PERSPECTIVES: NEUROBIOLOGY

Narcolepsy Genes Wake Up the Sleep Field

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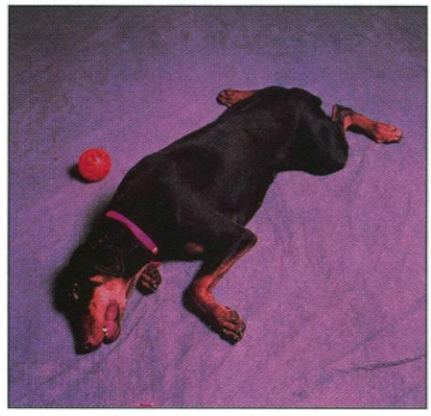
Although we spend one-third of our lives sleeping, most of us take it for granted. Unfortunately, for those individuals who either cannot sleep at night or cannot stay awake during the day, this primordial rhythm of life is all too apparent. Our understanding of the physiological basis of sleep and its relevance to medicine has matured over the last 25 years; however, the mechanism of sleep at the molecular, cellular, and systems levels has remained a mystery (*1*). Now, two remarkable papers published in recent issues of *Cell* are set to forever change our view of sleep (*2*, *3*). Mignot and colleagues (*2*) and Yanagisawa and his team (*3*)—working in dogs and mice, respectively—report a link between the hypocretin/orexin ligand and its receptors (known to be involved in feeding behavior) and the sleep disorder, narcolepsy.

Enhanced online at www.sciencemag.org/cgi/content/full/285/5436/2076

The modern field of sleep research traces its origins back to 1953 when Nathaniel Kleitman discovered rapid eye movement (REM) sleep and later made the association between REM sleep and dreaming (*4*). Kleitman's discovery was key because it demonstrated that sleep was more than a quiescent, inactive state. Rather, it became clear that sleep had an architecture and was characterized by different stages (*1*). REM sleep is associated with active dreaming, muscle atonia, and desynchronized electroencephalogram (EEG) activity. Non-REM sleep is characterized by deep sleep, partial muscle relaxation, and synchronized EEG activity. Both REM and non-REM sleep are vital functions and their complete deprivation leads to death in mammals (*5*). In addition to the REM and non-REM states of sleep, the 24-hour variation in sleep propensity is regulated by opposing homeostatic and circadian mechanisms (*1*). Simply stated, the homeostatic sleep system acts as a steady-state cumulative process that governs the probability of falling asleep. The longer one is awake, the higher the likelihood of falling asleep. Only sleep itself appears to discharge the cumulative need for sleep. The system is homeostatic because sleep deprivation leads to a rebound increased need for sleep as if the system were making up for a sleep debt. The circadian regulation of sleep is thought to promote wakefulness and to consolidate sleep into the characteristic 8-hour sleep bouts of humans. In general, the homeostatic mechanism primarily determines when we go to sleep and the circadian mechanism largely determines when we wake up (*1*).



Catnapping dogs. Narcoleptic Doberman in the midst of a cataplectic attack. Narcolepsy is characterized by cataplectic attacks, which are sudden episodes of muscle atonia triggered by emotions.

Disorders of sleep are quite prevalent and are becoming better recognized by clinicians with the emergence of sleep medicine as a specialty (*1*). The etiology of sleep disorders, however, is less clear and the underlying genetics appear complex in the few examples thus far examined. Among the various sleep disorders (insomnias, obstructive sleep apnea, and restless leg syndrome), narcolepsy is unique in that it is the only known neurological disorder that specifically affects the generation and organization of sleep (see the figure) (*6*). Human narcolepsy affects 1 in 2000 individuals and is a debilitating, lifelong disorder (*7*). It is characterized by excessive daytime sleepiness, cataplexy (a sudden weakening of the muscles triggered by emotions), and an alteration in the expression of and entry into REM sleep (*6*). Familial transmission of narcolepsy is rare; however, in families with affected individuals the risk for first-degree relatives is 10 to 40 times that seen in the general population (*7*). One of the predisposing factors is associated with specific class II human leukocyte antigen (HLA) haplotypes on human chromosome 6 (HLA DQB1*0602 and DQA1*0102 alleles are found in more than 85% of all narcoleptic patients). So far, the genetics of human narcolepsy have remained obscure. Thus, the discovery of Mendelian transmission of narcolepsy as an autosomal recessive trait in dogs two decades ago has remained a siren for geneticists and has promised a potential entrée into the molecular mechanism of sleep (*7*).

Now, after 10 years of effort, Mignot and his team have identified the canine narcolepsy gene (*canarc-1*) by positional cloning (*2*). This heroic effort was initiated by William Dement who had the foresight to maintain a colony of narcoleptic Labradors and Dobermans at great cost and effort at Stanford University. Over time, a large (by dog standards) set of genetic backcrosses was made and a linkage marker for the narcolepsy gene was identified. Ironically the linkage marker was an immune system candidate gene that turned out to be a red herring. By sheer brute force, Mignot and co-workers were able to clone much of the physical region in the vicinity of the gene in bacterial artificial chromosome (BAC) clones constructed from a heterozygous *canarc-1* Doberman (that carried one mutant and one normal copy of the gene) (*2*). The breakthrough came when they were able to identify a region of conserved synteny with a human gene and to use homology mapping with expressed sequences. Finally, a set of close recombinant animals narrowed the critical region containing the *canarc-1* locus, and a candidate gene emerged.

The *canarc-1* locus encodes an orphan receptor (*orexin$_2$ receptor* or *OX$_2$R*) that was shown by Yanagisawa and colleagues last year to be one of two receptors that bind the neuropeptide orexin (*8*), also called hypocretin (*9*). There are two peptide ligands for the orexin receptors that arise from a single polypeptide precursor. The structures of the mature peptides were determined to be a 33–amino acid peptide containing two disulfide bonds with an amino-terminal pyroglutamyl residue and carboxyl-terminal amide group (called orexin-A) and a 28–amino acid linear peptide with a carboxyl-terminal amide group (called orexin-B) (*8*). Orexin-A is similar but not identical to hypocretin-1, which was

CREDIT: EMMANUEL MIGNOT

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predicted from the cDNA encoding the hypocretin precursor protein (the predicted cleavage sites are not consistent with the analysis of the native peptide). Orexin-B is identical to hypocretin-2 (*8*, *9*). Both ligand and receptor loci bear the *hypocretin* moniker; however, given that the structure of hypocretin-1 was incorrect (*9*) and that the receptors were identified by Yanagisawa's team (*8*), the nomenclature of this ligand-receptor system should probably be reconsidered—"narcoleptin" perhaps?

The hypocretin/orexin neuropeptide system has been proposed to regulate feeding and energy metabolism based on the anatomical expression pattern of the peptides and their effects on feeding behavior (*8*). The neurons are located in the lateral and posterior hypothalamus and project widely within the forebrain, limbic system, thalamus, hypothalamus, brainstem, and spinal cord (*10*). Thus, one obvious question to be addressed is the effect that inactivating the hypocretin/orexin precursor gene will have in mice. Yanagisawa and colleagues introduced a null mutation into this gene and carefully examined the mice for behavioral abnormalities during the daytime (*3*). But nothing appeared unusual. Nevertheless, knowing that mice are nocturnal and feed at night, the investigators observed the mice at night using infrared video recording. Incredibly, the mice appeared to have frequent episodes of behavioral arrest that resembled cataplexy (*11*). These "narcoleptic attacks" were usually triggered by ambulating and grooming. To determine whether the episodes could be epileptic seizures, they recorded EEG and electromyogram (EMG) activity and found that the mice did not appear to have a seizure disorder, but rather appeared to enter into REM sleep prematurely. Taken together these results suggest that the *orexin* knockout mice have the mouse equivalent of narcolepsy. Classical sleep physiologists remain cautious about the mouse phenotype (*12*) but, as a geneticist, I am persuaded by the beauty of the ligand and receptor phenocopy that is so reminiscent of the *steel/c-Kit* and *obese/diabetic* pairs of mouse mutants.

How are the hypocretins/orexins affecting sleep? It is too early to tell, but it is clear that a new and unexpected pathway has been linked to sleep. These studies also highlight the lateral hypothalamus as an important site of sleep regulation. The fact that a mutation in a G protein–coupled orexin receptor can cause narcolepsy immediately opens up the possibility of new drug discovery efforts with the future hope of therapeutics for the hundreds of thousands of narcoleptic patients who would like to take sleep for granted like the rest of us.

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PERSPECTIVES: STRUCTURE

Function Is Structure

Anders Liljas

To understand the function of a biological system such as the ribosome—the factory in the cell cytoplasm that makes proteins—it is first necessary to know the structure of its component parts. The ribosome, however, has not readily yielded its secrets to x-ray diffraction analysis. Now, four papers—two in this issue of *Science* (*1*, *2*) and two in a recent issue of *Nature* (*3*, *4*)—present the crystal structures of the 70*S* bacterial ribosome and its two component subunits, providing molecular insights that go way beyond our previous knowledge.

The large (50*S*) and small (30*S*) subunits of the ribosome are together composed of three types of ribosomal RNA (rRNA) and 54 different proteins. These subcellular factories translate the genetic message of mRNA into the amino acid sequence of the particular protein it encodes. The substrate in this enzymatic translation process is transfer RNA (tRNA), which has an anticodon at one end (that interacts with the mRNA bound to the 30*S* subunit) and the corresponding amino acid at the other (the 3′ end, which interacts with the 50*S* ribosome). As the ribosome moves along the mRNA, the mRNA codon forms base pairs with the anticodon of the corresponding tRNA. An accepted codon-anticodon match allows the growing string of amino acids attached to the ribosome-bound tRNA to be transferred to the amino acid of an incoming tRNA, so that the polypeptide is elongated by one amino acid residue at a time. This activity occurs in the peptidyl transfer site in the 50*S* ribosomal subunit (see the figure). Protein synthesis is catalyzed by a range of different translation factors that are active in the initiation, elongation, and termination phases of polypeptide production.

The solving of the first tRNA structures by x-ray crystallography in 1974 began to unravel the mystery of how proteins are synthesized (*5*). The distance between the anticodon and the acceptor end of tRNA (where the amino acids are attached) was found to be very large (75 Å). Any new model explaining how the ribosome works had to accommodate this essential fact. Next came the structure (at 2.7 Å resolution) of tRNA bound to the polypeptide elongation factor Tu (EF-Tu) (*6*). In this complex, the amino acid at the acceptor end of tRNA was found to be firmly bound to and buried in the elongation factor and so could not be added to the polypeptide chain until its release from EF-Tu. Then came the discovery that the structure of the complex between tRNA and EF-Tu is mimicked by that of elongation factor G (EF-G)—the ribosomal translocase that moves the tRNA together with the mRNA to expose a new codon (*6*, *7*). The simple explanation for this similarity is that these elongation factors bind to the same site on the ribosome.

Despite these advances, solving the structures of the 50*S* and 30*S* subunits of the bacterial ribosome has been a challenge for more than 30 years. Electron microscopy, fluorescence resonance energy transfer, and neutron scattering, together with bifunctional cross-linking, affinity labeling, chemical footprinting, and other chemical techniques have enabled the ribosome to be analyzed at low resolution. Recently, cryo-electron microscopy (cryo-EM)—which can be thought of as crystallography performed on single particles—has improved the resolution of ribosomal structures dramatically (*8*), and other techniques have enabled the visualization of high-resolution details of some ribosomal components.

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