million years ago show many similarities to the vestigial teeth of the current day platypus, *Ornithorhyncus anatinus*.[53] Analyses of fossilized skull remains indicate remarkably little change in platypus morphology over at least 60 million years.[53,54] The low level of speciation throughout the fossil record is another indicator of the uniquely conservative lineage of monotremes. The 150 million years of platypus evolution has produced no species radiation, apart from the echidna line, and there are only two living and one extinct species of echidna. Although monotremes are distinctly mammalian, they do display a number of reptilian features, making study of their physiology a unique opportunity to determine the commonalities and divergences in mammalian evolution.[50,55,56]

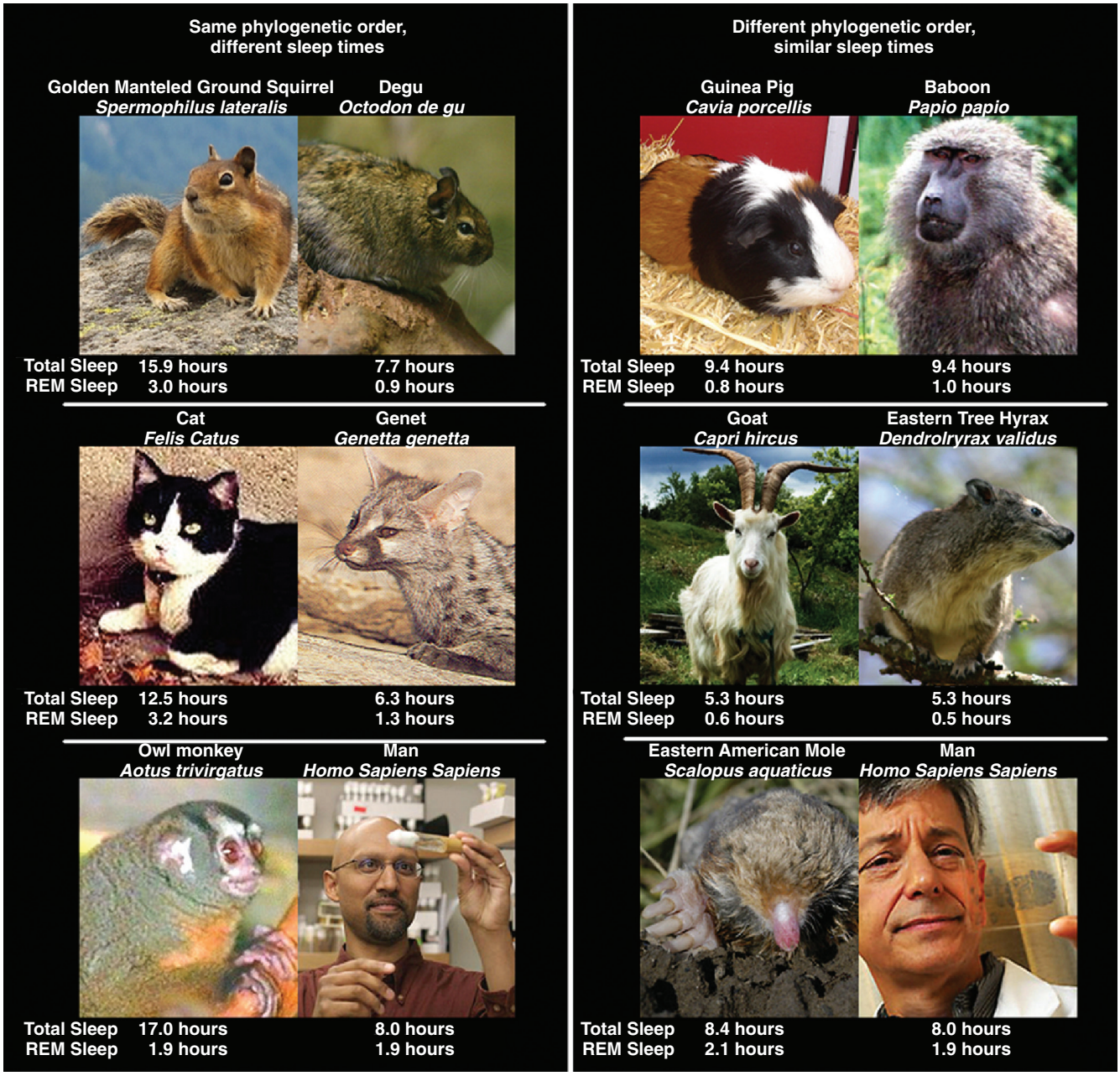


Figure 10-3 Mammalian phylogenetic order is not strongly correlated with sleep parameters. Despite similar genetics and physiology, sleep times within mammalian orders overlap extensively. On the *left* are three pairs of animals that are in the same order but have very different sleep parameters. On the *right* are three pairs of animals from different orders with similar sleep amounts. Mammalian sleep times are not strongly correlated with phylogenetic order. (From Allada R, Siegel JM. Unearthing the phylogenetic roots of sleep. Curr Biol 2008;18:R670-R679.)

This phylogenetic history led to an early study of the echidna to test the hypothesis that REM sleep was a more recently evolved sleep state. No clear evidence of the forebrain low-voltage EEG that characterizes sleep was seen in this study, leading to the tentative conclusion that REM sleep evolved in placentals and marsupials after the divergence of the monotreme line from the other mammals.[57] We reexamined this issue using single neuron recording techniques, in addition to the EEG measures employed in the prior studies. REM sleep is generated in the mesopontine brainstem (see Chapter 8) and is characterized by highly variable burst pause activity of brainstem neurons. This activity is responsible for driving the rapid eye movements, twitches, and other aspects of the REM sleep. We recorded from these brainstem regions in unrestrained echidnas to see if this activation was absent throughout sleep. We found that instead of the slow, regular activity that characterizes brainstem neurons in many nuclei during NREM sleep in placental mammals,[58,59] the echidna showed the irregular activity pattern of REM sleep throughout most of the sleep period (Fig. 10-4).[27,60] It appeared that the brainstem was in an REM sleeplike state, whereas the forebrain was in an NREM sleep state. Other investigators

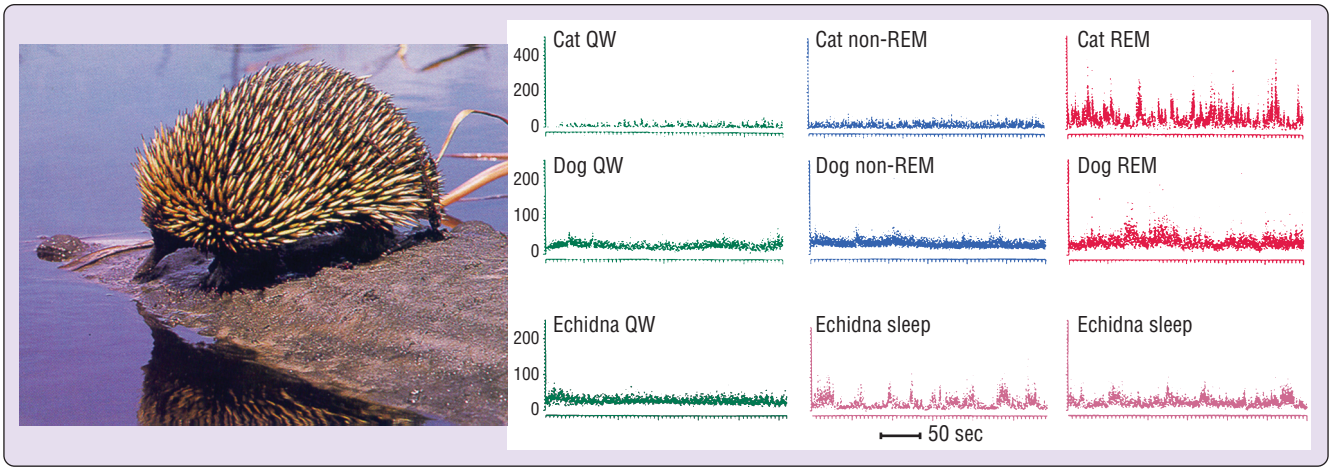


Figure 10-4 Brainstem activation during sleep in the echidna. Instantaneous compressed rate plots of representative units recorded in nucleus reticularis pontis oralis of the cat, dog, and echidna. Each point represents the discharge rate for the previous interspike interval. In cat QW and non-REM sleep, the discharge rate is low and relatively regular. The rate increases and becomes highly variable during REM sleep. A similar pattern can be seen in a unit recorded in the dog. In the echidna, sleep is characterized by variable unit discharge rates as is seen in REM sleep, but this occurs while the cortex is showing high-voltage activity. (From Siegel JM, Manger PR, Nienhuis R, et al. The echidna *Tachyglossus aculeatus* combines REM and non-REM aspects in a single sleep state: implications for the evolution of sleep. J Neurosci 1996;16:3500-3506.)

also concluded that the echidna had an REM sleeplike state.[61]

These findings encouraged us to perform electrophysiological studies of sleep in the platypus. We found that the platypus had pronounced phasic motor activity typical of that seen in REM sleep (see video on PPSM web-site and at http://www.npi.ucla.edu/sleepresearch/media.php). This intense motor activity could occur while the forebrain EEG exhibited high-voltage activity,[28] similar to the phenomena seen in the echidna. Not only was the motor activity during sleep equal to or greater in intensity than that seen in REM sleep in other animals, but also the daily amount of this REM sleep state was greater than that in any other animal. However, unlike the condition in adult placental and marsupial mammals, the signs of REM sleep were largely confined to the brainstem (Fig. 10-5). This bears some resemblance to the conditions in most mammals that are born in an immature (altricial) state, which do not show marked forebrain EEG activation during REM sleep early in life. The tentative conclusion reached in the initial studies of the echidna, that the monotremes had no REM sleep and that REM sleep was a recently evolved state, had to be reversed. It appears that a brainstem manifestation of REM sleep was most likely present in the earliest mammals, perhaps in very large amounts. It may be the brainstem quiescence of NREM sleep, and likely reduction in brain energy consumption that is the most recently evolved aspect of sleep in the mammalian line.

REINDEER

Reindeer are ruminants. Like other ruminants they appear to remain active over the entire circadian cycle to an extent not seen in most carnivores and omnivores. A recent study examined the activity of two species of reindeer living in polar regions where they experience periods of continuous darkness in the winter and continuous light in the summer. Activity was monitored for an entire year. This was the first such study and contrasts with the constant conditions of light and temperature usually employed in laboratory studies. It was found that the circadian rhythm of melatonin and circadian rhythms of behavioral activity dissipated in winter and summer. It was also reported that activity time was from 22% to 43% greater in summer than in winter (calculated from Figure 4 in van Oort and Tyler[62]).[63] EEG recording and arousal threshold tests were not done, however, the activity changes suggest that major changes in sleep duration occur seasonally.

BIRDS

Birds have REM sleep that appears physiologically very similar to that seen in mammals, although REM sleep values tend to be lower in comparison to total sleep values than in mammals.[30] Many bird species migrate over long distances. The effect of this migratory behavior on sleep has been studied in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). These birds, even when confined in the laboratory, decrease sleep time by two thirds during the periods when they would normally be migrating.[64] It should be noted that this is a common feature of cycles of adaptive inactivity; for example, a ground squirrel that normally hibernates in the winter will enter a state of torpor at the appropriate season even when maintained in a laboratory under constant conditions.[65]

During the migratory period, the sparrow's learning and responding was unimpaired or improved. Their sleep was not deeper by EEG criteria than that seen when they were not migrating, despite its greatly reduced duration. Their sleep latency did not differ from that during nonmigrating periods.[64]

The observations in monotremes and birds suggest that the reptilian common ancestor of both mammals and birds had REM sleep or a closely related precursor state, rather than the previously advanced speculation that REM sleep must have evolved twice based on the prior conclusion that monotremes did not have REM sleep. Although there

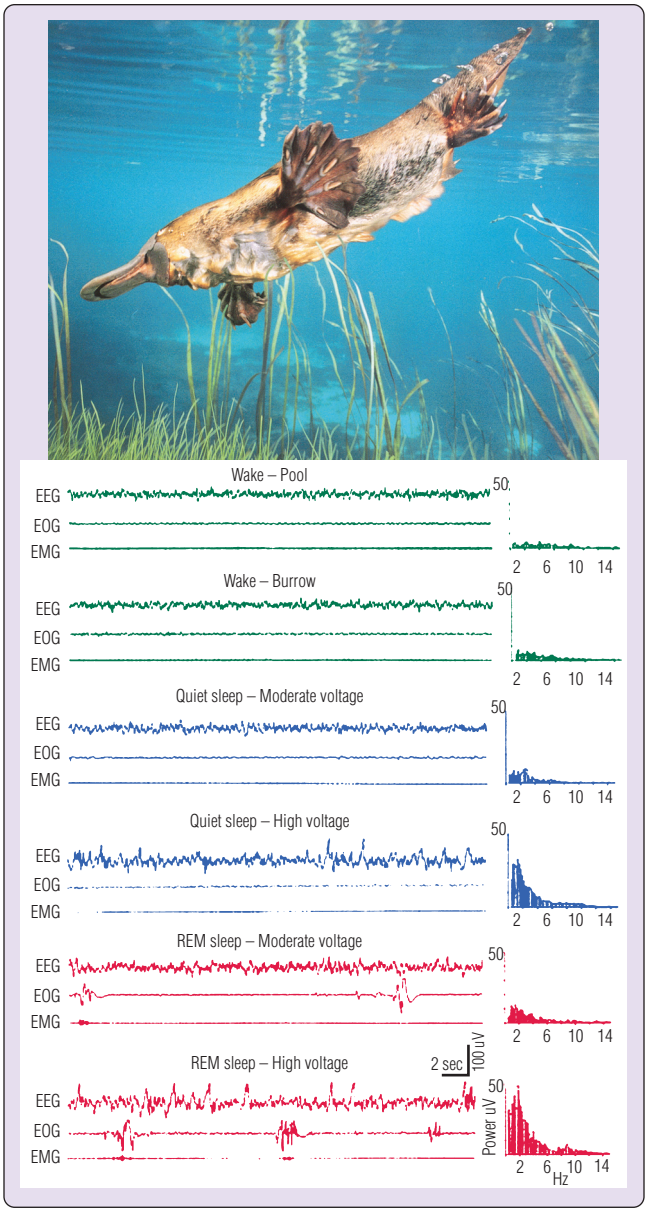


Figure 10-5 Brainstem REM sleep state in the platypus. Rapid eye movements and twitches can occur while the forebrain is showing a slow-wave activity pattern. EEG, EOG, EMG and EEG power spectra of samples shown of sleep–wake states in the platypus. (From Siegel JM, Manger PR, Nienhuis R, Fahringer HM, et al. Sleep in the platypus. Neuroscience 1999; 91:391-400.)

were scattered early reports claiming to have seen REM sleep in reptiles, these have not been replicated.[3] We applied the same recording techniques we had used in the echidna to the turtle in a search for evidence of REM sleep. We saw no evidence of phasic brainstem neuronal activity during quiescent states in this reptile.[66]

WALRUS

A recent study of the walrus revealed that these animals frequently become continuously active for periods of several days even when fed *ad libitum* and under no apparent stress.[67] Such behaviors have not been reported in terrestrial mammals, although they cannot be ruled out. Animals living in marine environments may not be as strongly affected by circadian variables because their evolution has been shaped by tidal and weather features that do not adhere to 24-hour cycles.

SLEEP IN CETACEANS (DOLPHINS AND WHALES)

REM sleep is present in all terrestrial animals that have been studied, but signs of this state have not been seen in cetaceans, which are placental mammals. These animals show only unihemispheric slow waves (USW), which can be confined to one hemisphere for 2 hours or longer. The eye contralateral to the hemisphere with slow waves is typically closed, although covering the eye is not sufficient to produce slow waves in the contralateral hemisphere.[28,68] They never show persistent high voltage waves bilaterally. Sometimes they float at the surface while showing USW. However, often they swim while USW are being produced (Fig. 10-6). When they swim while having USW, there is no asymmetry in their motor activity, in contrast to the behavior seen in the fur seal. Regardless of which hemisphere is showing slow wave activity, they tend to circle in a counterclockwise direction (in the northern hemisphere[69]). No evidence has been presented for elevated sensory response thresholds contralateral to the hemisphere that has slow waves. Indeed it seems that a substantial elevation of sensory thresholds on one side of the body would be quite maladaptive given the danger of collisions while moving. Similarly, brain motor systems must be bilaterally active to maintain the bilaterally coordinated movement. Therefore, forebrain and brainstem sensory and motor activity must differ radically during USW from that seen in terrestrial mammals during sleep (see Chapter 8).[58,59] The one study of USW rebound after USW deprivation in dolphins produced very variable results, with little or no relation between the amount of slow waves lost in each hemisphere and the amount of slow waves recovered when the animals were subsequently left undisturbed.[70] In another study it was shown that dolphins are able to maintain continuous vigilance for 5 days with no decline in accuracy. At the end of this period there was no detectable decrease of activity or evidence of inattention or sleep rebound such as would be expected of a sleep deprived animal.[1,71]

Unihemispheric slow waves would be expected to save nearly one half of the energy consumed by the brain that is saved during bilateral slow wave sleep (BSWS).[15,16] Unihemispheric slow waves are well suited to the dolphins' group activity patterns. Because dolphins and other cetaceans swim in pods, the visual world can be monitored by dolphins on each side of the pod and the remaining dolphins merely have to maintain contact with the pod. In routine "cruising" behavior this can be done with only one eye, allowing the other eye and connected portions of the brain to reduce activity as occurs in USW. This hypothesis needs to be explored by electroencephalographic observations of groups of cetaceans in the wild.

In some smaller cetaceans, such as the harbor porpoise[72] and Commerson's dolphin,[73] motor activity is essentially continuous from birth to death, that is, they never float or sink to the bottom and remain still. They move rapidly and

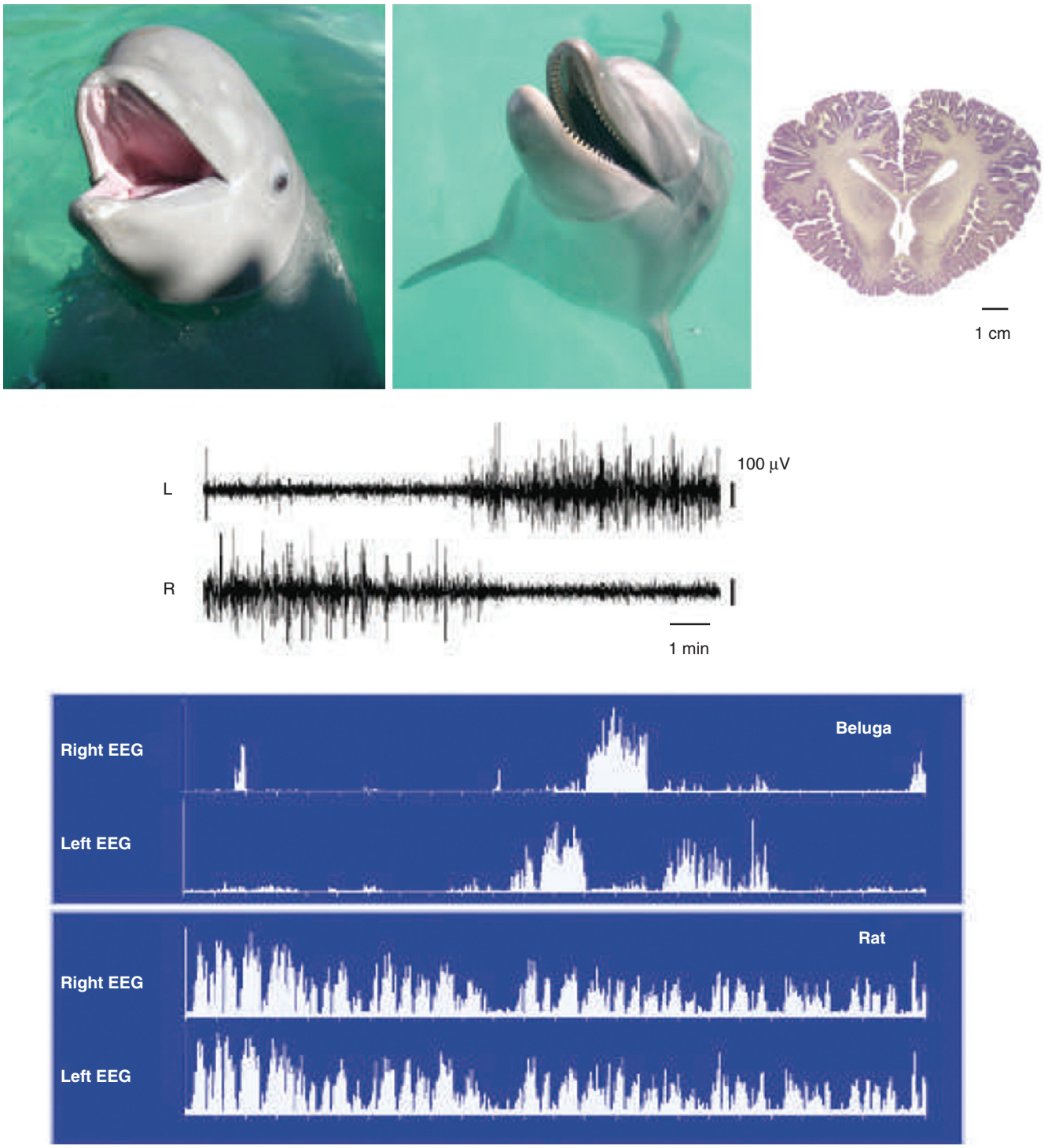


Figure 10-6 Cetacean sleep, unihemispheric slow waves in cetaceans. *Top*, photos of immature beluga, adult dolphin and section of adult dolphin brain. Electroencephalogram (EEG) of adult cetaceans, represented here by the beluga, during sleep are shown. All species of cetacean so far recorded have unihemispheric slow waves. *Top traces* show left and right EEG activity. The spectral plots show 1- to 3-Hz power in the two hemispheres over a 12-hour period. The pattern in the cetaceans contrasts with the bilateral pattern of slow waves seen under normal conditions in all terrestrial mammals, represented here by the rat (*bottom traces*). (From Siegel JM. Clues to the function of mammalian sleep [review]. Nature 2005;437:1264-1271.)

it is evident that they must have accurate sensory and motor performance and associated brain activation to avoid collisions. It is difficult to accept this behavior as "sleep" without discarding all aspects of the behavioral definition of sleep.[1]

All studied land mammals have been reported to show maximal sleep and maximal immobility at birth, leading to the conclusion that sleep is required for brain and body development. However, newborn killer whales and dolphins are continuously active. In captivity, they swim in

tight formation and turn several times a minute to avoid conspecifics in the pool and pool walls. During this period the calves learn to nurse, breathe and swim efficiently. Although some USWs might be present at these times, the eyes are open bilaterally when they surface at average intervals of less than 1 minute, indicating that any slow wave pattern could not last longer than this period.[74] Sleep interruption at such intervals can be lethal to rats,[75] and human sleep is not restorative if interrupted on such a schedule.[76] The cetacean mothers also cease eye closure at the surface and floating behavior and are continuously active during the postpartum period. No loss of alertness is apparent during the "migratory" period. In the wild, mother and calf migrate together, typically for thousands of miles from calving to feeding grounds. Sharks, killer whales, and other predatory animals target the migrating calves and a high level of continuous alertness is necessary for both mother and calf during migration. One could describe the maternal and neonatal pattern as "sleep" with well coordinated motor activity, accurate sensory processing, effective response to threats in the environment, and without the likelihood of any EEG slow waves or eye closure lasting more than 60 seconds.[77,78] However, this does not comport with the accepted behavioral definition of sleep.[79] Thus both cetaceans and migrating birds greatly reduce sleep time during migrations without any sign of degradation of physiological functions, sluggishness, loss of alertness, or impairment of cognitive function.

SLEEP IN OTARIIDS (EARED SEALS)

On land, sleep in the fur seal generally resembles that in most terrestrial mammals. The EEG is bilaterally synchronized and the animal closes both eyes, appears unresponsive and cycles between REM and NREM sleep. In contrast, when the fur seal is in the water, it usually shows an asymmetrical pattern of behavior with one of the flippers being active in maintaining body position, while the other flipper is inactive. The fur seal can have slow waves in one hemisphere with the contralateral eye being closed. The other eye is generally open or partially open (Fig. 10-7). Therefore, it appears that half of the brain and body may be "asleep" and the other half "awake." A microdialysis study showed that during asymmetrical sleep, the waking hemisphere has significantly higher levels of acetylcholine release than the sleeping hemisphere.[80]

SLEEP REBOUND

Sleep rebound is not always seen. When the fur seal goes in the water for extended periods, as they do in winter, REM sleep time is greatly reduced. There is little or no rebound of lost REM sleep when the fur seal returns to land, even after several weeks in the water.[81] In the cases of the dolphins and killer whales mentioned previously, a near total abolition of sleep for periods of several weeks during migration is followed by a slow increase back to baseline levels with no rebound. The same phenomenon is seen in migrating white sparrows, a species that has been carefully studied under laboratory conditions.[64] Manic humans greatly reduce sleep time for extended periods, and there is no persuasive evidence for progressive degradation of performance, physiological function, or sleep rebound during this period. Zebrafish can be completely deprived of sleep for three days by placing them in continuous light, but show no rebound when returned to a 12-12 light–dark cycle.[82] On the other hand, when they are deprived by repetitive tactile stimulation they do show rebound.

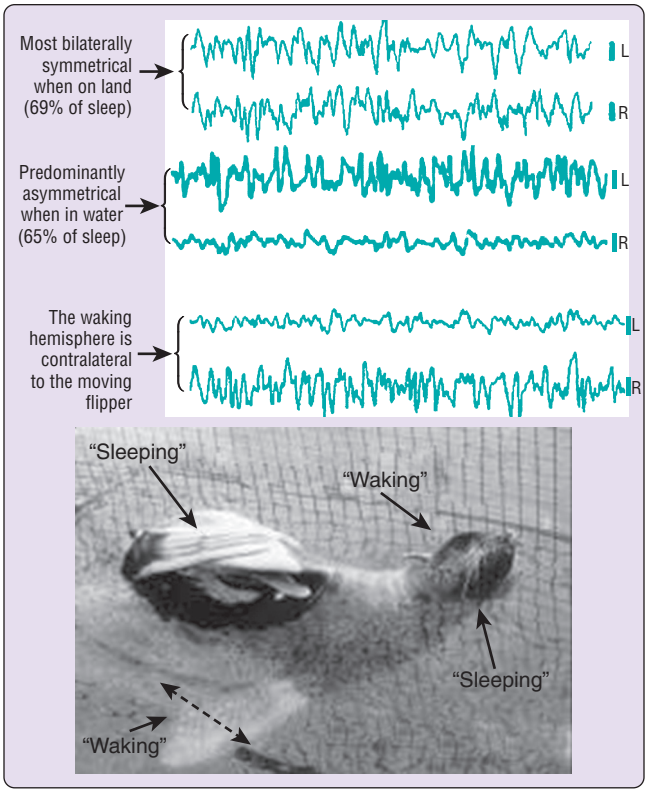


Figure 10-7 Fur seal sleep. On land fur seals usually sleep like terrestrial mammals, with bilateral EEG synchrony and REM sleep (not shown in the figure). However, when in water they typically show asymmetrical slow-wave sleep, with a sleeplike EEG in one hemisphere while the other hemisphere has a wakinglike EEG. Unlike the dolphin, the asymmetrical EEG of the fur seal is accompanied by asymmetrical posture and motor activity, with the flipper contralateral to the hemisphere with low-voltage activity used to maintain the animal's position in the water while the other flipper and its controlling hemisphere "sleep."

Typically 30% or less of lost sleep is recovered in the human and rodent studies, in which the phenomenon has been most extensively studied. A similar percent of rebound is seen in other species including some invertebrates (see Chapter 9). One may ask why, if sleep is essentially a maladaptive state, animals that have the ability to regain lost sleep in 30% of the time it would normally have taken have not evolved shorter sleep times to take advantage of the adaptive benefits of increased waking. However, if sleep is viewed as a form of "adaptive inactivity," then this paradox vanishes. A small sleep rebound may be necessary to compensate for processes that can only occur, or occur optimally, in sleep, but most sleep time is determined in each species by the evolved trade-offs between active waking and adaptive inactivity.

The variation in rebound within and across species needs to be more carefully studied. Some aspects of

rebound have been shown to be due to the deprivation procedure rather than the sleep loss. For example stressing rats by restraint can produce increased REM sleep even when no sleep has been lost. This is mediated by the release of pituitary hormones.[83,84] It is possible that in some species other aspects of rebound are driven by hormonal release linked to sleep rather than by some intrinsic property of sleep.

CONCLUSION

Sleep can be seen as an adaptive state, benefiting animals by increasing the efficiency of their activity. Sleep does this by suppressing activity at times that have maximal predator risk and permitting activity at times of maximal food and prey availability and minimal predator risk. It also increases efficiency by decreasing brain and body metabolism. However, unlike the dormant states employed in plants, simple multicellular organisms, and ectothermic organisms, and the hibernation and torpor employed in some mammals and birds, sleep allows rapid arousal for tending to infants, dealing with predators, and responding to environmental changes. A major function of REM sleep may be to allow this rapid response by periodic brainstem activation. Many organisms can reduce sleep for long periods of time without rebound during periods of migration or other periods in which a selective advantage can be obtained by continuous waking.

The big brown bat specializes in eating mosquitoes and moths that are active from dusk to early evening. The big brown bat typically is awake only about 4 hours a day.[21] Not surprisingly, this waking is synchronized to the period when flies are active. It is not likely that this short waking period, one of the shortest yet observed, can be explained by the need for some time consuming unknown process that occurs only during sleep and requires 20 hours to complete. It can be more easily explained by the ecological specializations of this bat. Similarly sleep in ectothermic animals is most likely determined by temperature and other environmental variables, rather than any information processing or physiological maintenance requirement. An approach that takes the environmental conditions in which each species evolved into account can better explain the variance in sleep time between mammals.

Many vital processes occur in both waking and sleep including recovery of muscles from exertion, control of blood flow, respiration, growth of various organs and digestion. Some may occur more efficiently in sleep, but can also occur in waking. It is highly probable that some functions have migrated into or out of sleep in various animals. Recent work has suggested that neurogenesis,[85] synaptic downscaling,[86] immune system activation[87] and reversal of oxidative stress[88,89] may be accomplished in sleep in mammals. It remains to be seen if these or any other vital functions can only be performed in sleep. However, this review of the phylogenetic literature suggests that such functions cannot explain the variation of sleep amounts and the evident flexibility of sleep physiology within and between animals. Viewing sleep as a period of well-timed adaptive inactivity that regulates behavior may better explain this variation.

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

❖ Clinical Pearl

Although sleep and sleep stages differ in amount between species, human sleep does not appear to be qualitatively unique. This factor makes animal models suitable for the investigation of many aspects of pharmacology and pathology.

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