NO SIMPLE SLUMBER

Exploring the Enigma of Sleep

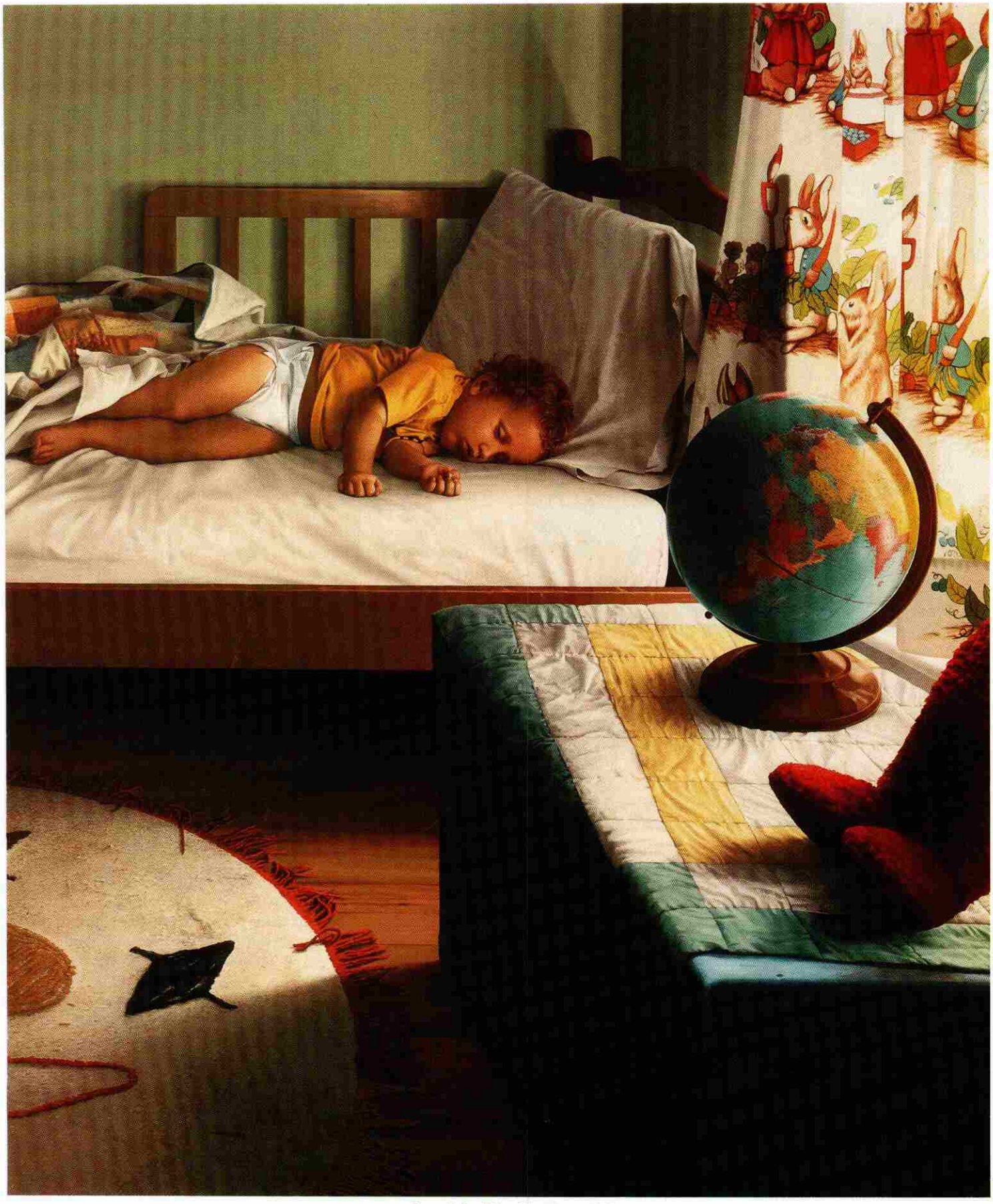
by JAMES M. KRUEGER

DURING THE SECOND ACT of Shakespeare's *Macbeth*, the usurping thane of Cawdor whispers to his wife that he has "done the deed"—that, according to their plan, he has murdered the sleeping King Duncan and thus has cleared his own path to the throne of Scotland. Though the remorseless Lady Macbeth implores her husband to "consider it not so deeply," regicide does not sit well with him. Guilt-ridden, he raves:

> Methought I heard a voice cry "Sleep no more!
> Macbeth does murder sleep"—the innocent sleep,
> Sleep that knits up the ravelled sleave of care,
> The death of each day's life, sore labor's bath,
> Balm of hurt minds, great Nature's second course,
> Chief nourisher in life's feast.

Throughout the play, Shakespeare portrays sleep's contradictory nature—at once soothing and haunting, restorative and tormenting (as in one of the more famous scenes, when Lady Macbeth, at last wracked by guilt, sleepwalks, rubbing her hands together, as if to wash the blood from them, and crying aloud: "Out, damned spot!"). Sleep in *Macbeth* is also a time of death, whether the death "of each day's life" or the murder of a king. Shakespeare was neither the first nor the last to link slumber and mortality; his reference to sleep as "death's counterfeit" echoes both Homer, who, in *The Iliad*, called sleep and death "twin brothers," and Ovid, who wrote, in *The Amores*, "What else is sleep but the image of chill death?" After Shakespeare, the seventeenth-century author and physician Thomas Browne mused, in his *Religio Medici*, that "sleepe... is that death by which we may be literally said to die daily." And, not quite two hundred years later, in *Queen Mab*, Shelley remarked, "How wonderful is Death,/Death and his brother Sleep!"

Intimations of mortality notwithstanding, it is common wisdom that sleep is, in fact, the "chief nourisher in life's feast"—the possessor of some essential, recuperative power. And, indeed, a good night's rest has for centuries been a cornerstone of preventive medicine—an observation based largely on the subjective experience that most people feel better when they have slept eight hours than when they have slept substantially less. There have been



Scott Prior, Max Sleeping, *1987*

Joseph Raffael, Lannis in Sieste VIII, *1988*

exceptions, of course. Thomas Edison is said to have resented sleep's hold and to have trained himself to get by on three or four hours of sleep a night—with the help of a midday nap. Similarly, Salvador Dali claimed to have cheated long-term sleep with short-term napping: He liked to doze off holding a spoon above a tin plate. At the moment the spoon slipped from his grasp and struck the plate, the surrealist would awaken, a new man.

But whether we view sleep as benign or malevolent, it draws each of us helplessly into its arms; the average person spends one-third of his existence (which, over the course of a normal life span, amounts to about twenty-five years) asleep. Yet, until recently, science understood surprisingly little about sleep's physiological cause and effects. There has been no shortage of theories—some involving the brain, some the circulation, and others a combination of both—but on the whole, sleep remains largely the same enigma described two hundred years ago by Samuel Johnson (who regularly slept until noon): "No searcher has yet found either the efficient or final cause; or can tell by what power the mind and body are thus chained down in irresistible stupefaction; or what benefits the animal receives from this alternate suspension of its active powers."

Still, a growing body of research is coloring in some of the blank spaces in our picture of sleep. Fittingly, considering the centuries of mystery that have shrouded it, sleep is no simple matter. Rather, as recent studies indicate, it results from a complicated series of biochemical reactions involving many parts of the brain, various kinds of cells, and the immune system.

SOME twenty-four hundred years ago, Hippocrates advised that a patient "should follow our natural habit and spend the day awake and the night asleep. If this habit be disturbed, it is not so good. . . . It is worst of all when he sleeps neither night nor day." Several times in writings attributed to the Greek physician, sleeplessness (as well as too much sleep) is cited as a sign that something is amiss. Noting the cooling of the limbs experienced by sleepers, Hippocrates concluded that sleep is caused by the retreat of blood and warmth into the body's inner regions. A century or so later, Aristotle proposed another hypothesis, based on humoralism, a medical doctrine that arose in Greece in the late sixth century B.C. and held sway for more than two thousand years.

The Greeks believed that each person's physical and emotional well-being depended upon maintaining a balance between four fluids, called humors, circulating throughout the body: blood, phlegm, black bile, and yellow bile. One of the factors thought to influence this equilibrium was the ingestion of food, a notion Aristotle seized on as an explanation for sleep. Sleep, he suggested, results from "the evaporation attendant upon the process of nutrition. The matter evaporated must be driven onwards to a certain point, then turn back, and change its current to and fro, like a tide-race in a narrow strait." In other words, vapors, emanating from food digesting in the stomach, were transported through the body via the humors, causing sleepiness. This, Aristotle posited, "explains why fits of drowsiness are especially apt to come on after meals."

Scant research was done on the subject during the two millennia after Aristotle, though a sensible routine of bed rest was acknowledged as crucial to a healthy, productive life. Maimonides, the twelfth-century Jewish physician and philosopher, recommended that a person sleep eight hours a night, "not . . . on his face nor on his back but on his side; at the beginning of the night, on the left side and at the end of the night on the right side," and that "he should not go to sleep shortly after eating but should wait approximately three or four hours after a meal." Four hundred years later, in *A Dyetary of Helth*, the English physician Andrew Boorde reflected a concern for psychological, as well as physical, influences on sleep when he offered this exhortation: "To bedwarde be you mery, or have mery company aboute you, so that, to bedwarde, no anger nor hevynes, sorrowe, nor pencyfulnes, do trouble or disquyet you."

During the nineteenth century, humoralism reasserted itself, with some variations (to this day, any theory that attributes sleep to the circulation, or lack thereof, of some substance in blood is referred to as humoral). The circulation of blood, which the ancients considered the most important humor, then lay at the foundation of two popular—albeit diametrically opposed—hypotheses of sleep. According to one, the cardiovascular system brings on sleep by flooding the brain with blood. The second theory suggested that, on the contrary, cerebral anemia—a *lack* of blood in the brain—induces slumber. Proponents of this view believed that, during sleep, blood is rerouted away

from the brain, to other organs. The prominence of two such antithetical notions made for quite a dilemma among insomniacs: whereas some physicians prescribed sleeping without pillows, to help draw blood back to the anemic cerebrum, others advised using as many cushions as possible, to divert blood from the congested brain.

Then, in the early twentieth century, René Legendre and Henri Piéron helped fuel a new idea: that during waking hours, the brain produces a variety of substances that combine to cause sleep. The two French physiologists conducted experiments involving pairs of dogs; one of each pair was made to stay awake for as long as two weeks, while the other was allowed to maintain its usual sleeping regimen. When the researchers injected cerebrospinal fluid from the sleep-deprived dogs into those that were well rested, the recipients fell into a deep, unusually long slumber.

Legendre and Piéron concluded that a sleep substance —which they dubbed hypnotoxin—was present in the cerebrospinal fluid and had accumulated in large quantities over the extended period during which the donor dogs had been awake. They were unable to isolate the substance, however, and by the 1920s a new line of inquiry—actually a variety of hypotheses generally classified as neural theories—had gained ascendancy, boosted, in no small measure, by the Russian physiologist Ivan Petrovich Pavlov.

At bottom, all neural theories hold that sleep results not from substances circulating in bodily fluids but from some characteristic change in the patterns of electrical impulses traveling between neurons in the brain. The most popular of these schemes was Pavlov's, according to which sleep originates in the cerebral cortex, the brain's furrowed outer layer. Once each day, Pavlov postulated, cortical neurons, exhausted as a result of overstimulation during wakefulness, become inhibited; then the inhibition spreads to other neurons, and sleep ensues. (Within the past twenty years, Pavlov's theory has been disproved: experiments have shown that laboratory animals sleep even after their cerebral cortices have been removed. Similarly, anencephalic babies, born without cerebral cortices, have sleep–wake cycles.)

Whereas Pavlov concentrated on cortical neurons, other investigators probed beneath the cerebral cortex, in search of an area deep within the brain geared specifically to regulating sleep. Recently, it became clear that there are, in fact, several such areas, including two clusters of neurons (the reticular formation network and the raphe nuclei) located in the brainstem and some parts of the hypothalamus (the small structure, tucked inside the base of the forebrain, that regulates the pituitary gland, along with hunger, thirst, and sexual appetite). It is just as apparent, however, that no one brain site is necessary for at least some sleep to occur: in laboratory animals, sleep continues, albeit impaired, even after any one of these areas is destroyed. Thus, the concept of a single sleep center is gradually being abandoned.

As some scientists groped in vain for clues to the cause of sleep, others made extraordinary progress in different areas of sleep research. Until fifty years ago, it had been assumed that sleep was a homogeneous, unified state, during which the brain is all but inactive. This notion was radically revised upon the development, during the thirties, of the electroencephalograph, the machine that charts the brain's electrical activity by recording rhythmic bursts of voltage oscillations (brain waves). It was then that researchers discovered that sleepers pass through two major stages, as well as a number of transitional phases.

The first stage of sleep was detected in 1935, by the physiologists Alfred L. Loomis, E. Newton Harvey, and Garret Hobart. It is characterized by high-amplitude brain waves of low frequency, during which the sleeper lies still and appears to be in his most restful state, and so came to be called slow-wave sleep. The second stage, discovered in 1952 by Nathaniel Kleitman, a physiologist at the University of Chicago, and his student Eugene Aserinsky, is marked by agitated high-frequency brain waves resembling those experienced during wakefulness; irregular heart rate, respiration, and blood pressure; muscular twitching; and the flurries of frenetic eye movement from which the stage derives its name, REM (rapid eye movement) sleep.

It is now known that sleep begins as a brief light slumber, lasting perhaps ten minutes, during which blood pressure, breathing, and body temperature ease into decline. This phase culminates in the first major sleep stage: deep slow-wave sleep. About an hour and a half later, there is a transition back to light slumber, followed by a shift to REM sleep. Over the course of eight hours, this cycle recurs four or five times; all told, about one-quarter of a night's sleep is spent in REM sleep, and the rest in slow-wave sleep and the transitional phases, all of which

Frederick Lord Leighton, Flaming June, *1895*

scientists group under the umbrella NREM (nonrapid eye movement) sleep.

Although advances in electroencephalopathy illuminated much about how we sleep, they said little about why. Only in the past two decades have scientists renewed the search for the cause—the somnogenic agent, or group of agents, that interacts with sleep centers in the brain to propel us from the structured, goal-directed, often stressful state of wakefulness to the less encumbered repose with which most of us bridge our days.

In the late sixties, John Pappenheimer, a physiologist at the Harvard Medical School, ushered in the modern era of sleep research with a series of studies on goats. Pappenheimer kept the animals awake for one or two days, after which—as did Legendre and Piéron, in their work with dogs—he extracted samples of their cerebrospinal fluid. Within the fluid was a substance that, when injected into rats that had been allowed to sleep normally, proved to be somnogenic.

After several years, Pappenheimer identified the substance as a peptide (a compound containing amino acids, the molecular building blocks of proteins), and in view of its marked sleep-producing ability, he named the compound factor S. Subsequently, other researchers confirmed his work, but because cerebrospinal fluid is available only in limited quantities—as opposed to, say, urine, which is more easily obtained—further characterizing of factor S has moved at a crawl. (We probably never will know whether Pappenheimer's peptide is the same as the mysterious hypnotoxin named by Legendre and Piéron in 1907.)

By 1980, however, Pappenheimer, Manfred Karnovsky, and I had observed that somnogenic substances similar—indeed, very likely identical—to the peptide found in goats are present in the brain tissues of sleep-deprived rabbits and in the urine of humans. These compounds have been further characterized as muramyl peptides, a class of glycopeptides (substances containing sugars as well as amino acids) contained in bacterial cell walls. When injected into laboratory rabbits, the muramyl peptides exert a potent effect: as little as one-billionth of a gram induces deep slow-wave sleep for several hours. Normally, rabbits spend about forty-five percent of their time in slow-wave sleep during daylight hours, but after an injection of muramyl peptides, this percentage jumps to about seventy.

All laboratory animals so far tested have responded to muramyl peptides by increasing the length and number of their sleep episodes. At the same time, their EEG readings have shown slow brain waves of very high amplitude, which are thought to indicate unusually deep slow-wave sleep similar to that which follows prolonged wakefulness. For reasons still unresolved, the effects of muramyl peptides on REM sleep vary from species to species.

Once it became clear that muramyl peptides are somnogenic, the task was to determine exactly how they induce sleep. One particularly intriguing possibility—that there is some link between sleep and the body's immune system—could justify centuries of medical wisdom. In the early seventies, scientists had discovered that muramyl peptides are immune adjuvants: they enhance the production of antibodies in the immune system and, therefore, are potentially valuable components of vaccines. Specifically, muramyl peptides stimulate the manufacture of lymphokines (including interleukin 1, tumor necrosis factor, and interferon)—chemicals involved in immune cell activation and proliferation. (In recent years, several research teams have discovered that one of these substances, interleukin 1, is not only a product of the immune system but also a constituent of the central nervous system.)

The search for a connection between sleep and the body's defenses against disease led us to ask whether lymphokines alter sleep. In fact, all three lymphokines tested—interferon, tumor necrosis factor, and interleukin 1—greatly enhanced slow-wave sleep in rabbits. What's more, the somnogenic effects of these substances were in many ways identical to those of muramyl peptides. But there was a crucial difference: the onset of sleep was much more rapid after the injection of lymphokines than after an injection of muramyl peptides, suggesting that lymphokine release occurs late in the sleep-activation process—that muramyl peptides exert their somnogenic powers only after some intermediate step that involves lymphokine production.

One of the ways in which lymphokines contribute to the immune response is by stimulating the production of prostaglandins—a family of compounds derived from fatty acids, some of which regulate the activities of macrophages, the immune cells that devour bacteria and viruses. Last year, the Japanese biochemist Osamu Hayaishi demonstrated that both muramyl peptides and lymphokines trigger the output of prostaglandin D2—which he had earlier shown to be somnogenic. Hayaishi's findings thus suggest that at least three biochemical events, involving both the immune system and the central nervous system, are associated with sleep: muramyl peptides induce an increase in the manufacture of lymphokines, which, in turn, give rise to prostaglandins.

That such key elements of the immune system as muramyl peptides and lymphokines are somnogenic seems to explain, at least in part, the sleepiness that often overpowers us when we are in the throes of an infectious disease. And because muramyl peptides are key components of bacteria, scientists have begun to suspect that the compounds also play a role in everyday sleep.

Bacteria, of course, are far more than couriers of disease: they contribute in a number of ways to mammalian physiological processes and have established symbiotic relationships with many forms of life. For example, in the rumen, one of the four stomach cavities in cattle and other cud-chewing animals, an ensemble of bacterial strains is essential to the breakdown of complex carbohydrates, which the animals' bodies otherwise would be unable to metabolize. Human skin harbors thriving colonies of the microorganisms, and most of us carry about a kilogram of bacteria in our intestinal tracts, where they help synthesize vitamin K and from which many of them pass through the intestinal wall.

As bacteria enter the body, they are devoured by macrophages, and as a by-product of this processs, muramyl peptides are released. That the macrophages' activity is continuous suggests that muramyl peptides influence not

only the excess sleep that often accompanies the body's immune response to infection but also everyday sleep, as a result of the normal metabolism of microbes.

DURING THE PAST SEVERAL YEARS, our understanding of these biochemical relationships has broadened considerably, in large part because of the discovery of close ties between the immune response, the endocrine network (the glands involved in hormone secretion), and the brain. Studies of their elaborate interactions have made it possible to sketch a rudimentary sleep-activation system, incorporating thirty chemicals that interact in various ways, working with—and sometimes against—one another, to both promote and inhibit sleep.

Consider, by way of illustration, some of the metabolic events set in motion when a muramyl peptide stimulates the production of interleukin 1, a key actor in this complex biochemical system of checks and balances. Interleukin 1 triggers an increase in the release of somatotropin —also known as growth hormone. (Exactly how this occurs is uncertain. One theory is that interleukin 1 stimulates the hypothalamus to secrete growth hormone–releasing factor, which regulates somatotropin release.) Somatotropin fosters the development of bone and muscle, aids in protein synthesis and tissue regeneration, and in humans is tightly coupled with sleep: most of the body's supply of the hormone is produced during slow-wave sleep. Moreover, somatotropin both enhances REM sleep, and, in large doses, inhibits NREM sleep. As it accumulates in the bloodstream, the hormone eventually suppresses the very substance that triggers its own synthesis, growth hormone–releasing factor—one of many examples of feedback (inherent self-regulatory "on–off switches") that appear to maintain a balance between the various sleep-inducing and sleep-preventing processes.

Another possible feedback loop underscores the synergism between the brain, the endocrine system, and the immune response that drives the sleep-activation network: Interleukin 1 signals the hypothalamus to secrete corticotropin-releasing factor, which stimulates the pituitary gland to secrete adrenocorticotropin hormone. Adrenocorticotropin, in turn, signals the adrenal gland to produce hormones called glucocorticoids. All the substances produced in this chain reaction—hypothalamic corticotropin–releasing factor, pituitary corticotropin hormone, and adrenal glucocorticoids—inhibit sleep, possibly via a feedback loop that reduces the synthesis of interleukin 1.

Though it is clear that most, if not all, sleep substances are involved in similar biochemical cascades, the timing of these events, and their impact on sleep, remain to be seen. We have yet to learn, for example, why certain of these chemicals promote both NREM and REM sleep in some doses but elicit contradictory results in others. Or why the effects of some substances seem to vary widely from species to species. Or exactly how many as yet unidentified sleep substances are circulating in our bodies. Indeed, at times it seems that for every layer of complexity we strip from sleep countless more lie still concealed. Certainly, to answer Samuel Johnson (and a great many other thinkers), a labyrinth of causes—at once hopelessly tangled yet remarkably synchronized—confine us to the "irresistible stupefaction" without which no mammal has been known to survive. ●

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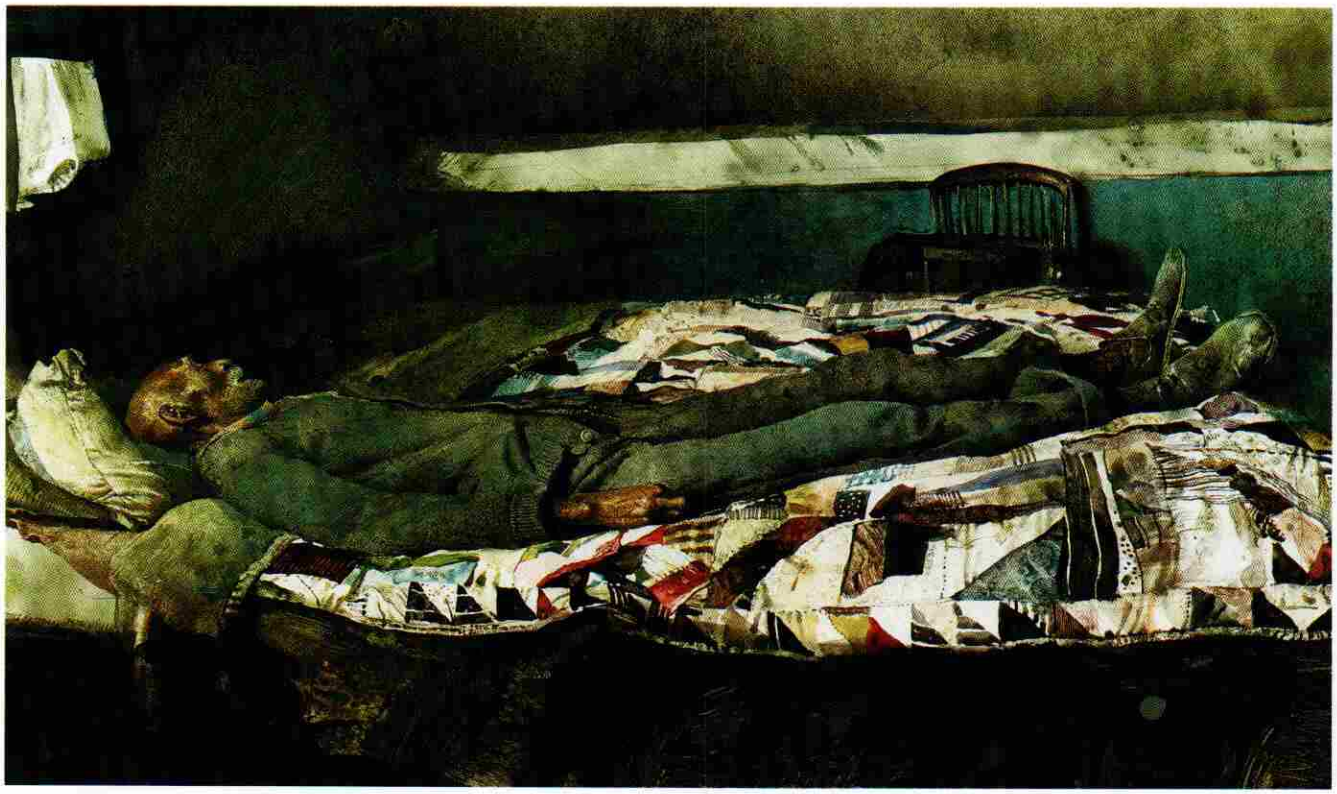

Andrew Wyeth, Garret Room, *1962*