Genotype-Dependent Differences in Sleep, Vigilance, and Response to Stimulants

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Abstract: To better understand the neurobiology of sleep disorders, detailed understanding of circadian and homeostatic sleep-wake regulation in healthy volunteers is mandatory. Sleep physiology and the repercussions of experimentally-induced sleep deprivation on sleep and waking electroencephalogram (EEG), vigilance and subjective state are highly variable, even in healthy individuals. Accumulating evidence suggests that many aspects of normal sleep-wake regulation are at least in part genetically controlled. Current heritability estimates of sleep phenotypes vary between approximately 20-40 % for habitual sleep duration, to over 90 % for the spectral characteristics of the EEG in nonREM sleep. The molecular mechanisms underlying the trait-like, inter-individual variation are virtually unknown, and the human genetics of normal sleep is only at the beginning of being explored. The first studies identified distinct polymorphisms in genes contributing to the endogenous circadian clock and neurochemical systems previously implicated in sleep-wake regulation, to modulate sleep architecture and sleep EEG, vulnerability to sleep loss, and subjective and objective effects of caffeine on sleep. These insights are reviewed here. They disclose molecular mechanisms contributing to normal sleep-wake regulation in humans, and have potentially important implications for the neurobiology of sleep-wake disorders and their pharmacological treatment.

Key Words: Candidate gene, genome-wide association, pharmacogenetics, *PER3*, adenosine, serotonin, dopamine, modafinil.

INTRODUCTION

Each day of human life is characterized by alternating periods of sleep and wakefulness. An endogenous oscillator with a period of approximately one day (from lat. *circa diem* = about a day) and an hourglass mechanism keeping track of sleep and wakefulness ("sleep homeostasis") interact to regulate timing and duration of sleep, sleep structure, rhythmic oscillations in the electroencephalogram (EEG), as well as predictable variation in cognitive functions and subjective state [1-7]. The molecular bases of normal human sleep and its physiological regulation are virtually unknown. It has recently been appreciated, however, that many aspects of sleep and sleep-wake regulation are highly variable among individuals, yet highly stable within individuals. Uncovering genetic factors contributing to these trait-like individual differences constitute one of the most promising avenues to foster our understanding of the neurobiology of sleep in health and disease. This review will discuss our current knowledge of genetic variation that contributes to inter-individual differences in sleep, the sleep EEG, and the response to sleep deprivation and stimulants primarily in healthy individuals. Accumulation of such insights into the molecular bases of normal sleep-wake regulation is likely to lead to improved and more personalized treatments of impaired sleep and vigilance associated with sleep and medical disorders, as well as modern life style.

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SLEEP AND SLEEP-RELATED PHENOTYPES

Large inter-individual differences are observed in many sleep and sleep-related variables including sleep timing, sleep duration, sleep structure, and preferred time of day for completion of distinct cognitive tasks. Age and sex may explain a large proportion of this inter-individual variance [4]. Nevertheless, each of these phenotypes is also under genetic control. Consistent with this notion, a moderate to high degree of heritability, i.e., the percentage of variance explained by overall genetic effects, has been demonstrated for several sleep and sleep-related variables. Twin studies are the classical approach to estimate and quantify the relative impact of genes compared with influences of the environment.

Sleep Timing

The timing of sleep, as well as many other circadian rhythms in physiology, behavior and neurological functions are strongly determined by a self-sustained circadian oscillator. This oscillator consists at the molecular level of a network of inter-locked transcriptional/translational feedback loops, which involve several clock-related genes including the transcription regulators *CLOCK*, *BMAL1*, *PER1-3*, *CRY1-2*, and *TIM* [8].

Nocturnal sleep time, but also peaks and troughs of alertness, are highly variable among healthy individuals. Self-rating scales such as the Horne-Östberg Chronotype Questionnaire [9] and the Diurnal Type Scale [10] show normal distribution of diurnal preference along an "eveningness – morningness" axis [11]. Such a distribution in a quantitative

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trait indicates the contribution of additive, small effects of many genes in combination with the environment. A recent study in a large number of monozygotic (MZ) and dizygotic (DZ) twins revealed that diurnal preference has a significant genetic component, which underlies roughly 50 % of the inter-individual variation [12].

Sleep Duration

Also sleep length shows large variation among adult individuals. Systematic investigations of the relative contribution of circadian and homeostatic factors determining inter-individual differences in sleep duration revealed that the biological night is shorter in short sleepers than in long sleepers [13]. Whether this difference is cause or consequence of the different sleep duration and associated changes such as light exposure, has not been established. While the dynamics of sleep homeostasis appear to be similar in both groups, sleep and waking EEG analyses suggest that short sleepers tolerate higher sleep pressure than long sleepers [14, 15].

An internet survey about self-rated caffeine sensitivity and sleep was recently conducted among 20'343 University students [16]. A total number of 2'308 men and 2'021 women responded (response rate: 21 %). Diurnal preference alike, habitual sleep duration (average between workdays and weekends) shows a perfect normal distribution (Fig. **1**), consistent with the influence of multiple, low-penetrance polymorphisms. Accordingly, several older studies reported for sleep duration moderate heritability estimates of 30-40 % [17-19].

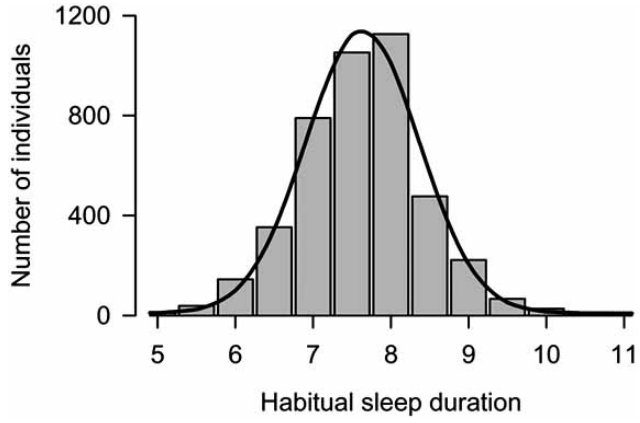

Fig. (1). Large variation and normal distribution in self-rated sleep duration (average over workdays and weekends) among 4'329 respondents to a brief internet questionnaire addressed to University students (age: 23.6 ± 3.6 years).

Sleep Architecture

Distinct oscillations in the EEG, together with information derived from the electrooculogram (EOG) and the electromyogram (EMG) serve to discriminate among non-rapid-eye-movement (nonREM) sleep, rapid-eye-movement (REM) sleep, and wakefulness [20]. Nocturnal sleep in healthy humans is typically initiated with stage 1. Reflecting increasing sleep depth, nonREM sleep is further subdivided into the stages 2 to 4. A normal sleep episode consists of four or more consecutive nonREM/REM sleep cycles with a period length of roughly 80-120 minutes.

Many variables characterizing sleep architecture demonstrate large variation among individuals and high stability within individuals [21-24]. This observation suggests the presence of trait-like, inter-individual differences in sleep physiology. Indeed, twin studies show striking similarity and concordance in visually defined sleep variables in MZ twins, yet not in DZ twins. Already the first polysomnographic sleep studies in MZ twins revealed almost complete concordance in the temporal sequence of sleep stages [25]. Subsequent work showed that in particular those variables, which most reliably reflect sleep need are under tight genetic control. They include total sleep time, duration of nonREM sleep stages, especially slow wave sleep (i.e., combined stages 3 & 4), and density of rapid eye movements in REM sleep [26-28]. Linkowski estimated that heritability of markers of sleep homeostasis is up to 90 % (REM density) [28]. Nevertheless, visual sleep state scoring relies on arbitrarily defined criteria [20] and can reveal only limited information about sleep physiology.

Sleep and Waking EEG

To obtain more detailed insights, quantitative analyses of the EEG signal recorded during sleep and wakefulness have to be performed. A powerful approach to quantify amplitude and prevalence of EEG oscillations with distinct frequencies is power spectral analysis based on Fast-Fourier Transform (FFT) [22, 29, 30]. NonREM sleep stage 1 is recognized by a pattern of theta (~ 5-9 Hz) activity. The EEG in stage 2 is characterized by the occurrence of phasic events representing sleep spindles (~ 11-15 Hz, sigma frequency range) and K-complexes. In slow wave sleep, high-amplitude, slow waves in the delta frequency range (< 4 Hz) are most prevalent. REM sleep is identified by low-amplitude EEG activity similar to stage 1. Finally, wakefulness with closed eyes is characterized in many individuals by regular alpha (~ 8-10 Hz) activity. The distinct EEG characteristics of nonREM sleep, REM sleep, and wakefulness are faithfully reflected in the EEG power spectrum of these vigilance states (Fig. **2**).

Recent studies strongly suggest that especially the sleep EEG, but also the waking EEG, are highly heritable traits in humans. All-night sleep EEG spectra derived from multiple recordings in healthy individuals show large inter-individual variation and high intra-individual stability [22, 23]. For example, Buckelmüller *et al.* [23] recorded in 8 young men 2 pairs of baseline nights separated by 4 weeks. While the spectra in nonREM sleep differed largely among the individuals, the absolute power values and the shape of each subject's spectra were impressively constant across all nights (for representative example, see Fig. **3A**). Hierarchical cluster analysis of Euclidean distances based on spectral values as feature vectors demonstrated that all 4 nights of each individual segregated into the same single cluster (Fig. **3B**). Similar results were obtained in REM sleep [23], and by other researchers in men and women of older age [22]. These data strongly suggest that the sleep EEG contains systematic and stable inter-individual differences, which are at least in part genetically determined. This conclusion is supported by

two recent studies comparing for the first time the spectral composition of the sleep EEG between MZ and DZ twin pairs. In nonREM sleep, the within-pair concordance in spectral power in the 2-13 Hz range is significantly higher in MZ twins than in DZ twins [31]. Especially alpha/sigma frequencies appear to reflect particularly strong genetic influences. Indeed, heritability in this frequency range may be as high as 96 % [32].

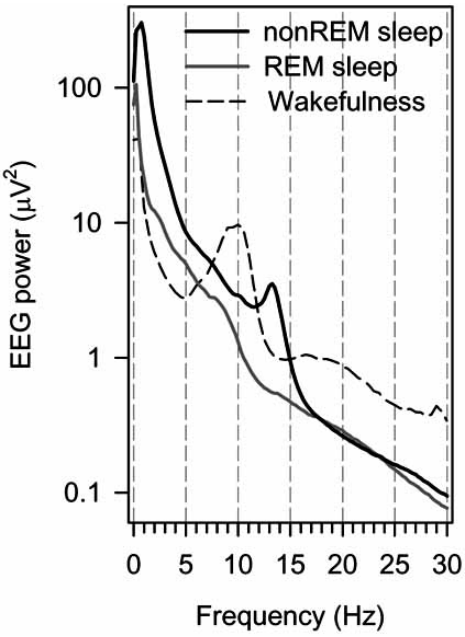

Fig. (2). Spectral characteristics of the EEG (derivation C3-A2) in nonREM sleep, REM sleep and rested wakefulness. NonREM sleep (combined stages 1-4) and REM sleep: All-night power spectra of an 8-hour baseline night (00:00 – 08:00) in 22 healthy men (age: 23.4 ± 0.5 years). Frequency resolution: 0.25 Hz. Wakefulness: Average over two, 3-min EEG recordings with closed eyes conducted at 08:15 and 11:00 in the morning following the baseline night. Frequency resolution: 0.5 Hz.

Whereas the first quantitative sleep EEG studies in twins are published only now, it has long been reported that the waking EEG has much higher resemblance between MZ twins than between DZ twins and unrelated persons [33-35]. Subsequent findings confirmed that genetic factors underlie pronounced inter-individual differences and high intra-individual test-retest correlation in spontaneous waking EEG activity [36, 37]. Van Beijsterveldt and co-workers estimated that the heritability of delta, theta, alpha and beta (> 12 Hz) frequencies in the waking EEG is over 75 % [37].

HOMEOSTATIC SLEEP-WAKE REGULATION

During wakefulness, sleep pressure accumulates, and during sleep, sleep pressure dissipates. Sleep homeostasis refers to the general principle of sleep-wake regulation that an accumulated sleep debt is highly predictably compensated [3, 38, 39]. In humans as well as animals, sleep after total or partial sleep deprivation - or rest deprivation in species in which sleep and waking cannot be unambiguously defined based on the EEG criteria - occurs with reduced latency, and is prolonged and more intense than baseline sleep. It is widely accepted that EEG delta/theta and spindle frequency activity (SFA) in nonREM sleep serve as physiological markers of sleep homeostasis in humans. The duration of slow wave sleep and initial low-frequency activity rise as a function of time awake, while SFA is typically reduced after sleep deprivation [29, 40-42].

An EEG correlate of sleep homeostasis can also be tracked during wakefulness. Power in the *theta/low-alpha*

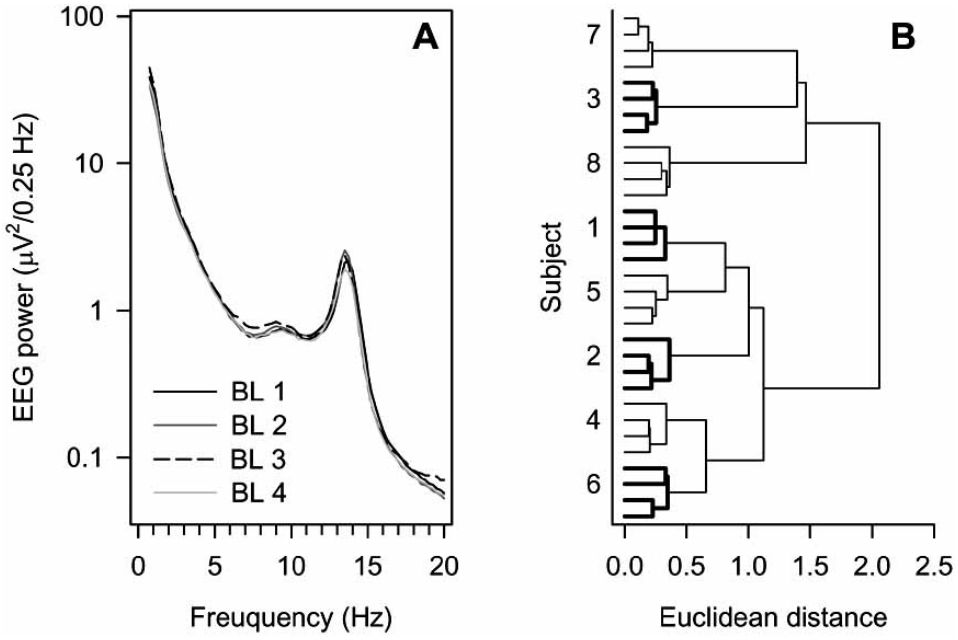

Fig. (3). Trait-like individual differences in nonREM sleep EEG in healthy men (age: 24.1 ± 0.6 years). (**A**) High intra-individual stability in nonREM sleep EEG power spectrum (0.75-20 Hz, derivation C3-A2). Two pairs of 2 consecutive baseline nights (23:00 – 07:00) separated by 4 weeks (BL 1 & BL 2 and BL 3 & BL 4). (**B**) Hierarchical cluster analysis allows separation of 8 individuals only based on Euclidean distances among feature vectors derived from EEG power spectra (0.75-20 Hz, frequency resolution: 0.25 Hz). All 4 nights of each individual segregated in the same cluster. Data adapted and replotted from Buckelmüller *et al.* [23].

band of the waking EEG increases during prolonged time awake [41, 43-45]. The time constant of this increase is similar to that of the wake-dependent increase of low-frequency activity in nonREM sleep [44, 45]. This finding suggests that the enhancement of delta activity in recovery sleep, and the rise rate of theta activity during waking are closely related. Both changes are larger over anterior than over posterior cortical areas [46]. In fact, the spatial distribution of EEG oscillations in nonREM sleep is highly stable across multiple recordings within the same individual. Thus, sleep EEG topography was proposed to reflect an individual "fingerprint", which is genetically determined [32, 47, 48].

Sleep deprivation not only affects sleep and waking EEG, but also alertness and performance on neurobehavioral and cognitive tasks. There exists, however, large inter-individual variation in performance decline during prolonged waking [49-51]. The differences are substantial and stable, even when sleep duration before sleep deprivation is experimentally varied (sleep restriction *vs*. sleep extension). The differences between vulnerable and resistant subjects do not reflect inter-individual differences in sleep need. In addition, the changes on distinct tasks show no clear relationships. More specifically, performance after sleep deprivation is clearly impaired in certain subjects on one task, while the same subject can even improve on another task [51]. Similarly, the evolution of wakefulness-induced changes in EEG theta/alpha activity, subjective sleepiness, and neurobehavioral performance can widely dissociate [49, 50, 52]. Taken together, accumulating data suggest that the magnitude of performance impairment from sleep loss reflects trait-like individual vulnerability [53, 54], yet the underlying neurobiological mechanisms are currently unknown.

HUMAN GENETICS OF NORMAL SLEEP-WAKE REGULATION

The manifestation and regulation of sleep, the sleep and waking EEG, as well as sleep-related waking functions reflect different aspects of complex behaviors. Each of these aspects is likely to be under the control of multiple genes, which may interact, and are also influenced by the environment. Considerable knowledge accumulated over the past years about the roles of distinct genes for sleep in model systems and patients with sleep disorders (e.g., [55-57]). By contrast, very little is known about the genes that contribute to the trait-like, individual "sleep-phenotypes". This lack of knowledge may reflect the fact that precise quantification of these phenotypes in healthy individuals requires controlled, long-lasting and cost-expensive experiments relying on high-tech methodology, as well as time-consuming data analyses.

Three main techniques for the genetic dissection of normal human sleep are currently available. The first is to examine the impact of candidate genes, for which evidence exists that they are implicated in sleep and sleep-wake regulation. With this method, individuals with distinct genotypes of known genetic polymorphisms are prospectively studied in the sleep laboratory. A limitation of this approach is that it precludes "sleep gene discovery". By contrast, genome-wide association studies may lead to the identification of novel "sleep genes". The weaknesses and strengths of these strategies were previously discussed in detail [58, 59]. Finally, a virtually unexplored, yet powerful approach to obtain insights into the molecular mechanisms of sleep-wake regulation is sleep pharmacogenetics.

In the following paragraphs, I shall summarize current concepts primarily derived from preclinical studies of neuroanatomy and neurochemistry of sleep-wake regulation, followed by an overview of the present state of knowledge of genes that influence normal human sleep. As a general remark, most current evidence suggesting a direct association between genetic variation in distinct "clock" genes and/or "sleep" genes and the physiological mechanisms underlying sleep-wake regulation may still be controversial and awaits confirmation.

NEUROANATOMY/NEUROCHEMISTRY OF SLEEP-WAKE REGULATION

It is well established that the "master" clock driving circadian rhythms is located in mammals in the suprachiasmatic nuclei (SCN) of the hypothalamus. By contrast, the neuroanatomical and neurochemical bases underlying sleep homeostasis are poorly understood. *In vivo* and *in vitro* electrophysiological studies revealed that the EEG characteristics of nonREM sleep, REM sleep, and wakefulness reflect the firing patterns of thalamo-cortico-thalamic and intra-cortical networks [60]. The firing of these cells is modulated by distinct neural systems originating in basal forebrain, hypothalamus and brain stem, which are thought today to importantly contribute to sleep-wake regulation [61-63].

Neural Systems Promoting sleep

Prominent roles for basal forebrain and rostral hypothalamic structures in generating and maintaining sleep have long been suggested from lesion studies in animals and clinical observations in humans [61]. More recent insights support this notion. For example, microdialysis experiments in cats and rats suggest that the extracellular adenosine concentration in basal forebrain is modulated by the sleep-wake cycle [64, 65]. Binding of adenosine to inhibitory A_1 receptors reduces excitatory neurotransmission and may contribute to sleep homeostasis [66]. However, a causal relationship between site-specific changes in extracellular adenosine and sleep homeostasis is controversial (see [67] for discussion). In humans, positron emission tomography (PET) indicated that sleep deprivation modulates adenosine A_1 receptor binding throughout cortical and subcortical brain regions, rather than in region-specific manner [68].

Another possibility for adenosine to promote sleep is via disinhibition of adenosine A_{2A} receptor-expressing neurons in basal forebrain and ventro-lateral preoptic (VLPO) area of the hypothalamus. Electrophysiological studies in freely moving rats have shown that the discharge rate of VLPO neurons is more than doubled during sleep when compared to wakefulness [69, 70]. Moreover, expression of the immediate early gene, Fos, in the VLPO is closely related to the amount of sleep obtained by the animal before FOS protein quantification [71]. In accordance with these animal studies, a relative increase in regional glucose metabolic rate in nonREM sleep compared to wakefulness was also found in basal forebrain/preoptic hypothalamus in humans [72]. The VLPO neurons form a dense cluster and a more diffuse extended

part containing the inhibitory neuromodulators galanin and γ-amino-butyric acid (GABA) [73-75]. Axons from the VLPO innervate cell bodies and dendrites of the noradrenergic locus coeruleus (LC), the serotonergic dorsal raphe nucleus (DRN), the histaminergic tuberomammillary nucleus (TMN), and also terminate within cholinergic cell groups of basal forebrain and the latero-dorsal/pedunculo-pontine tegmental (LDT/PPT) areas of the brain stem [75-78]. On the other hand, the VLPO receives afferents from each of the major monoaminergic systems [79]. These reciprocal connections suggest that VLPO activity is suppressed by the same arousal systems (see below) that it inhibits during sleep.

Electrophysiological recordings in rat brain slices demonstrate the existence of two distinct types - Type-1 and Type-2 - of sleep-active VLPO neurons [80, 81]. While stimulation of post-synaptic A_1 receptors inhibits both types of cells, pharmacological blockade of these receptors produces a reversible increase in spontaneous firing in Type-2 cells, which is further enhanced when adenosine is applied [81]. This effect reflects activation of A_{2A} receptors, because a selective A_{2A} receptor agonist excites Type-2, but not Type-1 neurons. It is possible that adenosine disinhibits both subtypes of sleep-active cells through pre-synaptic reduction of GABA release, and actively excites Type-2 neurons through post-synaptic activation of A_{2A} receptors.

In recovery sleep after 12-14 hours sleep deprivation, VLPO neurons display an even larger sleep-related increase in discharge rate than in baseline sleep [70]. Enhanced FOS immunoreactivity and cell firing in VLPO, however, are not increased in waking, but only after the animals enter sleep. This finding suggests that VLPO activity promotes sleep onset and/or consolidation, rather than underlies increased sleepiness and impaired waking functions associated with inadequate sleep [82]. The differences between Type-1 and Type-2 neurons may indicate that the latter are involved in sleep initiation, whereas the former contribute to sleep consolidation [81]. They are only activated when released from inhibition by monoaminergic and cholinergic arousal systems.

Neural Systems Promoting Wakefulness

Classical studies have shown that the brainstem reticular formation modulates cortical activation via a dorsal pathway through the thalamus, as well as a ventral, extra-thalamic pathway through the posterior hypothalamus and basal forebrain [83]. The main thalamic projection originates in LDT/PPT. Cell groups originating in LC, DRN, and TMN contribute to the extra-thalamic branch of the ascending arousal system. It is joined in posterior lateral hypothalamus by axons from orexin- (also known as hypocretin) and melanin-concentrating hormone-containing neurons, as well as in basal forebrain from acetylcholine-containing cells.

Cholinergic LDT/PPT neurons fire rapidly in wakefulness and REM sleep, whereas few cells are active in non-REM sleep. During this state, reduced cholingergic inhibition of GABA-ergic thalamic reticular neurons leads to hyperpolarization of thalamo-cortical cells resulting in the occurrence of delta waves and sleep spindles [60].

Clinical observations in neurologic patients and lesion studies in animals indicate that the noradrenergic system stimulates and maintains cortical activation and modulates the quality of arousal [61, 84]. The activity of the LC is high during wakefulness and low during sleep [85]. Destruction of nerve fibers originating in LC in rats with the neurotoxin DSP-4 reduces low-range (< 1 Hz) delta activity in nonREM sleep after both spontaneous and enforced wakefulness [86]. These findings support the hypothesis that noradrenaline release during wakefulness is causally related to the intensity of subsequent sleep.

Clinical symptoms of disorders such as Parkinson's Disease, which is associated with reduced dopaminergic neurotransmission, often include disturbed sleep and vigilance [87]. Dopaminergic neurons of ventral tegmental area and substantia nigra are involved in behavioral arousal [61, 88]. Moreover, amphetamine-like stimulants including methamphetamine and methylphenidate increase wakefulness by blocking catecholamine (i.e., adrenaline, noradrenaline and dopamine) re-uptake from the synaptic cleft and/or stimulating catecholamine release [89]. Genetically modified mice, in which the dopamine transporter (*Dat*) gene was deleted, exhibit reduced nonREM sleep and prolonged wakefulness when compared to wildtype littermates [90]. In addition, *Dat* knock-out mice are unresponsive to the normally robust wake-promoting action of catecholaminergic agents, yet hypersensitive to the adenosine receptor antagonist caffeine [90].

Though it has long been suggested that serotonin (5-hydroxy-tryptamine, 5-HT) is critical for sleep-wake mechanisms [91], the specific roles for this neurotransmitter remain uncertain [61, 92]. The serotonergic neurons of the DRN exhibit the highest discharge rate in waking, show diminished activity in nonREM sleep, and are virtually silent shortly before and during REM sleep [93, 94]. This firing pattern is mimicked by changes in the extracellular 5-HT concentration across sleep-wake alternations in rats and cats [95, 96], elevated 5-HT levels in the rat hippocampus during sleep deprivation [95], and ultradian variation of ventricular 5-HT concentration across nonREM/REM sleep cycles in humans [97]. These and other data may support the notion that serotonergic neurotransmission stimulates wakefulness and is partially (in nonREM sleep) or completely (in REM sleep) turned-off during sleep. Electrophysiological studies show that 5-HT inhibits or excites distinct VLPO cells *in vitro* [74, 81]. More specifically, not only adenosine, but also 5-HT excites type-2 cells in VLPO [81].

Taken together, while other neurotransmitter/neuromodulator systems are certainly also involved (e.g., histamine, orexin, GABA, glutamate, acetylcholine, neuropeptides), adenosinergic, catecholaminergic and serotonergic influences contribute to sleep-wake regulation.

CANDIDATE GENE APPROACH

Circadian Locomotor Output Cycles Kaput (CLOCK) Gene

The effect of a single nucleotide polymorphism (SNP) in the 3'-untranslated region (UTR) of the human *CLOCK* gene on diurnal preference as assessed with the Horne-Östberg questionnaire was first studied in middle-aged adults. Polymorphisms in this region affect stability and half-life of messenger RNA [98], and possibly alter the protein level that is

finally translated. Katzenberg *et al.* reported that homozygous carriers of the 3111C allele have increased evening preference for mental activities and sleep, with delays ranging from 10 to 44 minutes when compared to individuals carrying the 3111T allele [99]. A similar association was found in a Japanese population [11], but not confirmed in European and Brazilian samples [100, 101]. Sleep variables derived from nocturnal polysomnography did not differ between the genotypes [99]. Interestingly, clinical and rest-activity data in patients with mood disorders indicate that individuals with the 3111C allele have increased occurrence of disturbed sleep both during mood episodes as well as in remission, higher motor activity levels in the evening, and a different response to lithium than patients homozygous for the T allele [102-104].

Period (PER) Genes

In mice, *Per1* and *Per2* play important roles in the maintenance of circadian rhythmicity, whereas *Per3* functions outside the core circadian clock work [105]. Screening for missense mutations or functional polymorphisms in promoter and 5'- and 3'-UTRs of the human *PER1* gene in volunteers with extreme diurnal preference and patients with delayed sleep phase syndrome (DSPS) remained unsuccessful [106]. Moreover, a synonymous A to G substitution at nucleotide 2548 showed no association with eveningness-morningness tendencies [107]. By contrast, the distribution of the C and T alleles of a silent polymorphism in exon 18 differed between extreme morning and evening types [106]. Specifically, the frequency of the 2434C allele was significantly higher in subjects with extreme morning preference than in subjects with extreme evening preference.

A missense mutation in the human *PER2* gene provides today the most striking example of a direct link with changed circadian rhythms. Thus, familial advanced sleep phase syndrome (FASPS) is associated with altered amino acid sequences in PER2 and casein kinase I delta (CKIδ) proteins [108, 109]. A transgenic mouse model expressing the human FASPS mutation demonstrated that CKIδ can regulate circadian period through PER2 [110]. A C111G polymorphism located in the 5'-UTR of *PER2* was also reported to modulate diurnal preference in healthy volunteers [111]. Computer simulation predicted that the 111G allele, which may be associated with morning preference, has different secondary RNA structure than the 111C allele. It is possible that the two transcripts are differently translated [111].

A variable-number-tandem-repeats (VNTR) polymorphism in the human *PER3* gene may modulate multiple phenotypic variables related to sleep timing, sleep architecture and vulnerability to sleep loss. A 54-nucleotide sequence located in a coding region of this gene is either repeated in four or five units. This difference may alter the dynamics in PER3 protein phosphorylation. In European and Brazilian populations, the longer 5-repeat allele and the shorter 4-repeat allele were associated with morning preference and evening preference, respectively [112, 113]. Moreover, homozygous carriers of the long-repeat genotype showed increased slow wave sleep and delta activity in nonREM sleep, as well as higher theta/alpha activity in REM sleep and wakefulness than homozygous 4-repeat individuals [114]. The decline in cognitive performance during sleep deprivation was worse in the former than in the latter, in particular during late-night and early-morning hours and on tasks of executive functioning [115]. These data may indicate that the genetic variation in *PER3* affects sleep homeostasis. They support the notion that there are complex, mutual interactions between sleep-wake regulatory processes, and suggest a contribution of *PER3* to individual tolerance to shift work and jet-lag, which are highly prevalent in society.

Adenosine Deaminase (ADA) Gene

The adenosine-metabolizing enzyme, adenosine deaminase (ADA), plays an important role in regulating extracellular adenosine levels [116]. We recently found in humans that a functional variation in the *ADA* gene has a profound impact on sleep and the sleep EEG (Fig. **4**). More than 30 allelic variants of *ADA* are currently listed in the Online Mendelian Inheritance in Man (OMIM) database. Most of these variants represent non-functional alleles, which give rise to severe combined immunodeficiency. The most frequent allele that is asymptomatic in heterozygous carriers is caused by a G to A transition at nucleotide 22, leading to the substitution of asparagine for aspartic acid at codon 8 of the ADA protein [117]. The G/A genotype is found in roughly 10 % of healthy Caucasians, whereas the homozygous A/A genotype occurs in less than 1 % of the population [118]. Compared to individuals with the G/G genotype, individuals with the G/A genotype exhibit lower enzymatic activity in erythrocytes and leucocytes [119] and may be at elevated risk to develop

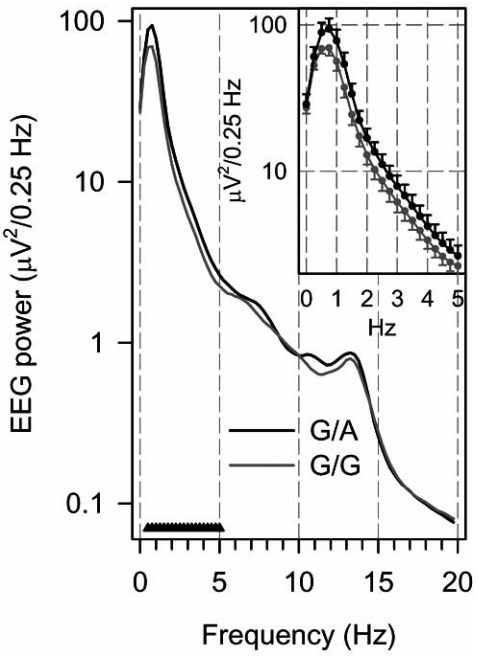

Fig. (4). The functional 22G>A polymorphism of the adenosine demanise (*ADA*) gene modulates nonREM sleep intensity in healthy individuals. All-night EEG power spectra in nonREM sleep (stages 1-4, 0.25-20 Hz, derivation C3-A2) in individually age- and sex-matched subjects with G/A (black lines, n = 8) and G/G genotypes (grey lines, n = 8). Mean absolute power values are plotted on a logarithmic scale. Triangles above the x-axis indicate frequency bins (0.75-5.0 Hz) that differed significantly between the genotypes ($p < 0.05$, two-tailed paired t-tests). The frequency range between 0 and 5 Hz was expanded for better visualization (inset, error bars indicate + or – SEM).

autism [120]. In accordance with the hypothesis that differences in extracellular adenosine levels affect markers of sleep homeostasis, baseline sleep in subjects with the G/A genotype was characterized by 30 minutes more slow wave sleep than in subjects with the G/G genotype [121]. Moreover, also the intensity of slow wave sleep as estimated from EEG delta activity was higher in the former than in the latter. While the differences associated with this polymorphism are similar in magnitude to the changes in sleep and the sleep EEG after one night without sleep, the consequences of sleep deprivation in individuals with distinct *ADA* genotypes are currently unknown. Based on data in animals, it may be expected that G/A and G/G genotypes behave differently during prolonged wakefulness. More specifically, QTL analyses in inbred mouse strains revealed that a genomic region including *Ada* modifies the rate, at which nonREM sleep need accumulates during wakefulness [122].

It is tempting to speculate that pharmacological interference with ADA might provide a possible target to promote sleep and its intensity, for example in patients with insomnia. Indeed, blocking ADA by local application of (deoxy)-coformycin in rats prolongs sleep and increases delta activity in nonREM sleep [123, 124]. However, elevated concentrations of adenosine and 2'-deoxyadenosine, another substrate of ADA, acting on adenosine receptors expressed on virtually all cells in the organism may lead to unwanted drug reactions.

Other Possible Candidate Genes

Catechol-O-Methyltransferase (COMT) Gene

Genetic evidence from patients with narcolepsy supports a role for noradrenergic/dopaminergic pathways in regulating sleep-wake functions. Distinct alleles and genotypes in the genes of monoamine oxidase type A (MAO-A) [125] – but see [126] – and catechol-O-methyltransferase (COMT) [126] are thought to be associated with the clinical manifestation of narcolepsy. In particular, the human COMT gene contains a functional SNP that alters the amino acid sequence of the COMT protein at codon 158 from valine (Val) to methionine (Met) [127]. Individuals homozygous for the Val allele show more COMT protein in post-mortem brain tissue than individuals with two Met alleles [128]. Moreover, the Val/Val genotype is associated with 3- to 4-fold higher COMT activity and presumably lower dopaminergic signaling in prefrontal cortex than the Met/Met genotype [128, 129]. The impact of this polymorphism on the severity of sleepiness as measured by multiple sleep latency tests (MSLT) supports the conclusion that sleep-wake symptoms in narcolepsy are associated with altered dopaminergic neurotransmission [126]. In addition, a more recent study found positive associations between narcolepsy and other genomic regions related to the monoaminergic system, including the genes of the dopamine D_2 and serotonin 5-HT_{2A} receptors [130].

Serotonin Transporter Gene (SLC6A4)

Serotonin may contribute to the build-up of sleep pressure associated with wakefulness. Extracellular 5-HT is catabolized in glia cells and non-serotonergic neurons by MAO-A to 5-hydroxy-indolacetic acid (5-HIAA). In addition, 5-HT is removed from the synapse by high-affinity serotonin transporters (5-HTT). The 5-HTT in brain is the principal site of action for many currently used antidepressants [131]. A functional VNTR polymorphism in the 5'-HTT promoter region of SLC6A4 has been consistently associated with psychiatric diagnoses and individual efficacy of antidepressant treatments. *In vitro* studies show that basal transcriptional activity of the long variant (L) allele is more than doubled when compared to the short (S) variant allele [132]. Human individuals homozygous for the L/L variant show higher 5-HTT messenger RNA levels in postmortem brain tissue than subjects carrying the S allele (L/S + S/S) [133]. It was speculated that reduced transcription associated with the S allele modulates serotonergic tone and 5-HT receptor-mediated neurotransmission [134]. Preliminary data indicate that the presence of the S allele affects subjective and objective measures of sleep in certain situations [135, 136]. While the functional relationship between the genetic variation in SLC6A4 and sleep-wake regulation has not been studied in healthy individuals, it appears that homozygous depressed patients with the L/L genotype respond more favorably to therapeutic sleep deprivation than carriers of the S allele [137].

Transgenic mice lacking functional 5-HTT and 5-HT_{1A} receptors show more REM sleep than wildtype littermates [138, 139]. A recent PET study demonstrated that the insertion/deletion polymorphism in the promoter region of *SCL6A4* affects 5-HT_{1A} receptor availability in humans [134]. More specifically, subjects with L/L genotype have higher 5-HT_{1A} receptor binding in all brain regions than carriers of the S allele. It is unknown whether this polymorphism also modulates availability of other 5-HT receptor subtypes. Notwithstanding, it may contribute to inter-individual differences in the efficacy of currently emerging hypnotics that improve sleep via a serotonergic mechanism of action [140, 141].

Genome-Wide Association Studies

Only one genome-wide association study of sleep-related phenotypes is currently available in humans [142]. Phenotypic and genetic analyses were conducted in 749 subjects and confirmed moderate heritability estimates for sleep duration (17 %) and habitual bedtime (22 %) as assessed by non-validated questionnaire, as well as subjective sleepiness as quantified by Epworth Sleepiness Scale (29 %) [143]. Some genetic loci containing circadian clock-related genes including casein kinase II alpha-2 (*CSNK2A2*), *CLOCK* and prokineticin 2 (*PROK2*) were identified to modulate habitual bedtime and sleep duration, respectively. None of the investigated phenotypes, however, was associated with previously suspected candidate genes for sleep and sleep-wake regulation. Methodological issues such as the used questionnaire and limited gene-chip resolution may underlie the discrepancy. Association tests suggested that "novel genes" encoding neuropeptide S receptor 1 (*NPSR1*) and phosphodiesterase 4D (*PDE4D*) may influence habitual bedtime on workdays and self-rated sleepiness, respectively.

Studies of Sleep Pharmacogenetics

At least some effects of the neuromodulator adenosine on sleep appear to be mediated via the adenosine A_{2A} receptor

(see [144] for recent overview). In rats, selective A_{2A} receptor agonists such as CGS21680 administered to the subarachnoid space adjacent to basal forebrain and lateral preoptic area reliably induce nonREM sleep, while infusion of A_1 receptor agonists produces weak and variable effects [145-148]. Local application of CGS21680 also increases *Fos* expression in VLPO [147]. Activation of A_{2A} receptors expressed in nucleus accumbens may underlie this finding. Moreover, Gallopin *et al.* demonstrated direct activation of sleep-promoting VLPO neurons via post-synaptic stimulation of A_{2A} receptors [81]. Interestingly, CGS21680 and the adenosine receptor antagonist, caffeine, are ineffective in mice lacking functional A_{2A} receptors [149, 150].

Caffeine is the most widely consumed stimulant in the World. To examine a role for A_{2A} receptors in human sleep-wake regulation, the interaction of caffeine with sleep deprivation was investigated in self-rated caffeine sensitive and insensitive individuals [16]. Alleles and genotypes of a synonymous 1083T>C polymorphism located in the coding region of the adenosine A_{2A} receptor gene (*ADORA2A*) were differently distributed between two groups of subjects who either reported or not disturbed sleep after caffeine consumption in the evening. This polymorphism is linked to a 2592C>T_{ins} polymorphism in the 3'-UTR of *ADORA2A*. The latter may modulate protein expression [151]. Supporting a role for A_{2A} receptors in human sleep, caffeine increased higher-frequency (> 16 Hz) EEG activity prominently in the C/C genotype (15.3 ± 3.1 %, n = 6), intermediately in the C/T genotype (6.9 ± 3.0 %, n = 10), and was ineffective in the T/T genotype (-0.2 ± 5.1 %, n = 3) [16]. Elevated high-frequency activity in nonREM sleep reflects reduced sleep intensity and may be characteristic of patients with primary insomnia when compared to healthy good sleepers [152].

Both stimulant and anxiogenic properties of caffeine contribute to inter-individual differences in the subjective response to the drug [153]. The 1083T>C polymorphism of *ADORA2A* not only modulates effects on the sleep EEG, but is also associated with symptoms of anxiety after moderate caffeine intake [151, 154] and habitual caffeine consumption [155]. Sleep disruption and anxiety, respectively, appear to be distinctly favored by the C and T alleles of *ADORA2A*. To obtain more information about a possible adenosinergic mechanism linking sleep and mood regulation, the effects of caffeine on sleep and anxiety symptoms should be studied simultaneously in individuals with distinct *ADORA2A* genotypes.

The *ADORA2A* gene is located on human chromosome 22q11.2, in close proximity to the gene encoding COMT. On the MSLT, female narcolepsy patients with high COMT activity (Val/Val allele carriers) fall asleep twice as fast as women narcoleptics with low COMT activity (Met/Met allele carriers) [126]. An opposite relationship is observed in men. Clinical data suggest that also the efficacy of the stimulant, modafinil, to control daytime sleepiness differs between women and men, and among *COMT* genotypes [156]. More specifically, the optimal daily dose of modafinil is 100 mg lower in female than in male narcoleptics, yet higher in patients (men and women) with Val/Val genotype than in patients with Met/Met genotype. Although the exact mode of action of modafinil is still controversial, the available data suggest that the drug stimulates wakefulness by promoting dopaminergic and (nor)adrenergic neurotransmission [157]. In healthy volunteers, the COMT genotype modulates the response of the prefrontal cortex to increased dopamine according to a inverted, U-shaped response curve [158]. It might, therefore, be expected that in healthy subjects modafinil would improve enhanced sleepiness after sleep loss more efficiently in the Val/Val genotype than in the Met/Met genotype.

CONCLUSION

Sleep and sleep-wake regulation in humans represent highly variable phenotypes, which are increasingly appreciated to be under moderate to strong genetic control. The molecular mechanisms underlying this variation are virtually unknown. Recent genetic studies, however, revealed first insights and led to exciting progress in our understanding of the neurobiology of normal sleep and sleep-wake regulation. These new findings have potentially important implications for the neurobiology of sleep-wake disorders and their pharmacological treatment.

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ABBREVIATIONS

NonREM sleep	=	Non-rapid-eye-movement sleep
REM sleep	=	Rapid-eye-movement sleep
EEG	=	Electroencephalogram
EOG	=	Electrooculogram
EMG	=	Electromyogram
CLOCK	=	Human circadian locomotor output cycles kaput gene
BMAL1	=	Human aryl hydrocarbon receptor nuclear translocator-like protein 1, brain and muscle gene
PER1-3	=	Human period genes 1-3
CRY1-2	=	Human cryptochrome genes 1-2
TIM	=	Human timeless gene
SFA	=	Spindle frequency activity
SCN	=	Suprachiasmatic nuclei
PET	=	Positron emission tomography
VLPO	=	Ventro-lateral preoptic area
GABA	=	γ-Amino-butyric acid
LC	=	Locus coeruleus
DRN	=	Dorsal raphe nucleus
TMN	=	Tuberomammillary nucleus
LDT/PPT	=	Latero-dorsal/pedunculo-pontine tegmental areas
Dat	=	Mouse dopamine transporter gene

5-HT	=	5-Hydroxy-tryptamine, serotonin
SNP	=	Single nucleotide polymorphism
UTR	=	Untranslated region
RNA	=	Ribonucleic acid
Per1-3	=	Mouse period genes 1-3
DSPS	=	Delayed sleep phase syndrome
FASPS	=	Familial advanced sleep phase syndrome
CKIδ	=	Human casein kinase I delta protein
VNTR	=	Variable-number-tandem-repeats
ADA, ADA	=	Human adenosine deaminase gene and protein
QTL	=	Quantitative trait locus
Ada	=	Mouse adenosine deaminase gene
COMT, COMT	=	Human catechol-O-methyltransferase gene and protein
MAO-A, MAO-A	=	Human monoamine oxidase type A gene and protein
MSLT	=	Multiple sleep latency test
SLC6A4	=	Human serotonin transporter gene
5-HIAA	=	5-Hydroxy-indolacetic acid
5-HTT	=	Serotonin transporter
CSNK2A2	=	Human casein kinase II alpha-2 gene
PROK2	=	Human prokineticin 2 gene
NPSR1	=	Human neuropeptide S receptor 1 gene
PDE4D	=	Human phosphodiesterase 4D gene
ADORA2A	=	Human adenosine A_{2A} receptor gene

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