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The Genetics of Sleep: Insight from Rodent Models

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Summary:

Sleep is a fundamental behavior in higher animals that has been firmly established to be under substantial genetic control. However, the identification of individual genes responsible for primary sleep-wake traits has largely eluded researchers. Genetic studies in animal models have uncovered a variety of genomic loci associated with specific traits, validated the role of key neurotransmitter systems (i.e., monoamines) in sleep-wake regulation, identified novel and unexpected genes responsible for controlling sleep-wake traits, and demonstrated substantial genetic overlap in the regulation of sleep and circadian rhythms. Future studies are expected to reveal additional genes and gene networks underlying certain sleep-wake traits, thereby advancing our understanding of the molecular basis of sleep, which may suggest answers to the ultimate question of why we sleep as well as provide unique insight into the relationship between sleep and chronic diseases.

Keywords

sleep; genetics; animal models; rodents; circadian rhythms

Substantial evidence demonstrates that many sleep-wake traits as well as several sleep disorders are under significant genetic control in organisms as diverse as flies, rodents and humans. Perhaps the most convincing evidence comes from twin studies in humans, where in addition to overall gross central nervous system (CNS) architecture and regional electrical activity patterns, complex electroencephalogram (EEG) traits exhibit a much higher concordance in monozygotic twins than in dizygotic twins (1-3). The EEG patterns in twins nearly match those recorded in the same individual on different occasions (4), highlighting the crucial role of genes in regulating complex EEG traits. Additionally, specific sleep traits, such as the total amount or timing of sleep, are also under significant genetic control: it has been estimated that about 50% of the variance of these traits is due to genetic factors (5) (see further contribution by Goel, this volume).

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These convincing data in humans are complemented by extensive work in rodents. Pioneering studies undertaken by Valatx and colleagues documented the segregation of sleep traits, mainly those related to rapid eye movement (REM) sleep, in inbred, recombinant-inbred, and hybrid mice (6-8). However, although the underlying role of genetics in the regulation of sleep-wake traits has been appreciated for decades, only recently have individual genes begun to be identified, beginning with the hypocretin-2 receptor gene, which was shown to be mutated in canine narcolepsy in 1999 (9). Surprisingly, the number of identified genes remains low, and the precise molecular basis of the function of these genes in sleep-wake regulation is unknown. The complexity and range of phenotypes underlying mammalian sleep suggest that many genes as well as complex networks of interacting genes working in unison will be integrated in the endogenous control of sleep and the converse state, wake. Indeed, the substantial phenotypic variability of specific sleep traits and the relatively significant influence of environmental factors on sleep-wake parameters have made the identification of specific genes difficult. In spite of these difficulties, technological advances in genomic sequencing, mapping and analysis, in combination with high-throughput sleep screening, offer great promise for the identification of the specific genetic components underlying sleep-wake traits in the near future. Additionally, sophisticated systems biology approaches can be integrated into these analyses in a powerful manner to uncover how interacting genetic networks contribute to the regulation of certain aspects of sleep. Together, these approaches offer tremendous potential for sleep researchers and are expected to further our understanding of which genes are involved in regulating sleep, and how these genes work together to form the molecular basis of the complex behavior of sleep.

In this article, we will summarize the literature addressing the genetic basis of sleep in rodents, particularly mice. We begin by describing studies exploring gene expression during wake and sleep as well as in response to sleep deprivation. We then review several comprehensive studies undertaken to identify genomic loci and candidate genes that regulate quantitative sleep traits in segregating mouse populations, including novel attempts to test the hypothesis that the regulation of gene expression underlies particular traits. Next, we highlight studies utilizing mutagenesis, transgenic and knockout technologies in the mouse to address the role of individual genes in sleep, with special emphasis on genes controlling circadian rhythms, including the sleep-wake rhythm. We discuss how these studies in mice indicate that the genetic control of sleep is highly integrated with the genetic regulation of circadian rhythms. It should also be noted that recent analyses of sleep in flies have demonstrated the validity of this invertebrate organism as a valid model system, and have contributed to our understanding of the genetic regulation of sleep, but this is beyond the scope of this review (see (10-12) for more information) (for further information, see contribution by Raizen and Zimmerman, this volume).

Both sleep and circadian rhythms are highly conserved, crucial physiological processes. Therefore, disruptions of sleep or circadian rhythms (or both) are expected to have significant detrimental effects on the organism. Indeed, a rapidly growing body of epidemiological and experimental evidence now demonstrates that sleep-wake and/or circadian rhythm disturbances are linked to a range of chronic diseases, both central (e.g., psychiatric and neurodegenerative disease (13)) and peripheral (e.g., metabolic and cardiovascular disease (14-16)), that have profound medical, public health and economic costs in humans. Thus, animal models demonstrating that sleep and circadian rhythms are regulated, to some extent, in unison at the genetic level will serve as important experimental tools necessary to uncover the mechanisms linking the genetic control of sleep and circadian rhythms to disease pathophysiology. This is expected to provide insight into the underlying basis of the widespread epidemiologic and experimental observations associating sleep-wake and circadian disturbances with disease in humans.

Analysis of gene expression across sleep and wake as well as after sleep deprivation

Initial studies examining variation in gene expression across sleep and wake identified activation of rapid response genes, also known as immediate early genes (IEGs), including *Fos*, during wake compared to sleep (17-19). Most IEGs, which are expressed in activated neurons, encode transcription factors that may lie upstream of critical signaling cascades. Thus, IEGs can be used to identify neuroanatomical regions activated by changes in arousal state. For example, while the majority of the cortex is active with higher levels of IEG expression during wake, there is a small group of gamma-aminobutyric acid-ergic (GABAergic) neurons also expressing neuronal nitric oxide synthase (nNOS) that exhibit increased *Fos* expression during non-REM (NREM) sleep (20). This set of sleep-active neurons may represent a cortical region important in regulating firing patterns necessary during NREM sleep, namely the synchronous firing underlying EEG delta power (slow-activity) (21).

Similarly, *Fos* expression is higher during sleep in the ventrolateral preoptic area (VLPO) (22), a hypothalamic region with extensive inhibitory GABAergic projections to wake-promoting centers (23), suggesting a critical role in the regulation of arousal and wake. The neuroanatomical studies of the VLPO, as well as a number of other hypothalamic nuclei, formed the basis of a model proposing hypothalamic regulation of sleep via a switch mediating alternating activity of arousal- and sleep-promoting nuclei (24).

Although these studies of IEG and *Fos* expression have contributed significant advances to our understanding of the neuroanatomical and physiological regulation of sleep, they have not provided clear insight into the genetic control of sleep. The expression patterns do not allow one to differentiate between genes controlling sleep-state changes and/or patterns of neuronal activation, as opposed to those merely responding to them. One possibility may be that expression differences between wake and sleep are indicative of compartmentalization of cellular functions to specific behavioral states: for example, transcriptional activity, as directed by the IEGs, may be performed preferentially during wake whereas protein synthesis may predominate during sleep. This hypothesis has some support from earlier studies finding a correlation between the amount of slow-wave sleep and incorporation of the radiolabeled amino acid leucine into protein in the brain (25, 26), however the data on protein dynamics related to sleep are quite limited. Thus, although analysis of differential gene expression across sleep and wake raises some interesting hypotheses about potential cellular functions during these states, it does not generate clear conclusions regarding the genetic regulation of sleep-state transitions or sleep-wake traits in general.

More recent studies of gene expression have utilized microarrays to perform comprehensive, unbiased analyses of global expression patterns across sleep and wake, often of thousands of expressed transcripts in a single brain region. The first such large-scale study compared transcripts collected from both undisturbed (and presumably sleeping) and sleep-deprived rats at 6pm, as well as from undisturbed rats at 6am that were awake most of the night (27). Approximately 10% of the greater than 15,000 transcripts detected in the cerebral cortex varied across day and night, and about half of these (corresponding to about 5% of the total) differed between sleep and wake regardless of the time of day, indicating that many genes respond directly to sleep-wake cycles. Of note, similar results were obtained in the cerebellum, a region that is not typically associated with sleep, suggesting that perhaps all neurons require cycles of rest and activity, although describing sleep at the level of single cells is premature and speculative at best (21).

Analyses of individual transcripts differentially regulated across sleep-wake states indicate that certain categories of genes tend to be increased during wake or sleep. The mRNAs upregulated during wake include, but are not limited to, those involved in energy-related processes (e.g., mitochondrial genes implicated in oxidative phosphorylation), transcriptional regulation, circadian rhythms, responses to stress, glutamatergic neurotransmission, and long-term potentiation (27). Those transcripts increased during sleep relative to wake include categories such as translational regulation, membrane trafficking, membrane potential and synaptic plasticity (27). These interesting results suggest that several broad classes of genes and expressed transcripts may be associated with or dependent on particular sleep-wake states, but again they do not provide clear insight into the genes underlying specific sleep-wake traits themselves.

Other microarray studies have explored patterns of gene expression across the entire day in both sleeping and sleep-deprived mice. One study found that many transcripts involved in biosynthetic processes and molecular transport were upregulated during sleep, a pattern particularly evident in genes for cholesterol synthesis and lipid transport (28). This observation is broadly consistent with the intuitive hypothesis that sleep is restorative at the molecular and/or cellular level within the central nervous system (CNS), although the precise details are unknown and, at present, convincing experimental support for this hypothesis is lacking.

Another large-scale study that included three different inbred strains of mice compared expression in sleep-deprived versus control animals every 4-hours throughout the 24-hour day (29). Interestingly, the majority of rhythmic transcripts in the brain (approximately 1600 out of about 2000) did not continue to cycle with sleep deprivation, suggesting that many diurnal patterns of gene expression may be dependent on sleep-wake state, as opposed to direct circadian factors. Also, many of these rhythmic transcripts were strain-specific. Therefore, analysis of the relatively few transcripts that cycled in a consistent manner across each of these strains is expected to highlight genes that are most functionally relevant for basic sleep processes. *Homer1a* (a truncated form of *Homer1*), a gene implicated in glutamate neurotransmission and believed to be important for cellular calcium homeostasis (30), demonstrated the most consistent changes across species. Importantly, this gene is located in a region of the genome that has been demonstrated to regulate, in part, the recovery from sleep deprivation (31) (see below).

Although these studies reveal interesting data regarding transcriptional changes associated with sleep states, there are several important limitations to consider. First, many of the observed changes may be species- or strain-specific and may be unique to the particular experimental conditions of each study. Second, it is impossible to determine directionality, namely whether these transcripts *cause* or simply *respond* to sleep state changes. Furthermore, sleep state changes may require only subtle alterations in expression levels that cannot be detected using conventional microarray approaches, although new sequence technologies, such as RNA seq (32) may partially address this limitation. In addition, although longer effects are possible, transcriptional regulation is expected to exert effects mainly on a time scale of minutes to hours while sleep state changes have the ability to occur over shorter periods of time. Therefore, there may be physiological processes that alter or affect neuronal function over a span of seconds, which may directly and rapidly regulate sleep state changes independent of differences in gene expression. For example, post-translational modification of proteins may underlie rapid alterations in protein function necessary for sleep state changes. Unfortunately, protein changes such as these are very difficult to measure *in vivo* in a high-throughput fashion in large numbers of animals, thus precluding comprehensive studies exploring how protein modifications are related to sleep states at this time.

Another important consideration is that studies including the measurement of gene expression during or following sleep deprivation require physical manipulation of the animals in order to maintain wakefulness. This intervention may itself induce changes in gene expression that are different from endogenous changes regulating natural sleep in an undisturbed environment (or sleep in humans). In spite of these potential limitations, the observation that a significant portion of mRNAs (about 5%) vary across sleep and wake indicates that sleep and transcription are linked. These data can be integrated with neuroanatomical and physiological information in order to determine the functional significance of the changes in expression and how they may relate to the regulation of sleep-wake physiology as well as to the general function(s) of sleep.

Identification of genetic loci controlling sleep-wake traits

The approach described above utilizes dynamic gene expression data across sleep-wake states and in response to sleep deprivation in an attempt to determine how global transcription alterations are associated with sleep state changes. An alternative approach exploits natural phenotypic variation among inbred strains of mice in order to determine which regions of the genome are responsible for the variation, and therefore regulation, of particular traits that differ between distinct strains. In this approach, two parental strains that vary with respect to the trait of interest are crossed, and their progeny (F1) are then intercrossed to create second generation offspring (F2), containing individuals that are mosaics of genetic material from each parental strain. Careful analysis of these F2 animals is expected to reveal the mode of inheritance of the trait of interest, which will be complex for behavioral phenotypes such as sleep. Genomic sequence analysis of these F2 offspring using polymorphic markers different between the two parental strains then allows for mapping of chromosomal regions associated with the trait of interest. The underlying assumption is that genetic markers that are physically linked to the gene(s) underlying the trait of interest will segregate with the trait. This approach has been especially successful for monogenic traits inherited in predicted Mendelian probabilities, and it has also become increasingly amenable for application to the study of complex traits arising from a combination of environmental factors and several genetic loci of relatively smaller effect sizes (33).

This approach, which is termed QTL (Quantitative Trait Loci) analysis, is a powerful tool to uncover the genomic basis for phenotypic traits. However, a major weakness of this approach regards the difficulty in identifying a particular gene(s) affecting the trait once the relevant genomic region is known. Often, the mapping strategy reveals a QTL containing dozens or even hundreds of candidate genes that must then be analyzed using conventional molecular biology techniques. Indeed, many QTL have been identified with regard to sleep-wake traits (see below), indicating substantial genetic control of sleep physiology, but for the most part, the individual genes underlying these traits have eluded identification. To address these limitations, strategies that involve the inclusion of outbred strains of mice, or animals from an ongoing breeding project called the “collaborative cross,” which involves several phenotypically and genetically diverse strains (34), is anticipated to greatly enhance the power of this approach. The wider genetic diversity in these animals is expected to permit more precise genetic mapping, resulting in smaller genetic loci that will contain only one or a small number of candidate genes, which can then be validated rapidly without the substantial commitment of time and resources needed to assess candidate genes in much larger genomic regions.

While there is tremendous promise concerning the use of these techniques to dramatically advance the field in the future, it should be noted that current approaches have already produced important results, yielding exciting and unexpected evidence for the role of specific components of the genome in regulating sleep-wake traits. It is beyond the scope of

this article to review all QTL studies in rodents (see (21) for more detail), but several prominent examples will be discussed in detail below to highlight the utility of this approach as well as the major conclusions that have resulted from it.

To identify loci implicated in the homeostatic regulation of NREM sleep, a recombinant-inbred approach was undertaken. Recombinant-inbred strains of mice are created with 20 generations of matings between F2 siblings derived from two inbred parental strains. This essentially achieves full homozygosity for the unique set of recombinations occurring in the original F2 progeny. In this way, a number of recombinant strains were generated from C57BL/6 and DBA/2 parental strains (BXD RI strains). The BXD RI strains underwent EEG analysis to identify QTLs associated with NREM EEG delta power achieved after 6 hours of sleep deprivation (31, 35). Analysis of NREM delta power responses to sleep deprivation revealed that about 37% of the total variance in this trait between the strains was explained by genetic factors. Furthermore, a QTL on chromosome 13 accounted for almost half of this genetic variance, suggesting the presence of an important gene. This locus was named *dps-1* (delta-power-sleep-1) and was subsequently shown to contain the *Homer1* gene, which, as discussed above, encodes a protein implicated in glutamate neurotransmission and intracellular calcium homeostasis (30). These results suggest that phenotypic variants of *Homer1a* may explain the effects of the identified QTL, highlighting *Homer1a* as an excellent candidate gene contributing to the homeostatic regulation of sleep.

A separate QTL that contributes to the slow delta oscillations (1-4 Hz) that are a hallmark of NREM sleep was also identified in the same BXD RI strains (36). The data from this study demonstrated that the majority of the variance could be explained by a single locus. A panel of 30 different inbred strains was then examined using a series of markers in the region of chromosome 14 corresponding to the site of the QTL. A single biallelic marker was identified and each of the different strains was classified as having one of the two possible alleles (termed B- and D-type). A group of divergent strains all having the D allele were subsequently analyzed for EEG activity and shown to possess the predicted EEG properties (power in the theta band (6-7 Hz) divided by the power in the delta band) associated with that particular allele. Additional polymorphic markers were then used across these strains to narrow this genomic region to about 350-kb, which was shown to contain the gene for retinoic acid receptor beta (*Rarb*) (36).

Interestingly, *Rarb* contains a restriction fragment length polymorphism (RFLP) that completely co-segregates with the genetic marker used to predict the trait. Molecular analysis, including sequencing and targeted deletion studies, was then used to clearly demonstrate that *Rarb* alleles were indeed responsible for the trait. Retinoic acid receptors are widely expressed in the brain and have been implicated in a range of neuronal functions, including long-term potentiation, control of locomotion and the regulation of dopaminergic and cholinergic neurotransmission (37). Different *Rarb* alleles may therefore have specific effects on aspects of neurotransmission that could potentially lead to altered patterns of neuronal firing that underlie synchronous cortical activity, which is ultimately responsible for the activity within the delta and theta bands.

Similar methodology was utilized by the same group of researchers to identify acyl-coenzyme A dehydrogenase (*Acads*) as a major gene involved in regulating the frequency of theta oscillations during REM sleep (38) (Figure 1). BALB/cByJ mice, which harbor a mutation in *Acads* (39), a gene encoding the enzyme that catalyzes the first step in the β -oxidation of C₄-C₆ fatty acids, exhibit a significant reduction in theta frequency specifically during REM sleep. Furthermore, these mice also have increased expression of *Glo1* (*Glyoxylase 1*), an enzyme that participates in metabolic byproduct detoxification (38). These surprising findings implicate metabolic pathways involving fatty acid β -oxidation in

regulating theta oscillations during sleep, providing an unexpected, yet profound, molecular link between cellular metabolism and REM sleep regulation.

In a different study recently undertaken, a comprehensive analysis of 20 different sleep-wake traits, grouped into 5 categories (fragmentation, REM sleep, state amount, power bands, and latency), in 269 mice from a segregating population was completed. At least 20 genomic loci, containing 52 significant QTL, were identified as regulators of many diverse sleep-wake traits (40) (Figure 2). Twenty eight of these QTL were specific for individual traits (e.g., duration of REM sleep) over the 24-hour day, while others influenced the trait during only the light or dark period, or had opposing effects on the trait during the light versus the dark phase. In order to accurately analyze this large data set, a systems biology approach was undertaken, and has revealed complex and unexpected results. For example, the seemingly different sleep-wake traits of REM latency and the number of arousals appear to be regulated, at least in part, by shared genetic mechanisms. Also, there is evidence suggesting that the number and duration of REM bouts, two traits previously expected to be highly related, are under differential genetic control (40).

Although identification of the individual genes underlying these QTL remains to be determined, these results clearly demonstrate that a complex genetic landscape underlies and regulates many sleep-wake traits. In addition, future studies can integrate gene expression data with these results through an extension of the QTL approach, termed expression QTL (eQTL) analysis, which treats variation in the amounts of expressed transcripts as quantifiable traits. By determining which genomic loci are responsible for the regulation of transcription levels of certain genes, eQTL analysis provides a powerful opportunity to examine the relationships between the genome, the transcriptome and the behavior or phenotype of interest. Furthermore, statistical testing can be utilized to infer the directionality of these relationships in order to predict which expressed transcripts causally regulate the phenotype of interest. These results and potential future experimental directions emphasize that a systems biology approach may be informative in developing a more complete understanding of how individual genes act as components of networks of interacting molecules that collectively give rise to complex behaviors, such as sleep.

In summary, QTL studies in mice have begun to uncover genomic loci and even individual genes implicated in the regulation of sleep-wake traits. It is important to recognize that in spite of decades of research exploring sleep-wake properties in rodents, much remains to be learned about which genes are involved in regulating sleep and how those molecules specifically affect sleep-wake physiology. The complexity of sleep, as well as the dramatic influence of many pharmacological agents and environmental factors on sleep-wake parameters, suggests that many genes and gene networks will ultimately be involved in controlling sleep. Technological advances in genetic mapping using different strains of mice, high-throughput sleep recording abilities, and complex computational biology approaches are expected to further our understanding of the genetic basis of sleep, which remains important from a medical as well as a biological perspective.

Knockout, transgenic and mutagenesis approaches in the mouse

Reverse genetics:

Strategies exploring the phenotypic effects of targeted, selective disruption of specific genes are referred to as reverse genetics approaches because the progression is from genotype to phenotype (41). Basically, this approach employs knockout and transgenic technologies in the mouse to specifically ablate the gene of interest, and the phenotype or behavior under investigation is then measured in animals lacking that particular gene (42, 43). Advances in transgenic technologies and targeted deletion of individual genes in mice have contributed

significantly to our understanding of the role of specific genes in sleep physiology (reviewed in (35, 44)). One important consideration that complicates the interpretation of studies using these techniques is developmental compensation. It may be possible for different genes, perhaps from the same or similar gene families, to perform the function of the missing protein (45). Thus, some studies may miss the effects of specific genes whose functions may be performed by similar genes still present. Also, there may be additional, nonspecific effects caused by removal of the gene of interest from tissues other than those directly controlling the trait under investigation.

To address these issues, the development and utilization of animals genetically engineered to eliminate genes at particular times and/or in specific tissues only (termed conditional- or inducible-knockout animals) will be important to make accurate conclusions about the precise functions of individual genes in certain tissues and/or structures of interest in relation to the regulation of sleep. Another concern that must be considered in these studies is the effect of genetic background. In spite of these limitations, these techniques remain integral experimental tools necessary for probing the molecular and genetic mechanisms underlying physiological processes, including complex behavioral traits such as sleep.

The first reports describing sleep abnormalities in transgenic mice were published nearly 15 years ago (46, 47). In general, the initial studies in transgenic and knockout mice focused on pathways and systems with previously described roles in the regulation of sleep: monoamine neurotransmitters, their receptors and their transporters (reviewed in (35, 44)). For example, mice without functional genes for the *serotonin-2C receptor* (*Htr2c*) were shown to have an altered NREM rebound after sleep deprivation, a finding suggestive of a role for this receptor in the homeostatic regulation of sleep (48). However, this difference has been shown to be attributable largely to decreased NREM sleep at baseline in *Htr2c* knockout compared to wild-type mice (21). Although this receptor may not directly regulate the response to sleep deprivation or the maintenance of homeostasis, it does appear to influence the endogenous regulation of sleep. Similarly, mice lacking the $\alpha 1A$ and $\alpha 1B$ subtypes of the serotonin receptor (*Htr1a* and *Htr1b*, respectively) exhibit increased levels of REM sleep at baseline (and after 6-hours of sleep deprivation) (49, 50), further implicating these receptors and the serotonergic system, in general. Additionally, a number of studies have conclusively demonstrated a role for cytokines (including interleukin-1, interleukin-10, tumor necrosis factor) and their receptors in sleep physiology (51).

Forward genetics:

In contrast to moving from genotype to phenotype, forward genetics entails a progression from phenotype to genotype (41). For example, the QTL approach for gene identification is considered a forward genetics approach because measurements of the variation in a phenotype of interest are taken first, and then used in conjunction with DNA sequence data to determine the underlying genetic loci involved in the regulation of that phenotype. Another forward genetics approach utilizes mutations induced randomly in the genome using techniques or compounds, such as chemical mutagens, that interfere with DNA in non-specific ways. The mutagen is applied to a population of animals who are then screened for particular abnormalities related to the phenotype of interest. Then, once “mutants” are identified, the animals are bred in an attempt to determine the mode of inheritance, map the location of the mutation in the genome and ultimately clone the underlying gene.

This approach has important advantages: 1) beginning with the phenotype ensures that any mutation identified will affect the physiological process of interest; and 2) the unbiased and random nature of the mutagenesis process provides the opportunity to identify genes without any a priori knowledge of gene function. A particularly successful application of this approach discovered *Clock*, the first mammalian gene described to be involved in the

generation of circadian rhythms (52). Importantly, this finding subsequently paved the way for the identification of many additional genes involved in the molecular generation of circadian rhythms in mammals (53).

Although the mutagenesis approach was instrumental in identifying the canonical gene *Clock*, it has not provided a similar result in the sleep field. There may be several reasons for this, including (but not limited to) the following: the technical difficulties in screening for sleep-wake traits (as opposed to circadian phenotypes for example) in large numbers of mice, the generated mutations may produce only subtle effects that are currently below the threshold of identification, the effects may be influenced by genetic background and/or epistatic interactions that prevent recognition of the altered phenotype, the inherent variability in the phenotype may mask the effects of certain mutations. The mutagenesis approach is probably most appropriate for mutations that are fully penetrant and behave according to Mendelian predictions. In contrast, the QTL approach is perhaps best suited to determine which genomic loci underlie natural phenotypic variation within populations of animals, and the networks of genes that collectively control sleep-wake traits.

Genetic disruption of circadian rhythms invariably leads to alterations in sleep

In conjunction with the transgenic and knockout studies described above, a large body of evidence from studies exploring sleep-wake traits in mice with mutations in or targeted knockout of canonical circadian clock genes have conclusively demonstrated an integral role for the circadian system in the regulation of sleep (54) (Table 1). Importantly, in addition to affecting temporal patterns of sleep, genetic components of the circadian system influence many other aspects of sleep, including its homeostatic regulation. For example, *Clock*-mutant mice have significantly reduced amounts of NREM sleep at baseline as well as a smaller compensatory increase in REM sleep after deprivation compared to wild-type animals (55). *Cry1*, *Cry2* double knockout mice exhibit signs of increased NREM sleep pressure, such as greater NREM sleep amount, increased NREM sleep consolidation, and elevated NREM sleep delta power, all under baseline conditions (56). These results indicate that *Clock* and the *Cry* genes have roles in regulating aspects of sleep duration and homeostasis in addition to their function in the generation and maintenance of circadian rhythms.

The expression of the *Per* genes (*Per1*, *Per2*, *Per3*) is controlled in part by activity of the *Cry* genes (53). *Per1* and *Per2* have also been shown to be upregulated in response to sleep deprivation (57), suggesting a potential role in sleep homeostasis. Independent experiments examining single *Per1*, *Per2* and *Per3* knockout mice, as well as double *Per1*, *Per2* knockout mice, documented a range of sleep-related changes in these animals, but the authors concluded that there were not any substantial effects on sleep homeostasis (58, 59). However, a number of the sleep alterations in these animals do not appear to be circadian in nature (e.g., *Per1*-knockouts and *Per1*/-2-double knockouts have a longer period of elevated delta power after sleep deprivation). Furthermore, *Per* mutations, in addition to affecting the timing of sleep, influence total sleep time as well as the effects of light and dark on sleep (21). Taken together, these results are consistent with a role for the *Per* genes in specific sleep-related processes in addition to their function in regulating circadian rhythms and the timing of sleep. Finally, it is important to remember that there are three mammalian *Per* genes, thus compensation for the specific gene(s) knocked-out may be sufficient to prevent more dramatic changes in sleep-wake regulation from being observed.

Additional evidence for the role of the circadian clock and circadian clock genes in the regulation of sleep homeostasis comes from studies in *Npas2*- and *Bmal*-deficient mice (60,

61), as well as from studies of animals with lesions of the central circadian clock, located in the suprachiasmatic nucleus (SCN) in the hypothalamus. While initial studies in rats suggested that the contribution of the circadian clock in the SCN to the homeostatic regulation of sleep was minimal, if even present (62, 63), subsequent work in SCN-lesioned mice (64) and monkeys (65) has demonstrated that the intact SCN can indeed influence the amount of sleep as well as the temporal control of sleep and wake. Further evidence implicating the circadian system and clock genes in specific sleep-wake processes comes from studies in *Drosophila* (10).

The profound connection between circadian rhythms and sleep is perhaps best understood from an evolutionary perspective. Circadian rhythms and sleep may have evolved together to coordinate and execute the temporal behavioral, biochemical, molecular and physiological program of the organism (66). Therefore, it should not be surprising that the genetic regulation of circadian rhythms overlaps significantly with that of sleep. The observation that significant sleep abnormalities are present in animals harboring genetic disruptions of the circadian system (Table 1) supports the hypothesis that sleep and circadian rhythms are under shared genetic control. It is important to point out that we are not arguing that all genes involved in controlling sleep-wake traits are circadian, nor are we arguing that all genetic components of the circadian system directly influence the sleep-wake cycle. We are simply framing these significant genetic relationships between circadian rhythms and sleep-wake physiology within their potential evolutionary basis.

The genetics of sleep offer a unique link to the genetics of chronic disease

The observation that circadian rhythms and sleep are highly intertwined at the genetic and molecular levels has special importance in the context of recent findings linking circadian rhythms to a range of chronic metabolic (15) and cardiovascular (14) diseases of critical importance to public health. Indeed, circadian mutant and knockout animals have emerged as important models for diseases such as the metabolic syndrome (67), diabetes (68) and hypertension (69), among others, implying that the common genetic mechanisms underlying sleep and circadian rhythms are intimately related to these pervasive chronic diseases. In addition, growing experimental and epidemiological evidence indicates that reduced and/or poor quality sleep is an independent risk factor for metabolic and cardiovascular disease (reviewed in (16)). However, in spite of these observations, there are few mechanistic hypotheses to explain exactly how disturbed circadian rhythms and/or sleep contribute to or influence the development of these diseases, which are traditionally considered to be peripheral in nature.

While dysfunction in peripheral organs (adipose tissue, pancreas, liver, etc.) is undoubtedly important to the pathogenesis of these diseases, we hypothesize that the CNS also serves a crucial role. Circadian rhythm and/or sleep disruption may negatively affect the CNS's ability to regulate metabolic processes necessary to maintain homeostasis and prevent disease development under certain environmental conditions. An intriguing possibility is that one function of sleep may be to monitor and regulate energy balance at the molecular level within the brain (70). Importantly, components of the circadian system have also recently been shown to be sensitive to redox state within the cell, potentially linking circadian rhythms and basic energy metabolism (71, 72). Therefore, disruptions of sleep and/or circadian rhythms may hinder the ability of the CNS to accurately regulate metabolic processes and energy balance within the CNS and possibly throughout the organism, which may predispose individuals to the development of metabolic and/or cardiovascular disease. While many details concerning the generation and regulation of circadian rhythms are understood at the molecular level (53), there are many outstanding questions regarding our understanding of the physiological and molecular processes underlying sleep that remain

to be answered. Studies that continue to elucidate the genetic basis of its regulation are expected to contribute to our understanding of how sleep is working at the molecular level, which, when integrated with our understanding of circadian rhythms, may provide insight into the pathogenesis and progression of certain metabolic and cardiovascular diseases shown to be linked to disturbed circadian rhythms and/or sleep.

Similarly, both circadian rhythm and sleep disturbances have been extensively implicated in psychiatric and neurodegenerative disease (13). For example, the *Clock* mutant mouse was originally shown to exhibit anxiety-like behavior (73), and, upon further investigation, has emerged as a potential model for mania-like behavior (74). Indeed, an understanding of the circadian system appears relevant for bipolar disorders in humans (75). Also, exciting recent evidence suggests that normalization of sleep-wake cycles and circadian patterns of activity may be beneficial in a transgenic mouse model of Huntington's disease (76). These are only a few of the now many examples of how the regulation of circadian rhythms, which are tightly coupled to the generation of normal sleep-wake cycles (66), at both the genetic and environmental levels, is implicated in psychiatric and neurodegenerative disease pathology and pathogenesis.

Perhaps the strongest evidence linking neuropsychiatric disease to sleep specifically occurs in depression (77). The overwhelming majority of patients with depression experience sleep-wake disturbances (as many as 90% in some studies (78)). Furthermore, periods of insomnia often precede episodes of depression (79), and successful treatment of insomnia often decreases the length, severity and incidence of clinical depressive episodes (80). Depressed patients also experience circadian rhythm disturbances (81, 82), which likely occur in unison with the sleep-wake abnormalities. Indeed, the intimate neuroanatomical, physiological and genetic connections between sleep and circadian rhythms (66) support the hypothesis that dysfunction of sleep-wake cycles will lead to reciprocal disturbances in the circadian system, which will then lead to increased sleep-wake abnormalities, creating a viscous cycle. There are several hypotheses about the mechanistic basis of the relationship between sleep, circadian rhythms and depression that are beyond the scope of this article (reviewed in (83-85)), but strong experimental support is lacking. Studies in animal models will be essential to refine and provide collaborative evidence and support for these hypotheses, and examination of the genetic basis of sleep-wake traits should provide unique insight into individual molecules, pathways, and genetic networks that may be implicated in the pathogenesis of depression as well as other psychiatric and neurodegenerative disorders.

The above represent just a few examples of specific disease classes associated with sleep and circadian rhythms. Due to the highly conserved evolutionary basis of these crucial processes, they are expected to be involved in nearly all aspects of central and peripheral physiology and, therefore, implicated in the pathogenesis of many other diseases as well. As a testament to just how highly intertwined sleep and circadian rhythms are, it should be noted that with few exceptions, most studies have failed to separate the effects of circadian rhythm disturbances specifically from those caused by sleep loss. For example, although it has traditionally been assumed that the increased incidence of chronic diseases in shift workers is due to circadian disruption (86-88), it has never been shown that it is not due to sleep disturbances, which are also exceedingly common in these individuals (89). Therefore, the fundamental connection between circadian rhythms and sleep should not be overlooked, and more work needs to be done to separate the effects of circadian disturbances versus sleep restriction as well as any synergistic effects that may arise.

Conclusions

In summary, animal models have played a significant role in our understanding of the genetic basis of sleep. In addition to verifying the roles of key neurotransmitter systems in sleep physiology, animal models have revealed important functions for previously unsuspected genes, such as those involved in fatty acid metabolism and β -oxidation. We have known for decades that many aspects of sleep are under substantial genetic control, and several studies have reported many specific genomic loci that are associated with individual traits. However, much work remains to conclusively identify the particular genes within those loci as well as the networks of interacting genes that together are responsible for the regulation of specific traits. Future work is expected to identify these individual genes and networks, which will lead to testable hypotheses that can be used by researchers to examine the effects of perturbations of these molecules and networks on sleep-wake regulation and physiology. This will allow scientists to probe the molecular mechanisms underlying sleep and perhaps begin to provide some satisfying answers to the ultimate question of why we sleep. Importantly, the recent reporting of successful targeted gene knockout strategies using embryonic stem cells in the rat (90) offers an exciting new opportunity to study genetics in an animal model more amenable to neuroanatomical manipulation and physiological experimentation than the mouse. Studies using animal models remain integral to the sleep field and will contribute to advances in our understanding of the genetic basis of sleep that may yield unexpected insight into the pathogenesis and development of diseases that have been associated with sleep abnormalities.

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References

1. Ambrosius U, Lietzenmaier S, Wehrle R, et al. Heritability of sleep electroencephalogram. *Biol Psychiatry*. 2008; 64:344–348. [PubMed: 18405882]
2. De Gennaro L, Marzano C, Fratello F, et al. The electroencephalographic fingerprint of sleep is genetically determined: a twin study. *Ann Neurol*. 2008; 64:455–460. [PubMed: 18688819]
3. Thompson PM, Cannon TD, Narr KL, et al. Genetic influences on brain structure. *Nat Neurosci*. 2001; 4:1253–1258. [PubMed: 11694885]
4. Stassen HH, Lykken DT, Propping P, et al. Genetic determination of the human EEG. Survey of recent results on twins reared together and apart. *Hum Genet*. 1988; 80:165–176. [PubMed: 3169741]
5. Tafti M, Maret S, Dauvilliers Y. Genes for normal sleep and sleep disorders. *Ann Med*. 2005; 37:580–589. [PubMed: 16338760]
6. Kitahama K, Valatx JL. Instrumental and pharmacological paradoxical sleep deprivation in mice: strain differences. *Neuropharmacology*. 1980; 19:529–535. [PubMed: 7402443]
7. Valatx JL. Possible embryonic origin of sleep interstrain differences in the mouse. *Prog Brain Res*. 1978; 48:385–391. [PubMed: 746165]
8. Valatx JL, Bugat R, Jouviet M. Genetic studies of sleep in mice. *Nature*. 1972; 238:226–227. [PubMed: 4558354]
9. Lin L, Faraco J, Li R, et al. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell*. 1999; 98:365–376. [PubMed: 10458611]
10. Allada, R. Principles and Practice of Sleep Medicine. 2010. Genetics of Sleep in a Simple Model Organism: *Drosophila*.
11. Andretic R, Franken P, Tafti M. Genetics of Sleep. *Annual Review of Genetics*. 2008; 42:361–388.
12. Shaw PJ, Franken P. Perchance to dream: Solving the mystery of sleep through genetic analysis. *Journal of Neurobiology*. 2003; 54:179–202. [PubMed: 12486704]

13. Wulff K, Gatti S, Wettstein JG, et al. Sleep and circadian rhythm disruption in psychiatric and neurodegenerative disease. *Nat Rev Neurosci.* 2010; 11:589–599. [PubMed: 20631712]
14. Durgan DJ, Young ME. The Cardiomyocyte Circadian Clock: Emerging Roles in Health and Disease. *Circ Res.* 2010; 106:647–658. [PubMed: 20203314]
15. Green CB, Takahashi JS, Bass J. The meter of metabolism. *Cell.* 2008; 134:728–742. [PubMed: 18775307]
16. Spiegel K, Tasali E, Leproult R, et al. Effects of poor and short sleep on glucose metabolism and obesity risk. *Nat Rev Endocrinol.* 2009; 5:253–261. [PubMed: 19444258]
17. Grassi-Zucconi G, Giuditta A, Mandile P, et al. c-fos spontaneous expression during wakefulness is reversed during sleep in neuronal subsets of the rat cortex. *J Physiol Paris.* 1994; 88:91–93. [PubMed: 8019528]
18. O'Hara BF, Young KA, Watson FL, et al. Immediate early gene expression in brain during sleep deprivation: preliminary observations. *Sleep.* 1993; 16:1–7. [PubMed: 8456228]
19. Pompeiano M, Cirelli C, Tononi G. Immediate-early genes in spontaneous wakefulness and sleep: expression of c-fos and NGFI-A mRNA and protein. *J Sleep Res.* 1994; 3:80–96. [PubMed: 10607112]
20. Gerashchenko D, Wisor JP, Burns D, et al. Identification of a population of sleep-active cerebral cortex neurons. *Proc Natl Acad Sci U S A.* 2008; 105:10227–10232. [PubMed: 18645184]
21. O'Hara, BF.; Turek, FW.; Franken, P. Principles and Practice of Sleep Medicine. 2010. Genetic Basis of Sleep in Rodents.
22. Sherin JE, Shiromani PJ, McCarley RW, et al. Activation of ventrolateral preoptic neurons during sleep. *Science.* 1996; 271:216–219. [PubMed: 8539624]
23. Sherin JE, Elmquist JK, Torrealba F, et al. Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. *J Neurosci.* 1998; 18:4705–4721. [PubMed: 9614245]
24. Saper CB, Scammell TE, Lu J. Hypothalamic regulation of sleep and circadian rhythms. *Nature.* 2005; 437:1257–1263. [PubMed: 16251950]
25. Nakanishi H, Sun Y, Nakamura RK, et al. Positive correlations between cerebral protein synthesis rates and deep sleep in *Macaca mulatta*. *Eur J Neurosci.* 1997; 9:271–279. [PubMed: 9058047]
26. Ramm P, Smith CT. Rates of cerebral protein synthesis are linked to slow wave sleep in the rat. *Physiol Behav.* 1990; 48:749–753. [PubMed: 2082375]
27. Cirelli C, Gutierrez CM, Tononi G. Extensive and divergent effects of sleep and wakefulness on brain gene expression. *Neuron.* 2004; 41:35–43. [PubMed: 14715133]
28. Mackiewicz M, Shockley KR, Romer MA, et al. Macromolecule biosynthesis: a key function of sleep. *Physiol Genomics.* 2007; 31:441–457. [PubMed: 17698924]
29. Maret S, Dorsaz S, Gurcel L, et al. Homer1a is a core brain molecular correlate of sleep loss. *Proc Natl Acad Sci U S A.* 2007; 104:20090–20095. [PubMed: 18077435]
30. Xiao B, Tu JC, Worley PF. Homer: a link between neural activity and glutamate receptor function. *Curr Opin Neurobiol.* 2000; 10:370–374. [PubMed: 10851183]
31. Franken P, Chollet D, Tafti M. The homeostatic regulation of sleep need is under genetic control. *J Neurosci.* 2001; 21:2610–2621. [PubMed: 11306614]
32. Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet.* 2009; 10:57–63. [PubMed: 19015660]
33. Flint J, Valdar W, Shifman S, et al. Strategies for mapping and cloning quantitative trait genes in rodents. *Nat Rev Genet.* 2005; 6:271–286. [PubMed: 15803197]
34. Churchill GA, Airey DC, Allayee H, et al. The Collaborative Cross, a community resource for the genetic analysis of complex traits. *Nat Genet.* 2004; 36:1133–1137. [PubMed: 15514660]
35. Franken P, Tafti M. Genetics of sleep and sleep disorders. *Front Biosci.* 2003; 8:e381–397. [PubMed: 12700094]
36. Maret S, Franken P, Dauvilliers Y, et al. Retinoic acid signaling affects cortical synchrony during sleep. *Science.* 2005; 310:111–113. [PubMed: 16210540]
37. Drager UC. Retinoic acid signaling in the functioning brain. *Sci STKE.* 2006; 2006:pe10. [PubMed: 16507818]

38. Tafti M, Petit B, Chollet D, et al. Deficiency in short-chain fatty acid beta-oxidation affects theta oscillations during sleep. *Nat Genet.* 2003; 34:320–325. [PubMed: 12796782]
39. Reue K, Cohen RD. Acads gene deletion in BALB/cByJ mouse strain occurred after 1981 and is not present in BALB/cByJ-fld mutant mice. *Mamm Genome.* 1996; 7:694–695. [PubMed: 8703125]
40. Winrow CJ, Williams DL, Kasarskis A, et al. Uncovering the genetic landscape for multiple sleep-wake traits. *PLoS One.* 2009; 4:e5161. [PubMed: 19360106]
41. Takahashi JS, Pinto LH, Vitaterna MH. Forward and reverse genetic approaches to behavior in the mouse. *Science.* 1994; 264:1724–1733. [PubMed: 8209253]
42. Capecchi MR. The new mouse genetics: altering the genome by gene targeting. *Trends Genet.* 1989; 5:70–76. [PubMed: 2660363]
43. Jaenisch R. Transgenic animals. *Science.* 1988; 240:1468–1474. [PubMed: 3287623]
44. Wisor JP, Kilduff TS. Molecular genetic advances in sleep research and their relevance to sleep medicine. *Sleep.* 2005; 28:357–367. [PubMed: 16173658]
45. Moran TH, Reeves RH, Rogers D, et al. Ain't misbehavin'--it's genetic! *Nat Genet.* 1996; 12:115–116. [PubMed: 8563742]
46. Tobler I, Gaus SE, Deboer T, et al. Altered circadian activity rhythms and sleep in mice devoid of prion protein. *Nature.* 1996; 380:639–642. [PubMed: 8602267]
47. Zhang J, Obal F Jr, Fang J, et al. Non-rapid eye movement sleep is suppressed in transgenic mice with a deficiency in the somatotrophic system. *Neurosci Lett.* 1996; 220:97–100. [PubMed: 8981482]
48. Frank MG, Stryker MP, Tecott LH. Sleep and sleep homeostasis in mice lacking the 5-HT_{2c} receptor. *Neuropsychopharmacology.* 2002; 27:869–873. [PubMed: 12431861]
49. Boutrel B, Franc B, Hen R, et al. Key role of 5-HT_{1B} receptors in the regulation of paradoxical sleep as evidenced in 5-HT_{1B} knock-out mice. *J Neurosci.* 1999; 19:3204–3212. [PubMed: 10191333]
50. Boutrel B, Monaca C, Hen R, et al. Involvement of 5-HT_{1A} receptors in homeostatic and stress-induced adaptive regulations of paradoxical sleep: studies in 5-HT_{1A} knock-out mice. *J Neurosci.* 2002; 22:4686–4692. [PubMed: 12040075]
51. Krueger JM, Obal FJ, Fang J, et al. The role of cytokines in physiological sleep regulation. *Ann N Y Acad Sci.* 2001; 933:211–221. [PubMed: 12000022]
52. Vitaterna MH, King DP, Chang AM, et al. Mutagenesis and mapping of a mouse gene, *Clock*, essential for circadian behavior. *Science.* 1994; 264:719–725. [PubMed: 8171325]
53. Lowrey PL, Takahashi JS. Mammalian circadian biology: elucidating genome-wide levels of temporal organization. *Annu Rev Genomics Hum Genet.* 2004; 5:407–441. [PubMed: 15485355]
54. Laposky AD, Turek FW. Circadian genes and the sleep-wake cycle. In: Squire, LR., editor. *Encyclopedia of Neuroscience.* Academic Press; Oxford: 2009. p. 909-914.
55. Naylor E, Bergmann BM, Krauski K, et al. The circadian clock mutation alters sleep homeostasis in the mouse. *J Neurosci.* 2000; 20:8138–8143. [PubMed: 11050136]
56. Wisor JP, O'Hara BF, Terao A, et al. A role for cryptochromes in sleep regulation. *BMC Neurosci.* 2002; 3:20. [PubMed: 12495442]
57. Wisor JP, Pasumarthi RK, Gerashchenko D, et al. Sleep deprivation effects on circadian clock gene expression in the cerebral cortex parallel electroencephalographic differences among mouse strains. *J Neurosci.* 2008; 28:7193–7201. [PubMed: 18614689]
58. Kopp C, Albrecht U, Zheng B, et al. Homeostatic sleep regulation is preserved in *mPer1* and *mPer2* mutant mice. *Eur J Neurosci.* 2002; 16:1099–1106. [PubMed: 12383239]
59. Shiromani PJ, Xu M, Winston EM, et al. Sleep rhythmicity and homeostasis in mice with targeted disruption of *mPeriod* genes. *Am J Physiol Regul Integr Comp Physiol.* 2004; 287:R47–57. [PubMed: 15031135]
60. Dudley CA, Erbel-Sieler C, Estill SJ, et al. Altered patterns of sleep and behavioral adaptability in *NPAS2*-deficient mice. *Science.* 2003; 301:379–383. [PubMed: 12843397]

61. Laposky A, Easton A, Dugovic C, et al. Deletion of the mammalian circadian clock gene BMAL1/Mop3 alters baseline sleep architecture and the response to sleep deprivation. *Sleep*. 2005; 28:395–409. [PubMed: 16171284]
62. Mistlberger RE, Bergmann BM, Waldenar W, et al. Recovery sleep following sleep deprivation in intact and suprachiasmatic nuclei-lesioned rats. *Sleep*. 1983; 6:217–233. [PubMed: 6622879]
63. Tobler I, Borbely AA, Groos G. The effect of sleep deprivation on sleep in rats with suprachiasmatic lesions. *Neurosci Lett*. 1983; 42:49–54. [PubMed: 6657146]
64. Easton A, Meerlo P, Bergmann B, et al. The suprachiasmatic nucleus regulates sleep timing and amount in mice. *Sleep*. 2004; 27:1307–1318. [PubMed: 15586783]
65. Edgar DM, Dement WC, Fuller CA. Effect of SCN lesions on sleep in squirrel monkeys: evidence for opponent processes in sleep-wake regulation. *J Neurosci*. 1993; 13:1065–1079. [PubMed: 8441003]
66. Turek, FW.; Dugovic, C.; Laposky, AD. Master circadian clock, master circadian rhythm. In: Kryger, MH.; Roth, T.; Dement, WC., editors. *Principles and Practice of Sleep Medicine*. 4th Ed.. Elsevier Saunders; Philadelphia, PA: 2005. p. 318-320. 4th Ed. ed.
67. Turek FW, Joshu C, Kohsaka A, et al. Obesity and metabolic syndrome in circadian Clock mutant mice. *Science*. 2005; 308:1043–1045. [PubMed: 15845877]
68. Marcheva B, Ramsey KM, Buhr ED, et al. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. *Nature*. 2010; 466:627–631. [PubMed: 20562852]
69. Doi M, Takahashi Y, Komatsu R, et al. Salt-sensitive hypertension in circadian clock-deficient Cry-null mice involves dysregulated adrenal Hsd3b6. *Nat Med*. 2010; 16:67–74. [PubMed: 20023637]
70. Scharf MT, Naidoo N, Zimmerman JE, et al. The energy hypothesis of sleep revisited. *Progress in Neurobiology*. 2008; 86:264–280. [PubMed: 18809461]
71. Reick M, Garcia JA, Dudley C, et al. NPAS2: an analog of clock operative in the mammalian forebrain. *Science*. 2001; 293:506–509. [PubMed: 11441147]
72. Rutter J, Reick M, Wu LC, et al. Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science*. 2001; 293:510–514. [PubMed: 11441146]
73. Easton A, Arbuzova J, Turek FW. The circadian Clock mutation increases exploratory activity and escape-seeking behavior. *Genes Brain Behav*. 2003; 2:11–19. [PubMed: 12882315]
74. Roybal K, Theobald D, Graham A, et al. Mania-like behavior induced by disruption of CLOCK. *Proc Natl Acad Sci U S A*. 2007; 104:6406–6411. [PubMed: 17379666]
75. McClung CA. Role for the Clock gene in bipolar disorder. *Cold Spring Harb Symp Quant Biol*. 2007; 72:637–644. [PubMed: 18419323]
76. Pallier PN, Morton AJ. Management of sleep/wake cycles improves cognitive function in a transgenic mouse model of Huntington's disease. *Brain Res*. 2009; 1279:90–98. [PubMed: 19450569]
77. Benca, RM. Mood disorders. In: Kryger, MH.; Roth, T.; Dement, WC., editors. *Principles and Practice of Sleep Medicine*. Saunders; Philadelphia: 2005. p. 1311-1326.
78. Thase ME. Antidepressant treatment of the depressed patient with insomnia. *J Clin Psychiatry*. 1999; 60(Suppl 17):28–31. discussion 46-28. [PubMed: 10446739]
79. Perlis ML, Giles DE, Buysse DJ, et al. Self-reported sleep disturbance as a prodromal symptom in recurrent depression. *J Affect Disord*. 1997; 42:209–212. [PubMed: 9105962]
80. Kupfer DJ. Pathophysiology and management of insomnia during depression. *Ann Clin Psychiatry*. 1999; 11:267–276. [PubMed: 10596741]
81. Germain A, Kupfer DJ. Circadian rhythm disturbances in depression. *Hum Psychopharmacol*. 2008; 23:571–585. [PubMed: 18680211]
82. Turek FW. From circadian rhythms to clock genes in depression. *Int Clin Psychopharmacol*. 2007; 22(Suppl 2):S1–8. [PubMed: 17917561]
83. Srinivasan V, Pandi-Perumal SR, Trakht I, et al. Pathophysiology of depression: role of sleep and the melatonergic system. *Psychiatry Res*. 2009; 165:201–214. [PubMed: 19181389]

84. Summa, KC.; Turek, FW. *Medicographia*. 2010. Circadian rhythm disturbances in Major Depressive Disorder.
85. Thase ME. Depression and sleep: pathophysiology and treatment. *Dialogues Clin Neurosci*. 2006; 8:217–226. [PubMed: 16889107]
86. Antunes LC, Levandovski R, Dantas G, et al. Obesity and shift work: chronobiological aspects. *Nutr Res Rev*. 2010; 23:155–168. [PubMed: 20122305]
87. Boggild H, Knutsson A. Shift work, risk factors and cardiovascular disease. *Scand J Work Environ Health*. 1999; 25:85–99. [PubMed: 10360463]
88. Knutsson A, Boggild H. Gastrointestinal disorders among shift workers. *Scand J Work Environ Health*. 2010; 36:85–95. [PubMed: 20101379]
89. Akerstedt T. Shift work and disturbed sleep/wakefulness. *Occup Med (Lond)*. 2003; 53:89–94. [PubMed: 12637592]
90. Hamra FK. Gene targeting: Enter the rat. *Nature*. 2010; 467:161–163. [PubMed: 20829783]

Figure 1.

A mutation in *Acads* is responsible for strain differences in theta peak frequency (TPF) during REM sleep. (a) Representative EEG samples during REM sleep in individual BALB/cByJ (C) and C57BL/6ByJ (B) mice. (b) Density analysis of EEG spectra (± 1 s.d.) in the theta range (5-9 Hz) indicates that TPF is 1 Hz faster in B mice ($n=10$ per genotype). (c) TPF during REM sleep (mean + s.d.) in parental and recombinant-inbred strains. C, BALB/cBy; B, C57BL/6ByJ. TPF in all C x B recombinant-inbred strains (and their parental C57BL/6ByJ and BALB/cBy strains) was faster than in BALB/cByJ mice (ANOVA, $F_{6,45} = 31.55$, $P < 2 \times 10^{-12}$; Scheffe's test, $P < 0.01$) (38), which are known to harbor a mutation in *Acads* (39). Thus, disrupted *Acads* is responsible for the decreased frequency of theta oscillations during REM sleep in these mice (see (38) for full details). Adapted, with permission, from (38).

Figure 2.

A comprehensive analysis of 20 sleep-wake traits, grouped into 5 categories (fragmentation, REM sleep, state amount, power bands, latency), in 269 mice from a segregating population reveals at least 20 genomic loci, containing 52 significant QTL, as responsible for variation in these traits. The colored bands represented the peak LOD score for each QTL and the fill of the bands indicates the time period for trait linkage as shown in the insert legend. See text and (40) for more detail.

Table 1

Sleep-wake patterns in animal models of circadian clock gene disruption

	Baseline 24 h wake percentage	Baseline 24 h NREM percentage	Baseline 24 h REM percentage	Baseline sleep fragmentation	Baseline NREM delta activity	Recovery response NREM activity ^a	Source ^b
<i>Cl/C1</i>	↑	↓	—	—	—	—	[5]
<i>Npas2</i>	↑	↓	↓	—	—	—	[1]
<i>Npas2</i>	↑	↓	—	—	—	↓	[2]
<i>Bmal1</i>	↓	↑	↑	↑	↑	↓	[4]
<i>Cry1,2</i>	↓	↑	↑	↓	↑	↓	[7]
<i>Per1</i>	—	—	—	—	—	↓	[3]
<i>Per1</i>	—	—	—	—	—	↑	[6]
<i>Per2</i>	—	—	—	—	—	↓	[3]
<i>Per2</i>	—	—	—	—	—	—	[6]
<i>Per3</i>	—	—	—	—	—	—	[6]
<i>Per1,2</i>	—	—	—	—	—	↑	[6]

Circadian clock mutant and deficient mice compared to wild-type mice. Empty cells indicate that information was not presented for the respective trait or could not be clearly interpreted. Many different sleep-deprivation paradigms and data analysis formats were used in these studies, making it difficult to directly compare the recovery sleep patterns among many of the animal models. ↑, increase; ↓, decrease; —, no change; EEG, electroencephalogram; NREM, non-rapid eye movement sleep; REM, rapid eye movement sleep.

1. Dudley CA, Erbel-Sieler C, Estill SJ, et al. (2003) Altered patterns of sleep and behavioral adaptability in NPAS2-deficient mice. *Science* 301:379-383.

2. Franken P, Dudley CA, Estill SJ, et al. (2006) NPAS2 as a transcriptional regulator of non-rapid eye movement sleep: Genotype and sex interactions. *Proceedings of the National Academy of Sciences of the United States of America* 103:7118-7123.

3. Kopp C, Albrecht U, Zheng B, and Tobler I (2002) Homeostatic sleep regulation is preserved in *Per1* and *Per2* mutant mice. *European Journal of Neuroscience* 16:1099-1106.

4. Laposky A, Easton A, Dugovic C, et al. (2005) Deletion of the mammalian circadian clock gene *BMAL1/Mop3* alters baseline sleep architecture and the response to sleep deprivation. *Sleep* 28(4): 396-409.

5. Naylor E, Bergmann BM, Krauski K, et al. (2000) The circadian clock mutation alters sleep homeostasis in the mouse. *Journal of Neuroscience* 20:8138-8143.

6. Shiromani PJ, Xu M, Winston EM, et al. (2004) Sleep rhythmicity and homeostasis in mice with targeted disruption of *mPeriod* genes. *American Journal of Physiology* 287: R47-R57.

7. Wisor JP, O'Hara BF, Terao A, et al. (2002) A role for cryptochromes in sleep regulation. *BMC Neuroscience* 3: 20 [online].

Reprinted, with permission, from (54).

^aIn the first few hours after sleep deprivation; recovery response in NREM EEG delta power is the standard measurement of sleep homeostatic drive.

^bData from: