Quantitative genetics of sleep in inbred mice

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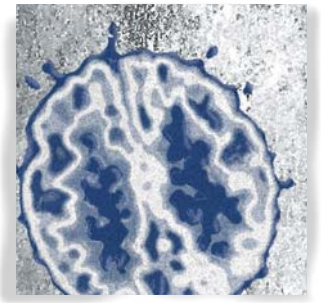

The timing and the organization of sleep architecture are mainly controlled by the circadian system, while sleep need and intensity are regulated by a homeostatic process. How independent these two systems are in regulating sleep is not well understood. In contrast to the impressive progress in the molecular genetics of circadian rhythms, little is known about the molecular basis of sleep. Nevertheless, as summarized here, phenotypic dissection of sleep into its most basic aspects can be used to identify both the single major genes and small effect quantitative trait loci involved. Although experimental models such as the mouse are more readily amenable to genetic analysis of sleep, similar approaches can be applied to humans.

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Vigilance states are classified, based on changes in brain electrical activity as indexed by the electroencephalogram (EEG), into wakefulness, non-rapid eye movement (NREM), and rapid eye movement (REM) sleep. Sleep occurs at specific times in phase with many other circadian variables such as core body temperature and endocrine hormone secretion. Within sleep, REM sleep also follows a circadian rhythm, reaching its maximum duration near body temperature minimum. The recovery process underlying sleep can be indexed by the intensity of NREM sleep as measured by the quantitative EEG within the delta (0.5-4.5 Hz) frequency range. Delta activity is high at sleep onset, in close relation with sleep need and depth, and increases over the baseline level after extended wakefulness. This intensity measure of sleep is relatively independent of the circadian process generated by the suprachiasmatic nuclei (SCN). Accordingly, SCN lesion results in arrhythmia in behavior and physiology, including sleep, but delta activity still follows a predictable variation as a function of prior wakefulness duration. The integrated interplay between circadian and homeostatic processes is mathematically described in the two-process model of sleep regulation,[1] which provides a framework for prediction and interpretation of a large body of experimental data.

The molecular clock is described as a transcriptional feedback loop with positive (eg, *Clock* and *Bmal1*) and negative (eg, *Per1-2* and *Cry1-2*) regulators responsible for 24-h periodicity.[2] During the last 20 years, different genetic elements of the circadian clock have been identified in experimental models and in humans, although the exact mechanisms through which the 24-h clock period is translated into cyclic changes in physiology and behavior is not fully understood. Also, mutations and polymorphisms of clock genes have been implicated in circadian sleep disor-

Selected abbreviations and acronyms

EEG	*electroencephalogram*
LTP	*long-term potentiation*
NREM	*non-rapid eye movement*
QTL	*quantitative trait loci*
REM	*rapid eye movement*
SCN	*suprachiasmatic nucleus*
TPF	*theta peak frequency*

ders such as familial advanced or delayed sleep phase syndromes or "morningness-eveningness" preferences.[3,4] In contrast, the molecular bases of sleep remain mostly unknown.

Sleep and the sleep EEG are complex phenotypes involving many genes in their expression and regulation. A systematic genetic approach is therefore needed for their identification.[5] Early work on human waking EEG recordings by Vogel[6] had strongly suggested the effect of single genes. Pioneering work by Valatx[7,8] in inbred mice had also indicated that several aspects of sleep are controlled by genetic factors.

Ten years ago, we reported the first evidence for the presence of quantitative trait loci (QTL) involved in the expression of REM sleep.[9] However, we have also argued that aspects such as the NREM-REM cycle might not be regulated at the molecular level (at least at the transcriptional level) and that many genes may change expression as a function of vigilance states instead of directly and causally inducing changes in vigilance states.[10] Although QTL analysis remains our best hope to dissect the complex genetics of sleep, single major genes may still be involved in specific and well-defined sleep features. Evidence for a major contribution of genetic factors to sleep and sleep disorders are reviewed elsewhere,[11,12] and here we will focus mainly on some EEG characteristics of sleep in inbred mice that have been shown to be affected by a major and/or single gene.

Genes regulating sleep amounts and organization

The regulation of sleep amount seems to be highly complex, and there is no model or empirical data available to predict the biologically needed amount of sleep. The amount of recovered sleep is poorly correlated with the amount of sleep loss, although NREM and REM sleep are differently affected. As mentioned before, NREM sleep is tightly regulated at the level of its intensity, which is represented by the EEG activity in the low-frequency range (delta activity). In contrast, the duration more than any intensity index of REM sleep is homeostatically regulated.[13] In our first attempt to map genes involved in sleep, we have used quantitative genetics (using the vigilance state quantities as the phenotypes for which gene mapping is performed) in a small set of recombinant inbred lines (BALB/cBy X C57BL/6By) and were able to localize four loci for the amount of REM sleep during the light period on mouse chromosomes 5, 7, 12, and 17.[9] In this preliminary study, we had already noticed that NREM and REM sleep, as well as their respective amounts during the light or dark cycle, are regulated by different genes, a finding replicated by others.[14] In a following study in 25 recombinant inbred lines derived from C57BL/6J and DBA/2J, QTLs were found to influence amounts of REM, NREM, and total sleep.[15] Among these, a single QTL (as a reminder, a QTL is defined as a genomic region containing naturally occurring allelic variations affecting a quantitative phenotype) on chromosome 5 was associated with all vigilance states, suggesting the presence of a gene affecting some basic aspects of sleep amounts. Total sleep time was associated with markers on chromosome 4, 5, 9, and 15, most of them showing also consistent association with the amount of NREM sleep, as these two parameters are highly correlated. REM sleep was associated with markers on chromosome 1, 17, and 19. The search for candidate genes within the identified regions indicated several interesting candidates: γ-aminobutyric acid (GABA)-A genes on chromosome 5 for all sleep parameters, several immune-related genes for REM sleep, and acetylcholine receptor genes for NREM and total sleep amounts. Also many of these chromosomal locations contained minor histocompatibility genes. However, sleep recordings in eight histocompatibility congenic strains resulted in conflicting findings, except that the congenic strain H24 (chromosome 7) confirmed the results of our first study, showing that a gene in the transferred region segregates with the amount of REM sleep during the light period.[15]

Overall, quantitative estimations indicated that between 40% and 60% of the variance in sleep amounts and distribution can be explained by the additive effects of between 6 and 15 loci, based on available data in CXB and BXD recombinant inbred lines, indicating, as for other complex traits, a polygenic basis.

Genes regulating the sleep EEG

By screening sleep in several inbred mouse strains to identify differences that could be related to genetic background, several EEG features appeared to be so tightly strain-specific that visual inspections of EEG recordings were enough to identify a strain fingerprint. These EEG characteristics can be quantified by spectral analysis (fast Fourier transform). Among these are the frequency of the EEG during REM sleep, the relative contribution of the delta activity to the NREM sleep EEG, and the delta power rebound after sleep deprivation.[16,17]

In addition to characterizing REM sleep, theta rhythm in rodents is selectively present during waking behaviors critical for the survival of the species, such as exploration.[18] It is also well-established that long-term potentiation (LTP) in the hippocampus is optimally elicited with priming stimulations delivered at theta frequency (5-8 Hz) range, and its strength increases linearly with increasing theta power.[19,20] Naturally occurring theta as well as LTP can induce synaptic changes of the type needed for memory storage.[21] The implication of theta in learning and memory is further demonstrated by the findings that selective elimination or facilitation of theta activity blocks or enhances the induction of LTP and overall memory.[22,23] Vertes and Kocsis[24] proposed that "The theta rhythm acts as a significant signal. Information arriving with theta (with a particular phase) is stored in the hippocampus, whereas information arriving in the absence (or phase shift) of theta is not encoded."

Although the implication of sleep in learning and memory has long been advocated,[25] there are as many studies that have failed to describe a link between sleep and memory as those that have claimed such a relationship. Based on the convincing evidence that theta is directly involved in mnemonic functions of hippocampus,[24] an important point is whether or not theta during active waking (exploratory behavior) and REM sleep serves the same function.[26] We have shown that theta frequency during exploratory behavior differed significantly from that during REM sleep,[16] either because of behavioral differences between inbred strains during waking, or because theta is controlled by different genetic mechanisms during sleep and waking. Because the link between theta and memory during sleep remains unknown and because theta is under strong genetic control, we believe that discovering its molecular basis could shed light on the theta rhythm function both during waking and sleep.

The theta peak frequency (TPF) during REM sleep varies greatly with genetic background. The TPF is significantly different between C57BL/6J (B) and BALB/cByJ (C) inbred mice during REM sleep, the first being slow (5.75-6.25) while the second fast (6.75-7.75). Over 80% of the inter-inbred strain variability can be explained by genetic effects. In BXC F1 mice the TPF is similar to that of B and significantly faster than C, suggesting that the C allele is recessive. We have mapped a highly significant locus linked to TPF on the mouse chromosome 5, suggesting the presence of an autosomal recessive gene. This single locus explained more than 65% of the variance. After narrowing down the identified region, different candidate genes were analyzed and the short-chain acyl-coenzyme A dehydrogenase gene (*Acads*) involved in the β-oxidation of short chain fatty acids, showed a spontaneous mutation in C mice. For comparison with REM theta, we have also analyzed TPF during waking episodes with clear theta activity (theta-dominated active waking). However, there was no correlation between waking and REM TPF and there was no significant linkage between the waking TPF and any markers of chromosome 5. These findings suggest that the *Acads* mutation has a highly specific effect on TPF during sleep only. Also waking TPF showed no significant difference between the mutant and the wild-type BALB/cBy, clearly indicating that the *Acads* mutation affects theta oscillations only during sleep.[27]

Mitochondrial fatty acid β-oxidation is the major source of energy for the heart and for skeletal muscle, but probably not for the brain. However, when the blood glucose level is low (eg, fasting), the liver β-oxidation is stimulated and provides ketone bodies, which are then an important source of energy for the brain.[28] Because a large body of evidence favors glucose as the principal energy source of the adult brain, little is known about the brain β-oxidation pathway. *Acads* deficiency in BALB/cByJ mice is accompanied by organic aciduria, suggesting that although these mice seem asymptomatic (as opposed to human subjects with *Acads* mutations) some toxic effects might occur in target organs, including the brain. Accordingly, further gene profiling experiments between *Acads* mutant and wild-type mice identified a single gene that was overexpressed in the brain of mutant mice.[27] The identified gene is glyoxalase 1 (*Glo1*), involved in a gluthatione-dependent metabolic detoxification pathway. *Glo1* overexpression has recently been linked to normal and pathological ageing[29] as well as to anxiety,[30] conditions where EEG changes are

believed to be a biological phenotypic marker.[31,32]
We have also noticed in some inbred mouse strains that during NREM sleep, when the EEG is normally dominated by delta activity, substantial activity may also be present in the theta frequency range.[16] In the DBA/2J (D2) strain, this activity actually exceeds delta activity during slow-wave sleep (SWS). Sleep is abnormally fragmented in D2 mice[17] and the rate at which sleep need accumulates is significantly reduced in this strain when compared with most other inbred strains.[33] Additionally, the D2 strain has long been used to dissect the genetic basis of susceptibility to audiogenic seizures.[34] The EEG of D2 mice in all three vigilance states presents spontaneous brief high voltage discharges (spindles) in the theta frequency range ([35] and personal observations) suggesting a basal EEG background congruent with seizure activity. To understand the mechanisms by which this dramatic change in the NREM EEG activity occurs in D2 mice, we undertook a systematic quantitative genetic analysis. A theta delta ratio (TDR) on relative power spectra was determined for B6 and D2 mice and differed by more than 5 standard deviations. The TDR of the F1s was similar to B6 and significantly different from D2, suggesting the presence of a recessive D2 allele. Quantitative linkage analysis in BXD recombinant inbred lines detected a single significant locus in the centromeric region of chromosome 14, which was responsible for more than 55% of the total variance, clearly confirming the presence of an autosomal recessive gene. Fine mapping revealed several single nucleotide polymorphisms in the 5' untranslated region of the retinoic acid receptor beta gene (*Rarb*). *Rarb* has four different transcripts, which were expressed at significantly higher levels in the brain of D2 mice compared with B6, indicating that the polymorphisms in the gene had an effect on the transcription *in vivo*. By testing six other inbred strains, we noticed that only *Rarb1* varied with delta power.[36]
Retinoic acid, the active derivative of vitamin A, plays a major role during ontogenesis and particularly during the development of the brain, most probably through dopaminergic pathways. The mammalian EEG and sleep are developmentally regulated. The best-studied model is the neonate rat recorded from postnatal day 12 (P12). The first type of sleep (also called active sleep) is an undifferentiated state from which both NREM and REM with their respective polygraphic features develop[37,38] at around P24. Other aspects of sleep, such as the amount of different sleep states or the amplitude of the EEG, do not stabilize before adulthood.[39-41] Aging is also accompanied by strong changes in both sleep organization and sleep EEG, with a major decrease in delta activity and increase in sleep fragmentation being the hallmarks of older age in humans.[42] On the other hand, sleep has long been suspected to be involved in remodeling neuronal connections and plasticity, and more so during critical periods of development.[43] Therefore, reciprocal interactions between the central nervous system development/remodeling and sleep may be critical for normal higher brain functions. Whether it is through brain development and plasticity or through dopaminergic pathways that *Rarb* regulates the contribution of delta activity during NREM sleep remains to be documented. Nevertheless, a recent study investigated the effects of a vitamin A-deficient diet on sleep and striatal monoamines and found that 4 weeks of deficiency in adult mice results in decreased delta power and the dopamine metabolite dihydroxyphenylacetic acid. [44]
Another highly genetically controlled sleep EEG characteristic is the typical delta power increase after sleep deprivation. The rate of accumulation of a need for NREM sleep (increase in delta power) varies greatly between inbred mouse strains.[17] QTL analysis was performed in 25 BXD recombinant inbred strains for the segregation of the rebound of delta power after a 6-hour sleep deprivation, starting at light onset. Results showed that additive genetic factors accounted for more than 67% of total variance.[33] By analyzing 788 polymorphic markers for a genome-wide scan, a significant QTL was identified on chromosome 13 and a suggestive one on chromosome 2. The QTL on chromosome 13 explained 50% of the total variance in delta power rebound, suggesting the presence of a major gene. Confirmation of the chromosome 13 QTL was obtained in baseline recordings in the same animals. This result suggests for the first time that sleep need is under strong genetic control, and genes can be identified underlying sleep homeostasis.

Conclusions

The functions of sleep remain elusive. Understanding the regulation of sleep at the molecular level represents a powerful step to gaining access to the enigma of sleep. Although evidence has accumulated to indicate a major role for genetic factors in normal and pathological sleep, the underlying molecular mechanisms have not been elucidated, except in a few rare sleep disorders. Like most

other complex traits, sleep is controlled by many genetic and environmental factors. New strategies are becoming available for genetic dissection of complex phenotypes. The hope of finding single genes that determine the presence or absence of any vigilance states in an all-or-nothing manner is highly unrealistic. However, as reviewed here, sleep-related endophenotypes, such as the sleep EEG features, can be controlled by single or major genes. A noteworthy discovery is that such genes indicate unpredicted pathways (eg, β-oxidation and vitamin A signaling) that are not only implicated in sleep but link sleep to other complex behaviors. ❑

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Genética cuantitativa del sueño en ratas consanguíneas

El sistema circadiano controla principalmente el ritmo y la organización de la arquitectura del sueño, mientras que la necesidad y la intensidad del sueño son reguladas por un proceso homeostático. No se conoce muy bien la independencia de estos dos sistemas en la regulación del sueño. En contraste con el impresionante progreso que ha tenido la genética molecular de los ritmos circadianos, las bases moleculares del sueño son poco conocidas. Sin embargo, como se resume en este artículo, los principales genes únicos y los loci que están involucrados en los efectos cuantitativos del sueño se pueden identificar mediante los aspectos más básicos de la disección fenotípica del sueño. Los modelos experimentales como el del ratón pueden ser más fáciles para el análisis genético del sueño, pero los mismos experimentos se pueden aplicar al hombre.

Génétique quantitative du sommeil chez la souris consanguine

Le système circadien est l'un des principaux régulateurs du rythme et de l'architecture du sommeil, alors que l'intensité et le besoin en sommeil sont plutôt régulés par un processus homéostasique. Nous ne connaissons pas très bien l'indépendance de ces deux systèmes dans la régulation du sommeil. Contrairement aux progrès importants qui ont été faits en génétique moléculaire des rythmes circadiens, les bases moléculaires du sommeil sont peu connues. Cependant, comme nous le résumons ici, aussi bien des gènes majeurs que ceux avec des effets mineurs (des locus des traits quantitatifs ou QTL), impliqués dans le sommeil peuvent être identifiés par dissection phénotypique du sommeil dans ses aspects les plus basiques. Les modèles expérimentaux comme celui de la souris se prêtent plus volontiers à l'analyse génétique du sommeil, mais les mêmes approches peuvent s'appliquer à l'homme.

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