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Discovery of hypocretin/orexin ushers in a new era of sleep research

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Abstract

Prior to the 21st century, genetic mechanisms that regulate sleep were largely unknown. In 1998, de Lecea et al. 1 and Sakurai et al. 2 reported the discovery of a gene they named hypocretin and orexin, respectively, which led to a revolution in our understanding of genetic and neuronal mechanisms that regulate sleep.

Keywords

Sleep; arousal; hypocretin; orexin

Sleep is an essential behavioral state that is conserved throughout evolution. Prior to the 21st century, much of our understanding of sleep came from pharmacology and lesion studies in humans and other mammals. These relatively blunt tools were used to identify neurotransmitters, neuromodulators and brain regions that affect sleep, but allowed for only limited types of experiments, resulting in slow progress when it comes to dissecting the functional contributions of specific cell populations. The year 1998 marked the publication of two papers [1, 2] that would eventually transform our understanding of mechanisms that regulate sleep and wakefulness, even though neither paper mentions sleep.

One paper [1], from J. Gregor Sutcliffe's lab at the Scripps Research Institute in La Jolla, California, was motivated by the notion that the hypothalamus is organized as a group of distinct nuclei that regulate a variety of homeostatic functions, including reproduction, feeding, circadian rhythms and sleep. It was hypothesized that specific genes are expressed in these nuclei and play key roles in their functions. Indeed, several such examples had already been identified. In the early to mid-1990s, to further test this hypothesis and identify additional genes that underlie the functions of specific hypothalamic nuclei, Kaare and Vigdis Gautvik (on sabbatical from the University of Oslo), together with Luis de Lecea and their colleagues in the Sutcliffe lab, undertook an mRNA subtraction screen to identify genes whose expression is enriched in the rodent hypothalamus compared to the

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hippocampus and cerebellum [3]. They identified several such genes, one of which was particularly intriguing, as it was exclusively expressed in a relatively small number of neurons in the paraventricular hypothalamus, representing the type of specificity that they had hoped to find.

Based on this initial discovery, de Lecea, Tom Kilduff and their colleagues in the Sutcliffe lab isolated a full-length cDNA corresponding to this clone from rat and mouse [1]. This cDNA encoded for a predicted preproprotein (a proprotein precursor) containing two putative peptides that had high amino acid similarity with each other. As expected from the previous study [3], *in situ* hybridization and immunohistochemistry showed specific expression of the mRNA and protein in the dorsal lateral hypothalamus, with fibers projecting both within the posterior hypothalamus and also widely throughout the brain. The protein appeared to be present in synaptic vesicles, and one of the peptides could stimulate cultured hypothalamic neurons, consistent with the function of a peptide neurotransmitter. This novel gene was termed hypocretin due to its *hypo*thalamic expression pattern and the similarity of the peptide sequences to members of the in*cretin* family of hormones. While the hypocretin expression pattern was intriguing, its function was entirely unknown, and the authors could only speculate that it might act by binding to a G-protein coupled receptor (GPCR), as do other members of the incretin family of peptides.

The second 1998 paper [2], published one month later, identified the same gene using a completely different approach and for an entirely different reason. Genomic studies had identified many so-called “orphan” GPCRs whose ligands were unknown. Identifying the ligands and functions of these GPCRs was considered important, in part because GPCRs were, and still are, the largest class of protein that is targeted by drugs. Thus, identifying the ligands of orphan GPCRs could lead to new drug treatments for a variety of diseases. In order to identify endogenous ligands for orphan GPCRs, the Yanagisawa group first biochemically fractionated rat brain extracts using high-performance liquid chromatography. They then applied these extracts to over 50 cell lines that each expressed a different orphan GPCR, and assayed for GPCR activation. This heroic effort resulted in the identification of 2 peptides, termed orexin-A and orexin-B (from the Greek word *orexis* for appetite), that activated cells expressing a specific GPCR, termed orexin receptor 1 (OX1R). Based on the peptide amino acid sequences, they isolated a cDNA that encodes for a preproprotein that contains both orexin-A and orexin-B. Unknown to them at the time, this was the same gene that the Sutcliffe group had discovered and named hypocretin. The Yanagisawa group next identified a GPCR similar to OX1R, which they termed OX2R, and showed that orexin-A and orexin-B can bind to both receptors. In agreement with the de Lecea et al. study, they found that orexin mRNA and protein were specifically expressed in the lateral and posterior hypothalamus, a brain region implicated by classical lesion studies in promoting feeding. Consistent with this observation, they found that central administration of orexin-A or orexin-B potently stimulated feeding, and that orexin expression was upregulated in response to fasting. Based on these results, the authors speculated that the orexin peptides may underlie the feeding-promoting role of the lateral and posterior hypothalamus.

The importance of these discoveries for sleep was revealed the following year, when the Yanagisawa lab reported that knocking out the hypocretin/orexin gene in mice results in a

behavioral phenotype similar to the human sleep disorder narcolepsy [4], and Emmanuel Mignot's group showed that a heritable form of canine narcolepsy is due to a mutation in the OX2R gene, which his group named hypocretin receptor 2 [5]. These findings were soon shown to be relevant to human narcolepsy, with the discoveries that the hypocretin/orexin gene was mutated in a case of severe and early onset narcolepsy [6] and that narcoleptic individuals are deficient in hypocretin/orexin [6, 7].

Subsequent studies showed that signaling by the hypocretin/orexin neuropeptides, as well as the activity of hypocretin/orexin-expressing neurons, plays a key role in promoting and maintaining wakefulness in mammals [8, 9] and other vertebrates such as zebrafish [10]. As mentioned, one of the motivations for the de Lecea et al. and Sakurai et al. studies was to identify new drug targets. This goal was recently achieved, with the development and FDA approval of a drug that inhibits the hypocretin/orexin receptors and provides an effective treatment for insomnia [11]. Hypocretin/orexin receptor agonists have also recently been reported [12], which have the potential to be therapeutic for narcolepsy and other disorders associated with excessive sleep.

While I focused here on the impact of the discovery of hypocretin/orexin on the sleep field, it should be mentioned that the hypocretin/orexin system has also been shown to regulate other homeostatic processes, including: feeding, as alluded to by the Sakurai et al. 1998 paper, and further explored in a vast body of follow up literature; energy homeostasis; reward-seeking; and more [13]. Back to the sleep field, the discoveries by de Lecea et al. and Sakurai et al. moved the field past anatomically defined brain regions and classical neurotransmitters into molecularly defined neuronal populations and the activity of specific genes within them. This shift, along with several technological advances, transformed the sleep field by enabling the interrogation of mechanisms that regulate sleep at the molecular and genetic levels. The discovery that a gene can profoundly affect sleep prompted the development of simpler invertebrate and vertebrate models to study sleep [14], including *Drosophila melanogaster*, *Caenorhabditis elegans*, and zebrafish, that allow for more sophisticated and powerful genetic approaches. These models have been used to perform large-scale screens for genes, neurons and drugs that affect sleep, which has led to the discovery of new mechanisms and revealed how some of these mechanisms interact. This approach recently came full circle with the publication by the Yanagisawa group of a heroic screen that identified novel sleep-regulating genes in mice [15]. The sleep field has recently undergone another revolution due to the use of cell-type-specific manipulation tools, such as optogenetics, to identify specific neuronal populations that promote sleep or wake states, and high-throughput sequencing methods to identify genes expressed in these neurons. These approaches, combined with new methods that enable large-scale recording of neuronal activity in the brain, have the potential to comprehensively identify the genetic and neuronal mechanisms that regulate sleep.

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