Handbook of Experimental Pharmacology 253

Hans-Peter Landolt
Derk-Jan Dijk *Editors*

Sleep-Wake Neurobiology and Pharmacology

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Volume 253

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Sleep-Wake Neurobiology and Pharmacology

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Preface

This volume of the *Handbook of Experimental Pharmacology* is the first handbook on sleep-wake pharmacology in which both sleep- and wake-promoting compounds are discussed and within the context of the neuroscience of sleep-wake regulation. We have organized the volume in five parts: (I) Basic Principles; (II) Optogenetics and Pharmacogenetics; (III) Sleep-Wake Pathologies; (IV) Current and New Targets, and Therapeutic Prospects; and (V) Outlook and Perspectives.

The alternation of sleep and wakefulness represents a fundamental biological rhythm, and undisturbed good quality sleep is indispensable for physical and mental health, cognitive functioning, and good quality of life. Although it is widely accepted that sleep must serve at least one basic function across a wide range of species, general consensus about the unique function(s) of sleep is lacking. Frank and Heller provide an overview of current hypotheses on sleep functions and categorize them into those serving higher order cognitive functions and restorative processes. They conclude that the strongest support for a primary function of sleep goes to learning and memory and the underlying process of synaptic plasticity. Furthermore, they suggest that impaired sleep-dependent brain energy reserve replenishment and clearance of brain metabolism-related waste products may contribute to cognitive decline with aging.

While significant gaps in the understanding of sleep-wake regulation remain, the knowledge base for a rational pharmacology of sleep-wake disorders is much stronger now than a decade ago. Luppi and Fort summarize the current understanding of the neuroanatomical and neurochemical bases responsible for the generation of wakefulness, non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep, as well as the putative networks responsible for the switch between wakefulness and NREM and REM sleep states. Then, O'Callaghan, Green, Franken, and Mongrain review the insights derived from powerful "omics" approaches applied to sleep regulation, including transcriptomics, epigenomics, proteomics, and metabolomics. They emphasize that the complexity of sleep regulation observed at the neuronal level also extends to the molecular level. Future integration of this accumulating knowledge at a systems level will eventually lead to an understanding of the information flow from the genome via molecules to networks regulating wakefulness and sleep in health and disease.

Additionally, important new concepts and model systems, such as astroglial regulation of sleep, sleep as a local-use dependent process, and occurrence of sleep-like states in vitro, have recently emerged. In the chapter dedicated to gliotransmission and sleep-wake regulation, Frank discusses the emerging evidence that not only neuronal but also glial brain cells play fundamental roles in the expression, regulation, and functions of wakefulness and sleep. McKillop and Vyazovskiy continue by focusing on recent advances achieved from using small neuronal networks as model systems to study the electrophysiology and pharmacology of ion channels, receptors, and intracellular pathways controlling and regulating the sleep-wake cycle. The convergent evidence suggests that neuronal-glial networks can exhibit wake- and sleep-like activity, which is consistent with the view that activity-dependent modulation of local networks underlies global behavioral states.

In the last decade, optogenetic, chemogenetic, and pharmacogenetic techniques have been established as powerful tools to interrogate sleep regulatory mechanisms. Optogenetics allows the remote, optical control of activity in genetically targeted neuronal circuits with physiologically relevant spatial and temporal resolution. Adamantidis and Lüthi provide a step-by-step review of optogenetic studies mapping the functional circuits underlying sleep-wake states and the switching between states and investigations of the neural substrates of neurophysiological sleep rhythms and their functions. Inspired by the introduction of optogenetics, pharmacosynthetics approaches such as DREADDs (Designed Receptors Exclusively Activated by Designer Drugs) offer pharmacological tools to selectively control neuronal activity and to probe causal roles of neuronal populations in regulating waking and sleep states. Varin and Bonnavion illustrate how DREADDs expand our understanding of discrete neuronal subpopulations in brain structures that are critical in controlling the vigilance state architecture. Their comprehensive review highlights the emergence of a large, complex network of strongly interconnected and heterogenous neuronal subpopulations controlling the sleep-wake cycle. It is a challenging task to decipher the complexity and unscramble the hierarchical organization of this sleep-wake regulatory network and to translate this knowledge into rational novel therapies of sleep-wake disorders. Nevertheless, as outlined by Landolt, Holst, and Valomon, based upon insights from opto-/chemogenetic strategies in animal models and human genetic studies, circuit mechanisms regulating distinct sleep-wake functions may also be identified in humans. Such an approach may reveal novel targets for the development of rational sleep-wake therapeutics.

Sleep-wake disorders rank third in the prevalence of brain disorders, which together cause an estimated economic cost of roughly 800 billion Euros per year in Europe. We have included two reviews summarizing clinical sleep-wake pathologies. While Baumann focuses on central disorders of hypersomnolence and sleep-related movement disorders and their current pharmacotherapies, Spiegelhalder, Nissen, and Riemann emphasize the high prevalence and pronounced disease burden associated with insomnia and circadian rhythm sleep-wake disorders in modern 24/7 societies, which are now present around the globe. Because of the

unknown neurobiology of insomnia, the current pharmacological treatments of insomnia disorders are almost entirely symptomatic. This is in contrast to the treatment of delayed sleep phase and jet lag disorder, which is based on an understanding of the circadian disruption underlying these disorders and the effect of melatonin and light on the circadian system.

A set of reviews covers current and new targets of sleep-wake pharmacology and discusses their therapeutic prospects. This volume highlights five neurochemical systems: GABA (γ-amino-butyric acid), melatonin, glutamate, dopamine, and adenosine. Based on pharmacogenetic evidence, Wisden, Yu, and Franks emphasize the possibility that the development of subunit-selective modulators of $GABA_A$ receptors could lead to novel hypnotics and anxiolytics. Alston, Cain, and Rajaratnam conclude that melatonin and melatonin receptor agonists provide a promising alternative option to pharmacologically treat sleep and mood disorders, particularly when the patient's circadian phase position is misaligned with the desired sleep-wake schedule. These authors discuss that the phase-shifting and sleep-promoting effects of melatonin, plus additional effects of certain melatonin receptor agonists on serotonin receptors, can provide an advantage over traditional sleep and depression treatments. Ketamine, a drug recently approved for rapid antidepressant treatment in a subset of patients with major depressive disorders (MDD), also appears to alter the timing and amplitude of circadian activity patterns in rapid responders *vs.* nonresponders with MDD. In addition, ketamine has well-described effects on slow wave sleep. The review by Duncan, Ballard, and Zarate emphasizes that ketamine treatment elevates extracellular glutamate in the prefrontal cortex, suggesting that central glutamatergic circuits may be targeted in the search for novel interventions to improve sleep-wake mechanisms and mood.

In contrast to the neuromodulators serotonin, noradrenaline, histamine, and hypocretin, dopamine has long been thought to play a minor role in the regulation of sleep-wake states. Wisor, however, summarizes opto- and chemo-genetic experiments in animal models and pharmacogenetic findings in humans that highlight a central role of dopaminergic signaling in the maintenance of wakefulness and individual responses to wake-promoting medications. Finally, Lazarus, Chen, Huang, Urade, and Fredholm present an overview of the current knowledge of the role of the widely accepted somnogen, adenosine, and its receptors in sleep-wake regulation. Although several aspects of the sleep-promoting action of adenosine are still unclear, there is an active search for natural compounds, including caffeine, that could interact with adenosine receptors for the treatment of sleep-wake disorders.

We conclude this volume with two informative reviews on two contemporary hypotheses of sleep function and their potential for pharmacotherapy, and a timely overview of recent findings on sleep-wake neurobiology and their relevance for the development of novel therapeutics. First, Hladky and Barrand provide background on the processes affecting elimination of metabolites created by brain cell activity and how these processes differ between wakefulness and sleep. They provide evidence that sleep increases clearance for amyloid-β, possibly suggesting that pharmacological agents promoting physiological sleep could have potential to reduce the formation of plaques and cerebral arterial deposits and their consequences

for neurodegenerative processes. Then, Heller and Ruby present convincing evidence that sleep and circadian rhythms functionally interact in the processes of learning and memory consolidation. Partly based on pharmacological studies in two distinct rodent models of learning disability, they posit the intriguing new hypothesis that the circadian system dampens neuroplasticity during the sleep phase, in order to stabilize labile memory transcripts during their transfer to long-term memory stores. Finally, Dijk and Landolt highlight in their concluding chapter that a better understanding of the neurobiology of sleep-wake regulation and circadian rhythmicity, and in particular its relation to the subjective experience of sleep and the subjective and objective quality of wakefulness, is necessary for the proper evaluation of sleep-wake therapeutics. Persistent societal demands and demographic changes will continue to be associated with a high prevalence of sleep-wake disturbances, and this will lead to a continued need for novel pharmacological and nonpharmacological therapeutic approaches.

We believe that the present volume provides an informative view on our current understanding of the neurobiology and pharmacology of wakefulness and sleep. It connects current ideas and concepts about sleep functions, sleep homeostasis, and circadian rhythms with the search for novel target-selective sleep-wake therapeutics. Towards this goal, it provides a timely overview of sleep-wake mechanisms in health and disease, ongoing developments in drug discovery, and their prospects for the clinical treatment of sleep-disordered patients. Special attention is given to the concept that sleep and wakefulness mutually affect each other. Thus, future therapeutic interventions with either sleep- or wake-promoting agents are expected to improve the quality of sleep as well as waking behavior, cognition, mood, and other sleep-associated physiological functions. We hope that the chapters in this book are helpful in identifying some directions for this important and exciting "work in progress."

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The Function(s) of Sleep

Marcos G. Frank and H. Craig Heller

Contents

Abstract

Sleep is a highly conserved phenomenon in endotherms, and therefore it must serve at least one basic function across this wide range of species. What that function is remains one of the biggest mysteries in neurobiology. By using the

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word neurobiology, we do not mean to exclude possible non-neural functions of sleep, but it is difficult to imagine why the brain must be taken offline if the basic function of sleep did not involve the nervous system. In this chapter we discuss several current hypotheses about sleep function. We divide these hypotheses into two categories: ones that propose higher-order cognitive functions and ones that focus on housekeeping or restorative processes. We also pose four aspects of sleep that any successful functional hypothesis has to account for: why do the properties of sleep change across the life span? Why and how is sleep homeostatically regulated? Why must the brain be taken offline to accomplish the proposed function? And, why are there two radically different stages of sleep?

The higher-order cognitive function hypotheses we discuss are essential mechanisms of learning and memory and synaptic plasticity. These are not mutually exclusive hypotheses. Each focuses on specific mechanistic aspects of sleep, and higher-order cognitive processes are likely to involve components of all of these mechanisms. The restorative hypotheses are maintenance of brain energy metabolism, macromolecular biosynthesis, and removal of metabolic waste. Although these three hypotheses seem more different than those related to higher cognitive function, they may each contribute important components to a basic sleep function. Any sleep function will involve specific gene expression and macromolecular biosynthesis, and as we explain there may be important connections between brain energy metabolism and the need to remove metabolic wastes.

A deeper understanding of sleep functions in endotherms will enable us to answer whether or not rest behaviors in species other than endotherms are homologous with mammalian and avian sleep. Currently comparisons across the animal kingdom depend on superficial and phenomenological features of rest states and sleep, but investigations of sleep functions would provide more insight into the evolutionary relationships between EEG-defined sleep in endotherms and rest states in ectotherms.

Keywords

Glycogen · Glymphatic system · Hippocampal place cells · Learning · Memory · Ocular dominance plasticity · Synaptic homeostasis · Synaptic plasticity

1 Introduction

Sleep researchers frequently begin talks with the statement that we spend one-third of our lives sleeping, and we don't know why. There is no other area of human biology that can make such a claim, but that is not a claim to fame. Many great minds and much excellent research have been focused on the question – what is the function of sleep? Reasonable hypotheses have been advanced, but a definitive answer still eludes us. In this chapter, we outline what we consider essential criteria for identifying sleep function, and we apply those criteria to several leading hypotheses.

Table 1 Criteria for identifying a sleep-like state

Characterized by an absence of voluntary movements
Spontaneous, occurring with a circadian rhythm
Reversible
Characterized by a species-specific posture and/or resting place that minimizes sensory stimulation
Have an increased arousal threshold
Regulated by a homeostatic mechanism that is modulated by circadian regulation
State-related changes in neural function, including those leading to decreased sensory input to the CNS
The state should be identifiable as a stable species characteristic

We should define sleep before discussing what its function is. Prior to about 2000, the word sleep was reserved for those animals – namely, mammals and birds – that shared certain EEG correlates of behavioral states. For all other animals, including invertebrates, rest state or sleep-like state was often the descriptor used (reviewed in Tobler 2005). But in 2000, two seminal papers appeared that made a strong case for rest in Drosophila being homologous with sleep (Hendricks et al. 2000a; Shaw et al. 2000). Also in 2000, Hendricks, Sehgal, and Pack published a paper titled: "The Need for a Simple Animal Model to Understand Sleep" (Hendricks et al. 2000b). They made a convincing argument that a phylogenetic approach would bring powerful molecular genetic tools to the investigation and identification of evolutionarily conserved mechanisms and functions of sleep. They proposed a list of criteria (Table 1) for identifying a sleep-like state in animals other than mammals and birds. However, all but one of these criteria are phenotypic features and are not mechanistic or functional characteristics. Their one mechanistic criterion, homeostatic regulation, could conceivably lead to a function through understanding the feedback signals that connect some functions to the expression of the homeostatic response.

Why is it critical to identify mechanistic and functional homologies between sleep-like states in different phylogenetic groups? Daily cycles of the physical environment are a feature of our planet and circadian rhythms of organisms are a ubiquitous adaptation to that fact. Daily cycles of rest and activity, whether circadian or not, can serve many functions, and they may share many characteristics such as quiescence, increased arousal thresholds, typical postures, and safe resting places. But, they may not serve the same essential function that sleep serves in mammals and birds. We therefore have a chicken and egg problem. If we knew a function of EEG-defined sleep, we could ask if sleep-like states in other organisms served that same function and are therefore truly homologous with avian and mammalian sleep. If so, we could use those simpler organisms to investigate the underlying mechanisms of that sleep function.

In this chapter, we review key findings that support different hypotheses of EEG-defined sleep function in mammals. For each hypothesis, we will apply four criteria that should be satisfied for it to be considered as defining a primary function of sleep:

1. Ontogeny: Does it account for changes in sleep throughout development?
2. Homeostasis: Does it explain the homeostatic regulation of sleep?
3. Necessity and sufficiency: Does it explain why having the brain "offline" during sleep is necessary and sufficient for the proposed function?
4. Two states: How does the proposed function explain the two extremely different EEG states of sleep, NREM and REM sleep?

We recognize, of course, that sleep in mammals and birds may serve more than one function, and sleep-like states on other organisms may serve a variety of functions that may be similar or different. However, if we want to take a phylogenetic approach to understand sleep, and if we want to make the case that sleep is a basic, primitive, evolutionarily conserved feature of animal life as are circadian rhythms, we must identify one or more core functions. We also focus on hypotheses that concern the brain rather than the body. This is because, as far as we know, the most evolutionarily conserved effects of sleep and sleep loss are neural and not somatic (Frank 2010). It is difficult to explain why the brain would have to be taken offline if the primary function of sleep were not neural.

Brain hypotheses of sleep function can be broadly subdivided into *cognitive* (*higher-order*) and *housekeeping* (*restorative and detoxification*) categories. Cognitive hypotheses propose that sleep serves functions such as memory or brain plasticity. Housekeeping hypotheses instead propose that the function of sleep is related to essential neural processes that support higher-order cognitive functions. Restorative hypotheses propose that sleep restores and repairs neural substrates degraded by wakefulness. Detoxification hypotheses propose that sleep detoxifies substances that accumulate during wake. As each of these putative functions of sleep have been extensively reviewed elsewhere (Rechtschaffen 1998; Frank 2006), only findings from selected studies are discussed below. We also emphasize that these different hypotheses are not mutually exclusive.

2 Learning and Memory

The importance of sleep for learning and memory has been abundantly documented in animals and humans (Stickgold 2005; Rasch and Born 2013), and virtually all of us can attest to that fact through personal experience. In recent years the neurophysiological mechanisms underlying the encoding of experience and its consolidation into long-term memory have been increasingly elucidated. The pioneering study of Wilson and McNaughton (1994) demonstrated in rats that ensembles of hippocampal "place cells," which fire in relationship to specific positions in a maze, repeat their patterns of firing when the rats were in subsequent NREM sleep. Those observations led the authors to the hypothesis, ". . .initial storage of event memory occurs through rapid synaptic modification, primarily within the hippocampus. During subsequent slow-wave sleep, synaptic modification within the hippocampus itself is suppressed, and the neuronal states encoded within the hippocampus are 'played back' as part of a consolidation process by which hippocampal information

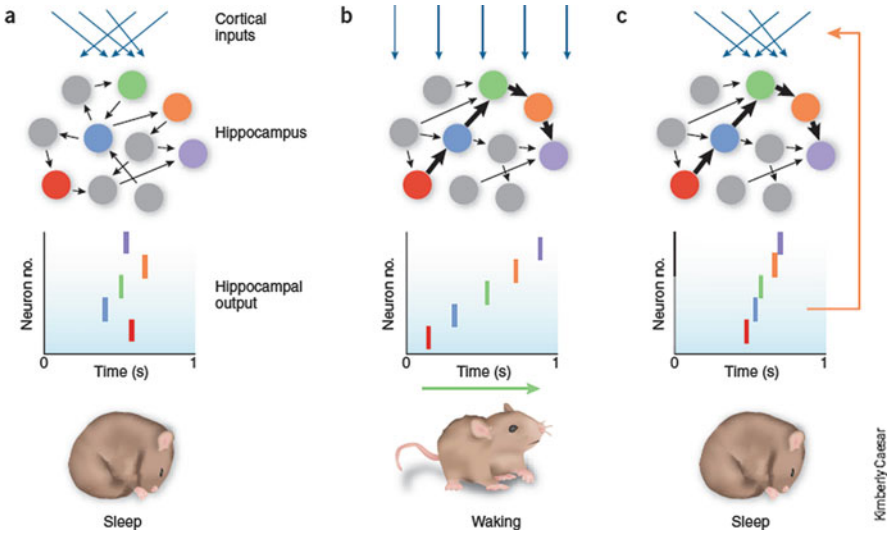

Fig. 1 Firing of "hippocampal place cells" corresponds to specific locations in space. (**a**) Recordings of a population of place cells during sleep in a naive animal show no obvious pattern of firing. (**b**) However, during wake activity running in a maze sequence patterns of place cell firing are observed. (**c**) During subsequent NREM sleep, those firing patterns are replayed but at a speed that is about seven times faster (reprinted from Mehta 2007 with permission)

is gradually transferred to the neocortex." Those original results have been nicely summarized by Mehta (2007) and reprinted as Fig. 1.

Much excellent work between 1994 and the present has supported the neural replay during sleep hypothesis and filled out details. The replay firing patterns of hippocampal CA1 ensembles, called low-probability sequences, occur during both quiescent wake and NREM sleep, and they run about fifteen 6–20 times the speed of the same sequence during active spatial experience (Davidson et al. 2009). These replay events are associated with hippocampal local field potentials (LFPs) called sharp-wave ripples (Lee and Wilson 2002). Thus, the replay sequences contained in sharp-wave ripples appear to spatially and temporally code information into short-term memory.

A little background information helps explain why replay events are associated with specific electrophysiological signatures recorded locally (LFPs) and more globally. Ripples are 100–200 Hz waves generated by local neuronal activity. Their detection denotes highly active neurons nearby. During waking and REM sleep (when acetylcholine is present), ripples occur at the depolarized peaks of hippocampal theta waves (6–10 Hz). During task-disengaged quiet wakefulness and NREM sleep (when acetylcholine is absent), ripples are associated with peaks of depolarization called sharp waves. Peaks of theta and sharp waves occur because of summed dendritic depolarization that brings neurons in the local field close to action potential threshold. The troughs following theta waves and sharp waves coincide with membrane hyperpolarization when neuronal spiking is least likely. Ripples do not appear at the troughs of slow waves (NREM sleep) or theta waves because the hyperpolarized membranes do not support high neuronal activity.

Communication of the information between the hippocampus and the cortex that is necessary for memory consolidation is enabled by coupling between ripple events in the hippocampus and the cortex. The hippocampus slightly leads the cortex, indicating directionality of information flow (Siapas et al. 2005; Khodagholy et al. 2017). During NREM sleep, another LFP, the slow oscillation (0–3 Hz), may organize the information exchange between the hippocampus and the cortex (Fujisawa and Buzsáki 2011). Sharp-wave ripples (100–200 Hz) are expressed during the up-states (depolarized phases) of the slow oscillation in both the cortex and the hippocampus. Thus, the slow oscillation appears to create sequential frames for the replay of information contained in the sharp-wave ripples. A single long replay sequence may span more than one frame. Importantly, the specific sequences expressed in the cortical and hippocampal ensembles during any one sharp-wave ripple correspond. Thus, it appears as if, during NREM sleep, there is a communication between the hippocampus and the cortex about the prior wake experience. Whereas the phase relationships of the theta oscillations of the hippocampus and the cortex during wake experience indicated a direction of information flow from hippocampus to cortex, the phase relationships between the slow (0–3 Hz) oscillations in these two structures do not clearly support a unidirectional flow of information (Ji and Wilson 2007). However, during the NREM state called NREM stage 2 when slow oscillations are interrupted by faster 10–15 Hz spindles lasting ~1.5 s, the direction of communication is clearly from the hippocampus to the cortex, and the cortex reverberates to hippocampal neuronal activity with a spindle frequency response (Wierzynski et al. 2009).

The very elegant studies of unit activity and LFPs in the hippocampus and cortex during experience and sleep support the model proposed by Born and Wilhelm (2012). This model proposes that both the cortex and the hippocampus acquire information about experience during wake with the cortical representation being weak and the hippocampal being strong. Then during sleep, the hippocampus tutors the cortex to strengthen or consolidate the information into long-term memory.

There are hippocampal replays of waking experiences during REM sleep as well (Louie and Wilson 2001; Poe et al. 2000), with the main difference being that REM replays occur without compression and the neuronal firing coincides with the peaks of the theta rhythm. One interesting feature of REM replay in the dorsal region of the hippocampus CA1 output region is that the firing of neurons associated with older memories that have already been consolidated to the neocortex is delayed so that they coincide with the troughs of the theta rhythm, a time consistent with the weakening of those familiar synapses in the hippocampus (Huerta and Lisman 1996), possibly to recycle the synapses so they may be free to encode novel memories in subsequent waking (Poe et al. 2000).

The idea that episodic memory encoding and consolidation involves the transfer of packets of information between the hippocampus and the cortex in the form of ripples gains support from studies showing that disruption of sharp-wave ripples during sleep following training impairs spatial learning and memory (Girardeau et al. 2009; Ego-Stengel and Wilson 2010). No one has yet attempted to disrupt theta ripple replay during REM sleep. Indirect evidence for the significance of replay

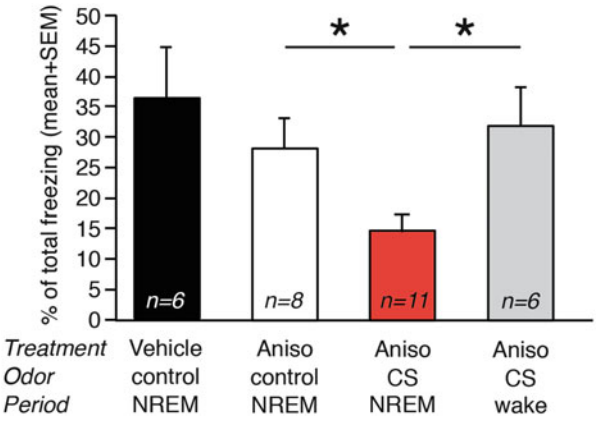

Fig. 2 Protein synthesis inhibitor (anisomycin) in combination with cued fear memory replay during sleep weakens the strength of that fear memory when cued during subsequent wake. In each case, the treatment (vehicle or anisomycin) was administered prior to the sleep phase, and during sleep the animal was exposed to the CS odor or a control odor. The strength of the cued fear response was determined during the subsequent wake phase as % of time displaying freezing behavior following the CS exposure. The anisomycin had no effect if the animal was only exposed to the control odor cue during sleep, but if the animal was exposed to the CS during sleep, the strength of the cued fear memory was considerably reduced during the subsequent wake phase. However, if the CS was delivered to the anisomycin-treated animals only when they were awake during the sleep phase, there was no effect on the strength of the cued fear memory during the subsequent wake phase

events comes from studies that used classical conditioning to reactivate memories during sleep. Memories that are reactivated in sleep are significantly strengthened (Rasch and Born 2007; Rudoy et al. 2009; van Dongen et al. 2012; Oudiette and Paller 2013; Rolls et al. 2013). Rolls et al. (2013) went on to demonstrate that the consolidation of the reactivated memory was an active process requiring protein synthesis (Fig. 2).

Using an odor as the conditioned stimulus (CS) and foot shock as the unconditioned stimulus (US), they showed that reintroduction of the CS during sleep resulted in a heightened, context-independent fear response to the CS during subsequent wake. In similar experiments, they injected a protein synthesis inhibitor (PSI) into the amygdalas of the mice following the fear conditioning and just prior to sleep. These animals were then exposed to the CS or a control odor stimulus during sleep. During the next wake phase, the mice that received the PSI injections and were exposed to the CS during sleep had decreased fear responses in comparison to mice that had received vehicle injections and also in comparison to mice that had received PSI injections but were exposed to a control stimulus during sleep. These results (first three bars in Fig. 2) were interpreted to mean that interfering with the active process of memory reactivation and consolidation during sleep reduced the strength of the memory. The very interesting result, however, was that when the conditioned stimulus was introduced during natural wake episodes during the sleep phase, the

PSI had no effect on the strength of the memory (Fig. 2, fourth bar, Rolls et al. unpublished). Thus, the influence of cued memory replay on the strength of the memory depends on sleep and not just on experiencing the CS during the sleep phase. Taken together, there is strong evidence that hippocampal encoded memories are reactivated during subsequent sleep and that reactivation involves communication with the cortex resulting in the formation and strengthening of a long-term memory transcript.

3 Synaptic Plasticity

Synaptic plasticity refers to changes in the strength of existing synapses, changes in synapse number or size, or changes in morphological structures that contain or form synapses (e.g., dendritic spines and synaptic boutons). Synaptic plasticity is thought to be the cellular basis of memory and also has historically been associated with sleep. Scientists have traditionally examined this relationship in two ways. "Top-down" approaches involve an organizing principle or hypothesis that attempts to explain the role of sleep in plasticity in a comprehensive way. "Bottom-up" approaches instead ask simpler questions about how sleep or sleep loss impacts classic models of plasticity in vivo or in vitro. The results of the latter investigations do not require that any particular "top-down" hypothesis be true. However, any "top-down" hypothesis must account for "bottom-up" results.

3.1 Synaptic Plasticity in the Hippocampus

The role of sleep in brain plasticity has traditionally been investigated using classic forms of tetany-induced Hebbian long-term synaptic potentiation (LTP) and long-term depression (LTD). Overall, sleep deprivation inhibits the induction or maintenance of LTP in vivo and in vitro. Sleep deprivation impairs hippocampal LTP in anesthetized or awake rodents (Romcy-Pereira and Pavlides 2004; Kim et al. 2005; Marks and Wayner 2005). Several studies also show that in vitro hippocampal LTP (either the induction or maintenance) is reduced in rodents that undergo varying amounts of REM sleep deprivation, total sleep deprivation, or sleep restriction prior to sacrifice (Campbell et al. 2002; Davis et al. 2003; McDermott et al. 2003, 2006; Chen et al. 2006; Ishikawa et al. 2006; Kopp et al. 2006; Ravassard et al. 2006, 2009; Tartar et al. 2006; Arrigoni et al. 2009; Vecsey et al. 2009; Florian et al. 2011). Interestingly, when REM sleep is restored (after prior deprivation) or increased in rodents, this reverses deficits in hippocampal LTP (Ravassard et al. 2009, 2015).

The underlying mechanisms mediating the effects of sleep loss on LTP and LTD are not understood. They do not appear to be simply due to indirect effects of the sleep deprivation procedures. For example, these deficits can be dissociated from changes in stress hormones (Kopp et al. 2006; Ravassard et al. 2009, 2015). Diminished plasticity may instead be linked to decrements in hippocampal NMDA receptor function (Chen et al. 2006; Kopp et al. 2006; McDermott et al. 2006;

Longordo et al. 2009) and ERK/MAPK activation (Ravassard et al. 2009) combined with reductions in hippocampal dendritic spines (Havekes et al. 2016), plasticity-related mRNAs or proteins (Davis et al. 2006; Guzman-Marin et al. 2006; Ravassard et al. 2015), and elevated concentrations of PDE4 (Vecsey et al. 2009) and extracellular adenosine (Arrigoni et al. 2009; Florian et al. 2011). This may also involve changes in protein synthesis, as the translational machinery in the hippocampus is suppressed during sleep deprivation but recovers with subsequent sleep (Havekes and Abel 2017) (Fig. 3).

3.2 Synaptic Plasticity in the Visual Cortex: Ocular Dominance Plasticity (ODP) and Stimulus-Selective Response Plasticity (SRP)

ODP refers to synaptic changes in visual cortical neurons in vivo triggered by monocular deprivation (MD) or other changes in patterned vision (Wiesel and Hubel 1963; Hubel and Wiesel 1970). ODP is more easily induced during a critical period of development, but it shares in common numerous mechanisms that mediate Hebbian and non-Hebbian plasticity in the adult hippocampus and non-sensory cortex. ODP is considered physiological for the following reasons. It occurs in the intact, unanesthetized brain in response to changes in sensory input that animals actually experience. The resulting plasticity involves naturally occurring changes in synaptic proteins and molecules as part of an adaptive response to this change in vision. Third, the underlying plasticity governs cortical adjustments to visual input that normally occur during the critical period. These adjustments are thought to be essential for the development of binocular vision, acuity, and other visual response properties in cortical neurons (for review see Spolidoro et al. 2008; Smith et al. 2009; Tropea et al. 2009; Espinosa and Stryker 2012).

In the cat during the peak of the critical period, sleep significantly enhances the effects of MD on cortical neurons, a process that does not occur when animals are instead sleep-deprived (Frank et al. 2001). The precise mechanisms governing this process are similar to those that mediate LTP. For example, both acute (Aton et al. 2009a, b) and chronic recording (Aton et al. 2013) of single neurons show responses to the non-deprived eye become stronger after sleep. In comparison, sleep has little to no effect on the magnitude of depression observed in the deprived-eye pathway. This process is activity-dependent (Jha et al. 2005), and inhibiting the *N*-methyl-D-aspartate receptor (NMDAR), protein kinase A (PKA), the extracellular-regulated kinase (ERK), or the mammalian target of rapamycin (mTOR) during post-MD sleep inhibits this potentiated response (Aton et al. 2009a, b; Seibt et al. 2012). In addition, post-MD sleep is accompanied by activation of several kinases implicated in LTP and phosphorylation of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) that lead to trafficking and insertion of this receptor into the post-synaptic membrane (Aton et al. 2009a, b). Post-MD sleep also promotes the synthesis or phosphorylation of several proteins implicated in LTP (Seibt et al. 2012; Dumoulin et al. 2015) (Fig. 4).

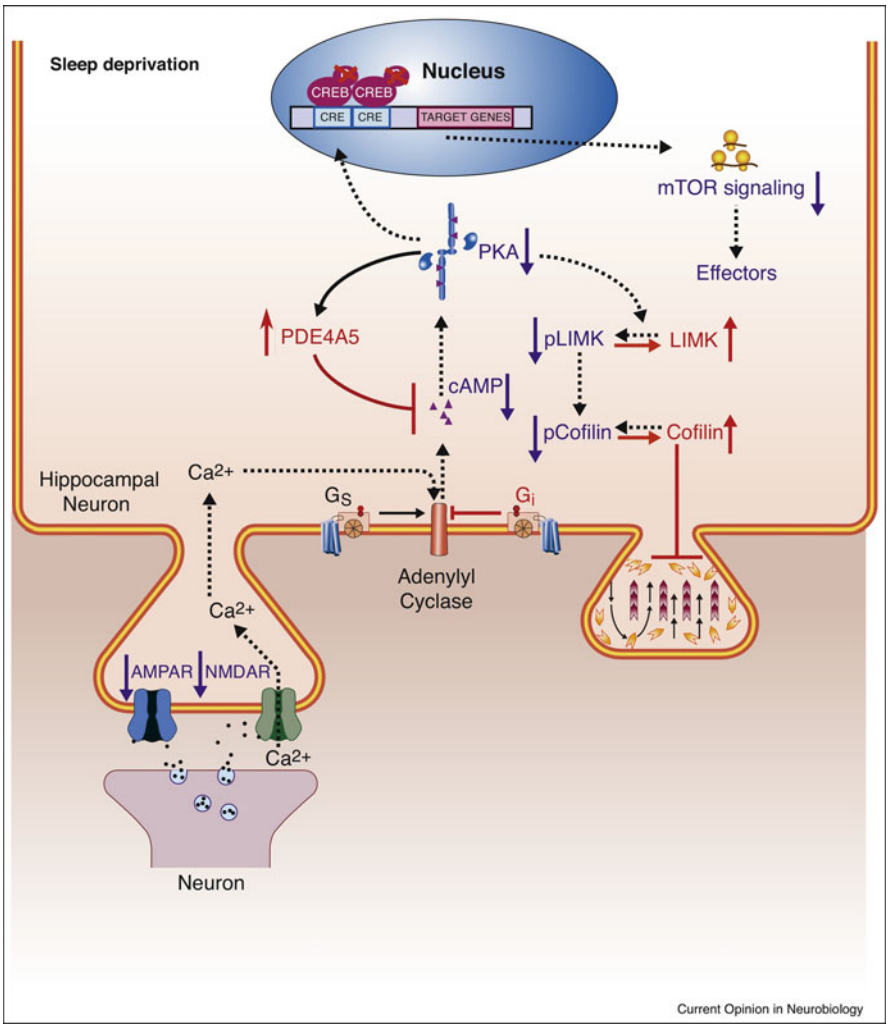

Fig. 3 The molecular impact of sleep deprivation. A schematic overview of hippocampal signaling pathways whose modulation by sleep deprivation may contribute to effects on memory formation. Sleep deprivation has been reported to reduce glutamatergic signaling while increasing adenosine levels. Sleep deprivation also attenuates cAMP signaling, CREB-mediated gene transcription, translational processes through mTOR signaling, and structural plasticity through modulation of the PKA-LIMK-cofilin pathway. All of these molecular events are shown in a single connected pathway in order to demonstrate how the effects of sleep deprivation could potentially interact to impact learning and memory. Dashed black lines and blue arrows pointing down indicate attenuation of the signaling pathway. Red lines and upward pointing arrows indicate an increase of the signaling pathway. Reproduced with permission from Havekes and Abel (2017)

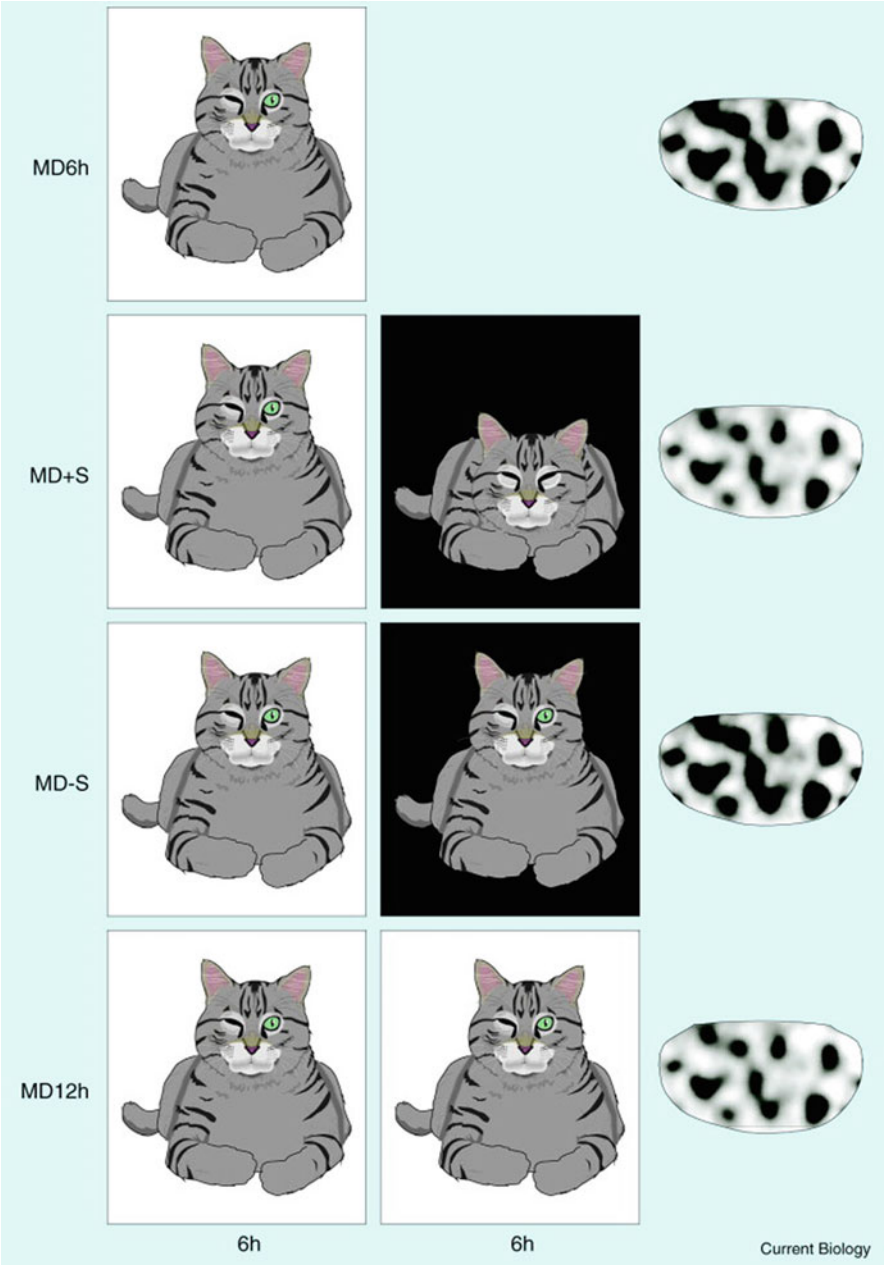

Fig. 4 Effect of sleep on the magnitude of the ocular dominance shift induced by monocular deprivation. The first two columns depict the rearing conditions of kittens employed by Frank et al. (2001). The right-most column schematically shows ocular dominance maps obtained from primary visual cortex under the various conditions. All kittens were monocularly deprived for 6 h, and one group was tested immediately afterward (MD6h). A second group was allowed to sleep as much as

Stimulus-selective response plasticity (SRP) is a form of in vivo LTP also induced by changes in visual input but occurring in the developing and adult visual cortex. In mice, brief exposure to a visual stimulus (phase-reversing, oriented gratings) results in enhanced cortical (V1) responses to stimuli of the same orientation (Frenkel et al. 2006). SRP is considered an in vivo form of LTP of cortical glutamatergic synapses because it requires the same cellular mechanisms as LTP in vitro (Frenkel et al. 2006) and occludes tetany-induced thalamocortical LTP (Cooke and Bear 2010). SRP is not present immediately after training in an awake mouse. It is only observed after a subsequent period of sleep and suppressed by sleep deprivation (Aton et al. 2014). A follow-up investigation (Durkin and Aton 2016) showed that these changes could not be explained as a form of synaptic weakening in excitatory synapses, as recently suggested (Cirelli and Tononi 2015). Instead they require thalamocortical spindles and likely involve mechanisms implicated in classic LTP (Durkin et al. 2017). In support of this interpretation, calcium in cortical dendrites is elevated during NREM spindles in a manner that may promote Hebbian synaptic modifications (Seibt et al. 2017) (Fig. 5). Nevertheless, there are several unknown mechanisms that likely are important in this process, including changes in intracortical inhibition (Kaplan et al. 2016).

3.3 The Synaptic Homeostasis Hypothesis (SHY)

SHY proposes that sleep promotes global (or "net') synaptic weakening that offsets global synaptic strengthening that occurs during wake (Tononi and Cirelli 2003, 2006, 2014). This global synaptic weakening in sleep preserves the relative strength between synapses, allows for further synaptic changes, and prevents maladaptive metabolic costs associated with excessive synaptic maintenance. These are intuitively appealing aspects of SHY. If indeed all or most learning results in synaptic strengthening (but see Frank 2012), then eventually the brain's ability to learn or store information would saturate at some point. There should be other forms of plasticity that restore a set point of synaptic strength to the network. This problem was recognized many years before SHY was proposed and several mechanisms including heterosynaptic adjustments (e.g., a sliding threshold for plasticity) and synaptic homeostasis were proffered as solutions (reviewed in Turrigiano 2007; Hulme et al. 2014).

Fig. 4 (continued) they liked during the following 6 h (MD + S), while a third group was kept awake in the dark (MD − S). A fourth group was deprived for 12 h and then tested (MD12h). The ocular dominance maps obtained by intrinsic signal imaging (Bonhoeffer and Grimwald 1996) display cortical regions dominated by the deprived eye in black and those dominated by the non-deprived eye in white. The MD + S group shows a loss of territory dominated by the deprived eye well beyond that is observed in the MD6h group, while the sleep-deprived group (MD − S) does not. In fact, the consolidation of the MD shift in the MD + S group amounts to about the same magnitude as is observed after 12 h of monocular deprivation (MD12h). Reproduced with permission from Sengpiel (2001)

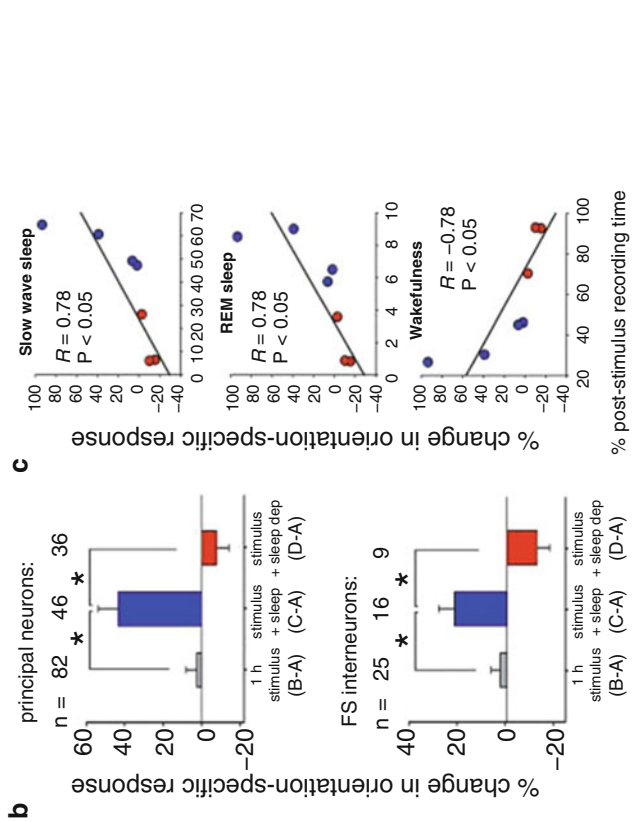

Fig. 5 Stimulus-specific response potentiation (SRP) is consolidated during poststimulus sleep. (**a**) V1 neurons were recorded across a baseline period of ad libitum sleep and wakefulness, a 1-h period of oriented grating stimulus presentation in the awake mouse (starting at lights-on), and a 6-h period of either ad libitum sleep or sleep deprivation in the dark. Visual responses were recorded during presentation of a series of gratings (four orientations plus a blank screen) in the contralateral visual field at the intervals indicated (arrows): timepoint A, after baseline recording; timepoint B, after stimulus presentation; and timepoint C, after subsequent ad libitum sleep, or timepoint D, sleep deprivation. (**b**) Orientation preference for the presented stimulus did not change after 1-h stimulus presentation but was enhanced after subsequent sleep in both non-fast-spiking (principal) neurons and in fast-spiking (FS) interneurons. OSRP was blocked by sleep deprivation. *$P < 0.05$, Holm-Sidak post hoc test. (**c**) OSRP was proportional to sleep time and negatively correlated with wakefulness. Adapted with permission from Aton et al. (2014)

Therefore the core concept of SHY is not new; what is new is the idea that this renormalization of synaptic weights predominantly occurs in sleep and that it should manifest as a global reduction in synaptic strength. Although the most recent formulation of SHY allows for subsets of synapses to be preserved against the downscaling process ("selective down-selection"), the latter does not involve synaptogenesis or new synaptic strengthening during sleep.

A number of changes in proteins, synaptic efficacy, and synapse and dendrite morphology are consistent with predictions of SHY (Vyazovskiy et al. 2008; Liu et al. 2010; Maret et al. 2011; de Vivo et al. 2017). In homogenized tissue, markers of synaptic potentiation (e.g., changes in AMPAR subunit number or phosphorylation) are higher in rats sacrificed at the end of the active phase or after sleep deprivation, compared to animals sacrificed at the end of the rest phase (Vyazovskiy et al. 2008). Similar results are reported for measures of synaptic efficacy (EPSPs and mini EPSPs) and neuronal firing rates, which are also elevated at the end of the active phase (or after sleep deprivation) relative to sleep (Vyazovskiy et al. 2009; Liu et al. 2010). Two imaging studies of cortical dendrite spine morphology showed that the ratio of spines eliminated to those formed was greater after sleep (Maret et al. 2011; Yang and Gan 2011). However, these results were restricted to stages of development when there is an overall pruning of synapses and were not detected in adult mice (Maret et al. 2011). It was also shown using electron microscopy in fixed mouse tissue (layer 2–3 of the cortex) that many synapses shrink in size when examined after a long period of sleep, relative to sleep deprivation or the wake phase (de Vivo et al. 2017). These studies were also conducted in juvenile mice; therefore, it is unclear if this reflects a general sleep-dependent process that occurs in adult animals. It is also reported that hippocampal sharp waves during sleep lead to synaptic downscaling (Norimoto et al. 2018), findings which are surprising considering the role of sharp-wave ripples and replay in synaptic potentiation (Sadowski et al. 2016).

There are a number of important caveats to SHY. The first is that the effects of sleep on synaptic plasticity are not uniform. They vary based on a number of factors, including the brain region under examination, the age of the animal, the types of waking experience that precede sleep, and circadian phase (Ribeiro 2011; Frank 2012; Frank and Cantera 2014; Areal et al. 2017; Puentes-Mestril and Aton 2017; Timofeev and Chauvette 2017). For example, the decrease in neuronal firing rates during sleep (Vyazovskiy et al. 2009) does not occur in the visual cortex in juvenile and adult rodents (Aton et al. 2014; Hengen et al. 2016) or developing cats (Aton et al. 2013). In the frontal cortex of rats, neuronal firing rates across bouts of sleep are inconsistent with only "selective down-selection." Instead, sleep appears to promote firing rate adjustments consistent with a preservation of the weaker synapses (Watson et al. 2016). Sleep has also been shown to increase *or* decrease cortical dendritic spines in adult mice, depending on the type of learning that precedes sleep and the cortical region under examination (Yang et al. 2014; Li et al. 2017). In contrast to what is reported in rodent cortex, extended wakefulness reduces morphological and biochemical markers of hippocampal synapses, events that are reversed during recovery sleep (Havekes et al. 2007, 2016; Hagewoud et al. 2009). Changes

in cortical AMPAR subunits reported after sleep deprivation in rats (Vyazovskiy et al. 2008) are not found in mice (Diering et al. 2017) or cats (Seibt et al. 2012). The conclusion from these various studies is that SHY does not accommodate several basic findings from "bottom-up" approaches.

A second caveat is that some findings cited in support of SHY are based on nonphysiological conditions and/or are rely heavily on ex vivo preparations. As discussed elsewhere (Holscher 1999; Albensi et al. 2007), plasticity is considered nonphysiological when it involves forms of stimulation not naturally experienced by the intact brain or measurement conditions that do not reproduce the conditions of the intact brain (Holscher 1999; Albensi et al. 2007). Studies cited in support of SHY employ nonphysiological approaches, including exogenous, transcallosal electrical stimulation (Vyazovskiy et al. 2008), intracranial infusions of chemicals that cause cortical spreading depression (Faraguna et al. 2010), intracortical infusions of neurotrophins and antibodies (Faraguna et al. 2008), transcranial electromagnetic fields (Huber 2007), and measurements in vitro that require the use of tetrodotoxin and picrotoxin (Liu et al. 2010). A recent study reporting sharp-wave-mediated synaptic downscaling relies heavily on in situ preparations and not actual direct measurements of spines or synapses in vivo (Norimoto et al. 2018).

The third caveat is that virtually nothing is known concerning the sleep-dependent mechanisms that purportedly weaken synapses during sleep (Frank 2012, 2013). Homer 1a has been implicated in synaptic downscaling during sleep, but this study did not examine sleep per se. It instead measured changes in synapses or proteins at two different times of day in a strongly circadian species (mice) in the absence of quantitative measures of sleep or wakefulness or controls for circadian influences (Diering et al. 2017). Therefore the results may be due to sleep or circadian rhythms.

NREM SWA has been proposed to directly weaken synapses in SHY (Tononi and Cirelli 2003, 2006). However, there is no direct evidence that SWA in vivo weakens synapses (Steriade and Timofeev 2003; Frank 2012) while several studies indicate that SWA might strengthen synapses (Tsanov and Manahan-Vaughan 2007; Watson et al. 2016; Timofeev and Chauvette 2017). As mentioned above, SWA appears to be critical in the transfer of information from the hippocampus to the cortex (Fujisawa and Buzsáki 2011), which seems to be incompatible with a synaptic weakening function. If, as suggested, there is extensive transfer of information between the hippocampus and cortex during sleep in support of memory consolidation, and that those communications are organized by specific local field potentials makes it unlikely that those LFPs are functioning to weaken synapses (and see above discussion).

A final caveat is that there is no direct evidence for a functional significance of the synaptic weakening associated with SHY (Tononi and Cirelli 2014). Currently, evidence supporting a functional significance comes primarily from computational models (Hill et al. 2008; Olcese et al. 2010; Nere et al. 2013). Computational models depend critically on what variables are included and the assumptions made about how actual neurons operate in vivo. Other computational models of memory consolidation during sleep do not employ "selective down-selection" or "renormalization" as described in SHY (O'Donnell and Sejnowski 2014; Blanco et al. 2015). A

remaining challenge is the need for direct in vivo evidence for adaptive functions (behaviorally or otherwise) of synaptic down-selection during sleep.

To summarize, it appears that sleep does more than simply weaken synapses. Rather, during sleep, there is a mixture of synaptic weakening and strengthening that is circuit-specific and determined in large part by the experience that precedes sleep (Frank 2015).

4 Restorative Functions

We commonly refer to a good night of sleep as "restorative sleep," but we don't know what is being restored. The most ubiquitous conceptualization of a sleep restorative function in the sleep literature is Process S based on the changing expression of EEG slow-wave activity (SWA) as a function of prior wake and subsequent sleep duration (Borbely and Achermann 1992). Process S is quantified by the EEG spectral power in the 0.5–4.5 Hz range, which is highest following prolonged wake and decays exponentially during subsequent sleep. Varying the duration of wake prior to sleep indicates that Process S builds as an exponentially saturating curve. The dynamics of Process S reflect a negative feedback mechanism – some condition accumulates during wake and that condition produces a signal that controls the intensity of subsequent sleep and presumably the restorative process that returns the condition to its normative state. Identifying the feedback signal should lead to identification of that condition and the restorative process.

4.1 Brain Energy

The prevalent and age-old use of adenosine A1-receptor antagonist caffeine and theophylline to promote wakefulness presaged the scientific demonstration that adenosine A1-receptor agonists promote sleep (Radulovacki et al. 1984; Benington et al. 1995). Moreover, in sleep satiated rats, adenosine agonists promote dose-dependent increases in SWA that have spectral profiles identical to those following different durations of prior wake, and these increases in SWA show a monotonic decline similar to that seen in recovery from prolonged prior wake (Benington et al. 1995). Adenosine concentrations and the activity of adenosine synthetic and degradative enzymes show diurnal variations in the rat brain with adenosine highest during the rest phase and lowest during the active phase (de Sanchez et al. 1993). Microdialysis studies in cats revealed increases in adenosine in the basal forebrain and cortex with prolonged wake and decline during subsequent sleep (Porkka-Heiskanen et al. 1997). The mechanisms whereby adenosine can regulate SWA are established. Acting through adenosine A1 receptors throughout the thalamus and cortex, adenosine promotes increased K+ conductance, hyperpolarization, and de-inactivation of low-threshold Ca++ channels that are the basis for the synchronized bursting that produces the slow waves in the cortical EEG (reviewed in Benington and Heller 1995). These observations and many more

(Palchykova et al. 2010; Greene et al. 2017) clearly support adenosine as being a critical feedback variable in the homeostatic regulation of SWA.

What does the identification of adenosine as the critical feedback variable for control of SWA suggest as to the function of sleep? Adenosine is a central player in energy exchanges. When metabolic demand reduces the ATP/ADP ratio, excess ADPs are scavenged to produce ATP with adenosine being a leftover. Thus, increased adenosine release reflects energy depletion. The hypothesis presented by Benington and Heller (1995) was that the major brain energy reserve, glycogen, is regionally depleted during wake resulting in local transient energy deficits and adenosine release. Adenosine release promotes NREM sleep with increased SWA during which glycogen reserves are restored. The hypothesis was supported by a study in rats showing sleep deprivation depletes brain glycogen and recovery sleep restores brain glycogen (Kong et al. 2002). However, attempts to replicate those findings in mice produced equivocal results (Gip et al. 2002; Franken et al. 2003). Supporting molecular genetic data came from a study by Petit et al. (2002) showing that 6 h of sleep deprivation in mice elevated expression of glycogen synthase-a and protein targeted to glycogen which serves as a scaffolding bringing glycogen and glycogen metabolic enzymes together. However, many subsequent studies reviewed by Petit et al. (2015) have shown that the relationships between sleep-wake and brain energy metabolism are more complex with both glycogen synthesis and degradation occurring during sleep or wake. Thus, measures of rate of turnover might be more informative than time point measures of glycogen concentrations.

Whether or not glycogen replenishment is a major function of sleep, adenosine is clearly an important controlling element and perhaps a feedback signal. The role of adenosine in modulating the SWA response to prior waking activity was demonstrated in a study in which the ability of astrocytes to release ATP was reversibly impaired by means of a conditional double negative SNARE transgene. The release of ATP by astrocytes is a major factor in control of extracellular adenosine. This study showed that baseline sleep was normal in the mice expressing the dnSNARE, but these mice did not show the enhanced SWA response to sleep deprivation (Halassa et al. 2009). These results provide further evidence that adenosine is the feedback signal controlling the sleep homeostatic response, but if that adenosine is the result of ATP release from astrocytes, what could the restorative function be?

4.2 Macromolecular Synthesis

Sleep may also serve a restorative process by promoting the synthesis of proteins, peptides, or lipids necessary for normal waking function. NREM sleep has historically been viewed as the "restorative" sleep state (Benington and Heller 1995). Though far from conclusive, there are a number of findings that support this view. NREM sleep amounts are positively correlated with cerebral protein synthesis in adult rats, monkeys, and the ovine fetus (Ramm and Smith 1990; Nakanishi et al. 1997; Czikk et al. 2003; Vazquez et al. 2008). Studies in rabbits show positive

correlations between RNA synthesis in purified nuclear fractions of neocortical neurons and EEG synchronization during sleep (Giuditta et al. 1980a, b). In cats and rodents, NREM sleep promotes the synthesis of a number of synaptic proteins and neurotrophins (Seibt et al. 2012; Vecsey et al. 2012; Tudor et al. 2016).

Molecular studies show that recovery sleep after total sleep deprivation upregulates cortical and medullary expression of genes that may play a role in protein biogenesis in the endoplasmic reticulum (ER) (Terao et al. 2003). Complementary results have been reported after 6 h of total sleep deprivation in mice, which induces cellular events that decrease protein synthesis (Naidoo et al. 2005). Other studies have found sleep-related increases in several genes implicated in cholesterol synthesis, membrane trafficking, and vesicle maintenance and transport (Taishi et al. 2001; Cirelli et al. 2004; Basheer et al. 2005; Mackiewicz et al. 2007). Total sleep deprivation is also reported to reduce cell proliferation in the hippocampus (Guzman-Marin et al. 2003, 2005; Hairston et al. 2005; Tung et al. 2005). This latter effect does not appear to be simply due to stress accompanying sleep deprivation because it persists even when stress hormones are clamped (Mueller et al. 2008).

The evidence for macromolecule synthesis in REM sleep is not as clear. REM sleep deprivation also reduces hippocampal neurogenesis (Guzman-Marin et al. 2008), but it has inconsistent effects on protein synthesis, with some investigators reporting no effects (Bobillier et al. 1971) and others showing reductions, chiefly in non-cortical structures (Denin et al. 1980; Shapiro and Girdwood 1981).

An important caveat applies to all studies that employ selective REM sleep deprivation. Even very short-term REM sleep deprivation on the order of hours compromises the quality of NREM sleep as the attempts to enter REM sleep come at increasingly shorter intervals (Benington and Heller 1994). Nevertheless, selective REM sleep deprivation has continued to be used in many studies, so this caveat has to be kept in mind while attempting to interpret the results of these studies.

REM sleep deprivation alters the expression of several genes associated with REM sleep mechanisms, but there is little evidence that REM sleep enhances the expression of genes other than those located in REM sleep circuits (Merchant-Nancy et al. 1992; Toppila et al. 1995; Maloney et al. 2002). Although REM sleep is accompanied by reduced monoaminergic activity (Hobson 1999), the significance of this interaction in terms of neuro-regeneration is unclear. For example, short-term REM sleep deprivation (96 h) has been shown to increase noradrenergic activity and downregulate beta-adrenergic receptors (Pedrazzoli and Benedito 2004; Andersen et al. 2005), but extended total sleep deprivation or REM sleep deprivation minimally impacts monoamine levels and receptor number (Porrka-Heiskanen et al. 1995; Farooqui et al. 1996; Hipolide et al. 1998; Rechtschaffen et al. 2002) and only modestly affects neuronal morphology in cholinergic and noradrenergic neurons (Majumdar and Mallick 2005). However, REM sleep deprivation has been shown to profoundly reduce the activity of the kinase extracellular signal-related kinase (ERK). ERK works synergistically with the mammalian target of rapamycin (mTOR) to activate protein synthesis in neurons (Dumoulin Bridi et al. 2015; Dumoulin et al. 2015). Studies in cultured cortical neurons also show that conditions that simulate the biochemical environment present in REM sleep can lead

to pulses of protein synthesis (Soulé et al. 2012). Intriguingly, oligodendrocytes (a glial cell that manufactures myelin) proliferate during REM sleep, suggesting that myelination may be promoted by this sleep state (Bellesi et al. 2013).

5 Neural Detoxification

Restoration can involve replacement of something depleted as in the energy hypothesis above, or it can involve the elimination of something accumulated above a desirable level. Elimination of waste products of metabolism is the focus of a relatively new hypothesis on sleep function – the glymphatic clearance hypothesis (Xie et al. 2013). The term glymphatic was introduced in 2012 in a description of the newly characterized system in the brain for exchange of cerebral spinal fluid (CSF), interstitial fluid (ISF), and blood. In summary, the evidence supports a model in which subarachnoid CSF enters the brain through perivascular spaces around penetrating arteries (Fig. 6).

These spaces are bounded by the end-feet of astrocytes and the endothelium and smooth muscle of the vessel walls (Iliff and Nedergaard 2013). Water and small molecular solutes enter the astrocytes through aquaporin (Aqp4) channels in the astrocyte end-feet membranes. From the astrocytes the water and small molecular solutes are distributed to the ISF. The ISF along with its solutes leaves the brain parenchyma through the perivascular spaces around venules and veins draining into cervical lymphatics and venous blood in the dural sinuses. This drainage of ISF carries with it waste products of brain metabolism such as beta amyloid, soluble proteins, lipids, ions, and small molecules such as lactate (Lundgaard et al. 2016).

The connection of the newly described glymphatic system with sleep comes from the observation that the perivascular spaces and therefore the flow through them expand dramatically (up to 60%) during sleep in comparison to wake facilitating the flow of ISF through the brain parenchyma. The volume of the interstitium and hence the flow of ISF appear to be controlled by at least one neuromodulator that is high during wake and low during sleep – norepinephrine (Xie et al. 2013).

The glymphatic clearance hypothesis for the function of sleep has possible connections with the brain energy restoration hypothesis discussed above. The brain depends on glucose and its breakdown product lactate for energy, and the astrocytes mediate the delivery of these energy substrates to the neurons. First, glucose is transferred to astrocytes from the blood via glucose 1 transporters (Glut1). The astrocytes deliver glucose to the ISF and thereby to neurons via Glut1. Second, astrocytes also synthesize glucose into glycogen by means of series of enzymatically controlled steps notably including glycogen synthase. Third, astrocytic glycogen is an important and rapidly activated energy reserve, but the process of glycogenolysis produces glucose-6-phosphate moieties that cannot leave the astrocyte. Instead, they enter glycolysis producing lactate molecules that can leave the astrocyte and be an energy source for neurons (reviewed in Falkowska et al. 2015).

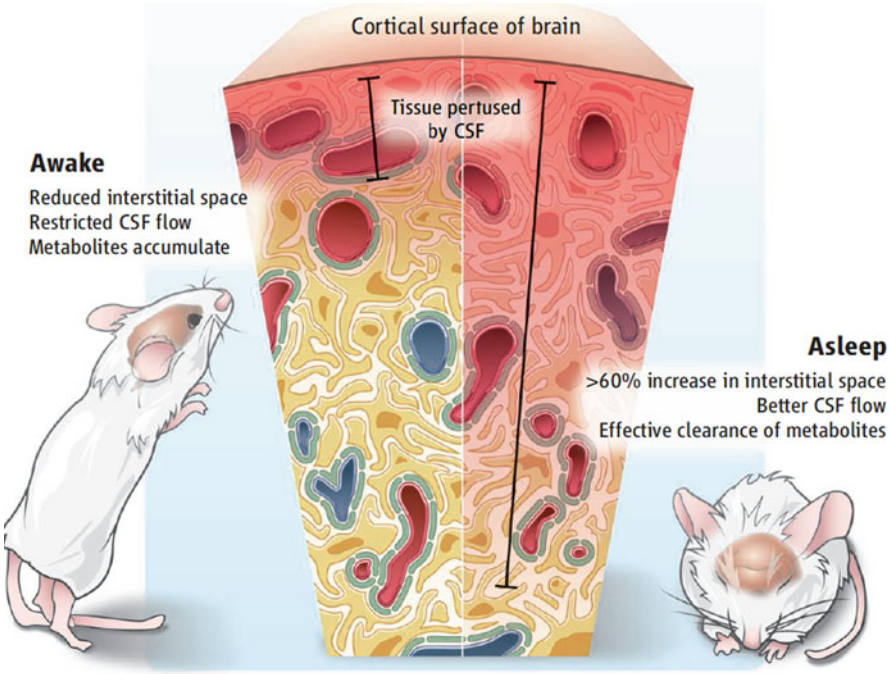

Fig. 6 The glymphatic hypothesis for clearance of brain metabolic waste involves the flow of CSF from the subarachnoid space into the brain along the periarterial spaces around penetrating arteries and arterioles. Aquaporins in the end-feet of the astrocytes lining the blood vessels allow water and small molecular solutes to enter the astrocytes and from the extensions of the astrocytes into the interstitial fluid (ISF) of the brain parenchyma. The ISF along with metabolic wastes and toxins leaves the parenchyma through perivascular spaces around the venules and veins. This ISF flow increases by about 60% during sleep due to widening of the perivascular spaces (figure from Herculano-Houzel 2013)

The high-energy demands of neural activations during wake call upon the glycogen reserves of the astrocytes causing increases in lactate levels in the ISF, and indeed studies have shown higher brain lactate levels during wake than during sleep (Lundgaard et al. 2016). In addition, neuronal activations are associated with transient rises in glycolysis resulting in neuronal contributions of lactate to the ISF (Prichard et al. 1991). Thus, sleep-related clearance of lactate from the brain is one potential function of the glymphatic system (Lundgaard et al. 2016) and that clearance function would pertain to many other components of the ISF as well.

6 Discussion

There are two main categories of brain-based hypotheses of sleep function. Cognitive hypotheses propose that sleep is important for complex brain functions such as learning, memory, and plasticity. Housekeeping hypotheses (restoration and

detoxification) instead propose that sleep governs more basic, homeostatic functions necessary for normal operation of neurons. How do these hypotheses fare when one considers our criteria for a core function of sleep?

6.1 Ontogeny

Both categories of hypotheses appear to explain many aspects of sleep across the lifespan. Early development is a time of rapid and extensive learning. If sleep is necessary to consolidate and strengthen the effects of experience on the brain, it is reasonable that sleep amounts should be high in young animals. Similarly, if sleep plays a critical role in brain plasticity, then one might expect the amount of sleep to parallel developmental changes in brain plasticity. This is indeed the case across a wide range of animal species (Frank 2005). Sleep is maximal during periods of development when the brain is most plastic. With respect to SHY, it might explain the need for sleep function during specific developmental windows when overall pruning exceeds synaptogenesis. These periods of rapid growth, learning, and plasticity are likely accompanied by greater metabolic demands and increased rates of macromolecule synthesis. This in turn could theoretically require greater amounts of glymphatic clearance of waste products.

6.2 Homeostasis

Not all hypotheses offer explanations for homeostatic regulation. Cognitive hypotheses have yet to provide a convincing link between learning or plasticity and Process S. There is no clear feedback variable resulting from memory consolidation or brain plasticity per se that then determines sleep need or expression. It has been suggested that BDNF could play that role. BDNF concentrations in the brain are higher during wake reflecting the level of neural activity. Unilateral applications of BDNF into the rat brain promote higher slow-wave activity in that side of the brain during subsequent sleep. However, these studies rely on nonphysiological manipulations of BDNF (Faraguna et al. 2008).

There is a link between cortical slow-wave activity and memory consolidation. The expression of hippocampal sharp-wave ripples that are believed to be the conduit for information transfer between the hippocampus and the neocortex during memory consolidation is linked to the up-states of the slow-wave oscillations. Therefore, the cortical slow-wave activity appears to synchronize the neocortical-hippocampal interplay that is fundamental to the formation of long-term memories (Molle et al. 2006). There is elegant evidence that regional brain activation during wake influences SWA in that region during sleep, and the subsequent improvement in the task related to that activation is proportional to the change in SWA (Huber et al. 2004). However, no feedback signal related to cognitive demand related to memory consolidation has been identified.

Stronger linkages exist between some housekeeping hypotheses and Process S. As proposed by Benington and Heller (1995), wake promotes regional energy deficits that result in a negative feedback signal, adenosine, that promotes sleep and in particular the EEG delta power that is the primary measure of Process S. During sleep energy reserves are restored, and the negative feedback signal is decreased. The higher energetic demands during waking could be related to regional synaptic activity, maintaining ion gradients, and/or macromolecule turnover; all are energetic processes.

It is also conceivable that the synthesis of macromolecules during sleep includes molecules that enable sustained wakefulness; an interruption of this process would then lead to heightened sleep pressure.

At present, there is no direct evidence linking glymphatic clearance with Process S; however, a clearance hypothesis for sleep function implies that there is a buildup during wake of one or more substances that are cleared during sleep, and any such substance could serve as a feedback variable in the homeostatic regulation of sleep. There is a potential connection between glymphatic clearance and brain energetics that could relate to Process S. During wake, brain lactate levels are higher than during sleep (Lundgaard et al. 2016). When regional metabolic demand of neurons exceeds the available blood glucose supply, the astrocytes break down glycogen and through glycolysis supply lactate to the interstitial fluid to serve the metabolic needs of the neurons. Thus, wakeful brain activities could generate the need for extracellular lactate clearance during sleep, but that lactate production could be coincident with energetic demands resulting in adenosine release.

6.3 Necessity and Sufficiency

Very few brain hypotheses for sleep function demonstrate why sleep is necessary and sufficient and why the brain must be offline for the proposed function. The best case can be made for sleep being necessary for learning and memory. New information is coded during wake, and significant information is consolidated and integrated during subsequent sleep. Although studies have shown that simple cued fear memory may not require sleep (Graves et al. 2003; Cai et al. 2009), their strengthening and integration may require sleep. Rolls et al. (2013) showed that the intensity of cued fear memories is dependent on replay occurring during sleep. The interesting dichotomy between the wake and sleep response to the cue is that repeated exposure to the cue during wake leads to extinction but repeated exposure during sleep results in intensification. Clearly working memory and short-term memory do not require sleep. Certain forms of replay also occur in quiet waking (Foster 2017), and emotional valence may extend the duration of those memories. Sleep, however, is necessary for higher-order memory consolidation and integration.

The necessity of sleep for learning and memory consolidation may involve the importance of stabilizing memory transcripts during hippocampal/neocortical interactions, thus separating the encoding of new information from the consolidation of prior information (Heller et al. 2014). The vulnerability of those memory

transcripts has been demonstrated by the ability to alter their emotional valence (Rolls et al. 2013) and by the ability to manipulate them during sleep to create false memories (Ramirez et al. 2013). Having the brain offline during higher cognitive processing blocks new inputs and protects the fidelity of the memories being consolidated.

Few restorative and detoxification hypotheses have been able to demonstrate necessity and sufficiency and the need for offline brain states. Offline states would seem to promote glycogenesis. The neuromodulators promoting wake activate glycogen phosphorylase promoting glycogenolysis, and the neuromodulators promoting sleep activate the enzymes of glycogen synthesis. Short-term sleep loss has been shown to reduce cerebral glycogen content in rats (Kong et al. 2002) but not in other rodent studies (Gip et al. 2002; Franken et al. 2003). In Drosophila, if sleep loss is maintained for longer periods, glycogen synthesis resumes even though the animals are awake (Zimmerman et al. 2004). Thus, sleep may be sufficient, but not necessary, for glycogen synthesis. Similarly, it is not yet clear if sleep is necessary and sufficient for glymphatic clearance or why the brain must be offline for this to occur. It is conceivable that because the control of the perivascular resistance to flow is under adrenergic control, and adrenergic tone is high during wake, sleep is needed to decrease adrenergic tone. Yet, it is unknown what happens to clearance if wakefulness is extended. Given that clearance does occur during wakefulness, but at a lower rate (Xie et al. 2013), it is possible that during sleep deprivation, clearance rates simply increase even in the absence of sleep.

There is some evidence that sleep may be necessary and sufficient for the synthesis of certain types of cerebral proteins. This has been demonstrated in the hippocampus and visual cortex. In both brain regions, activation of some enzymes important for mRNA translation (and protein synthesis) only proceeds in sleep (Seibt et al. 2012; Tudor et al. 2016). Offline brain states may be necessary for optimal protein synthesis because waking may inhibit the upstream activation of mTOR. The higher consumption of ATP during waking states may shift the AMP/ATP ratio toward AMP thus activating AMP-kinase, leading to inhibition of mTOR (Inoki et al. 2012).

6.4 The Presence of Two Sleep States

No current hypothesis of sleep function has adequately explained the presence of REM and NREM sleep. Several proposals have been made that posit that the interplay between the two states is required for memory consolidation (Giuditta et al. 1995; Smith 2001). However, the supporting evidence is not strong (Ackermann and Rasch 2014). There is evidence that supports a role for both brain states in brain plasticity and macromolecular synthesis, but it is not clear if they govern the same or different plastic processes.

Other proposals are largely mute when it comes to REM sleep (Xie et al. 2013; Tononi and Cirelli 2014). This may in part stem from the difficulty in examining the role of each state in isolation. Experimentally, this is very hard to do as selective

REM sleep deprivation (which is commonly used to probe the role of REM sleep) disrupts NREM sleep (Endo et al. 1997, 1998). In addition, REM sleep periods in mice (which are the more commonly used animal model) are very short in duration, which makes measurements selectively in this state difficult.

The problem of accommodating both sleep states in a hypothesis of sleep function only exists if we assume that the functions of both sleep states are in relationship to wake. Therefore, the problem disappears if we view REM sleep as serving a need generated during NREM sleep as proposed by Benington and Heller (1994). They produced convincing evidence for a homeostatic relationship between NREM and REM sleep with the need for REM sleep building up during episodes of NREM sleep until that need forces a transition to REM to dissipate that need.

7 Synthesis and Concluding Remarks

None of the hypotheses of sleep function that we have discussed convincingly satisfy all of the criteria we set forth at the beginning of this review. The least satisfactory challenge for all hypotheses is explaining the two different states of sleep. Perhaps this difficulty is due to a wrong assumption that all sleep functions are in relationship to waking. If REM sleep serves a need created by NREM sleep rather than waking (Benington and Heller 1994), then all hypotheses of sleep function explain the existence of the two sleep states. The question converts to what conditions or needs created by NREM sleep require the alternations of that state with REM sleep.

The macromolecular synthesis hypothesis appears to accommodate the ontogeny criterion well since early life is a time of rapid and extensive growth and remodeling of the nervous system and also a time of maximum sleep amounts. It also has the potential of satisfying the homeostasis criterion since it postulates that many gene products are depleted during wake and replenished during sleep. However, no specific feedback signal has been identified that relates biosynthetic functions to Process S. Problems arise for the macromolecular synthesis hypothesis with respect to necessity and sufficiency with respect to gene transcription. Although thousands of genes have been shown to alter expression levels between wake and sleep, the observed changes in levels are subtle (Mackiewicz et al. 2007). It is difficult to explain why the brain must be taken offline to achieve those subtle changes. There is also a logical problem in postulating that biosynthesis serves as the primary function of sleep. All of the other hypotheses of sleep function could be expected to be facilitated by changes in gene or protein expression, so it seems that the evolution of those functions would have preceded the associated changes in macromolecular synthesis.

The other two restorative hypotheses, brain energy reserve replenishment and brain clearance of waste products, are compatible with increased demands during early life. Decrease in these functions reflected in less sleep in the aged could also be factors in cognitive capacity senescence. A strong feature of the brain energy hypothesis is that it identifies a feedback molecule (adenosine) that explains Process

S and a mechanism (glycogen depletion) that relates the production of that feedback molecule to events during waking. However, it is difficult to conclude that the brain energy hypothesis meets the necessity and sufficiency criterion. Although glycogen metabolism is strongly affected by the neuromodulators associated with sleep/wake regulation, it has been shown that glycogen synthesis occurs in both sleep and wake (Petit et al. 2015). Conversely, the glymphatic clearance hypothesis does not identify a feedback molecule that explains Process S, but mechanistically it satisfies the criterion of sufficiency. It is tempting, however, to see a link between the energy and the clearance processes. Lactate is a significant component of the glymphatic clearance process, and its accumulation in the interstitial fluid should correlate with the energy demands placed on the brain.

Our conclusion is that the strongest support for a primary function of sleep hypothesis goes to learning and memory along with the requisite underlying processes of synaptic plasticity. Obviously synaptic plasticity is a feature of wake; otherwise, new information could not be encoded, and practice would not have an effect on subsequent recall/performance. However, sleep surely involves unique properties of synaptic plasticity that underlie complex memory consolidation and integration that are fundamental to higher cognitive functions. Evidence shows that sleep is necessary and sufficient for these higher cognitive functions. A reason for why the brain must be offline for these functions to occur likely resides in the brain processes that communicate memory transcripts from one brain region to another. The fidelity of these memory transcripts could be compromised by intrusion of new information that is constantly being encoded during wake. A feedback signal controlling sleep homeostasis in response to accumulation of information during wake has not been identified, but a major feature of sleep homeostasis, cortical slow-wave activity, may play an important role in coordinating the information processing involved in memory consolidation.

As we learn more about the complex neural mechanisms underlying sleep and its relationships to wake, we are likely to find that the final answer to what is the primary function of sleep involves a combination of aspects of the current leading hypotheses. A final consideration is that pharmacologically induced brain states resembling sleep (e.g., hypnotic induced sleep) may not accomplish the normal functions of sleep. For example, hypnotic agents that have relatively modest effects on sleep architecture can profoundly inhibit sleep-dependent brain plasticity in animals (Seibt et al. 2008; Aton et al. 2009a, b) and reduce sleep-dependent memory in humans (Hall-Porter et al. 2014). Therefore, the secret of sleep function must lie beyond the superficial metrics such as EEG that we use to measure its outward appearance. Conversely, as we learn more about specific functions normally subserved by sleep, it may be possible to stimulate them or improve them without replicating the superficial manifestations of sleep.

We end this brief review of the search for sleep function with a quote from our esteemed colleague who engaged in that search for many years, Allan Rechtschaffen: “If sleep does not serve an absolutely vital function, then it is the biggest mistake the evolutionary process has ever made.”

References

Ackermann S, Rasch B (2014) Differential effects of non-REM and REM sleep on memory consolidation? Curr Neurol Neurosci Rep 14(2):430

Albensi BC et al (2007) Electrical stimulation protocols for hippocampal synaptic plasticity and neuronal hyper-excitability: are they effective or relevant? Exp Neurol 204(1):1–13

Andersen ML et al (2005) Endocrinological and catecholaminergic alterations during sleep deprivation and recovery in male rats. J Sleep Res 14(1):83–90

Areal CC et al (2017) Sleep loss and structural plasticity. Curr Opin Neurobiol 44:1–7

Arrigoni E et al (2009) Long-term synaptic plasticity is impaired in rats with lesions of the ventrolateral preoptic nucleus. Eur J Neurosci 30(11):2112–2120

Aton SJ et al (2009a) The sedating antidepressant trazodone impairs sleep-dependent cortical plasticity. PLoS One 4(7):1–10

Aton SJ et al (2009b) Mechanisms of sleep-dependent consolidation of cortical plasticity. Neuron 61(3):454–466

Aton SJ et al (2013) Visual experience and subsequent sleep induce sequential plastic changes in putative inhibitory and excitatory cortical neurons. PNAS 110(8):3101–3106

Aton SJ et al (2014) Sleep promotes cortical response potentiation following visual experience. Sleep 37(7):1163–1170

Basheer R et al (2005) Sleep deprivation-induced protein changes in basal forebrain: implications for synaptic plasticity. J Neurosci Res 82(5):650–658

Bellesi M et al (2013) Effects of sleep and wake on oligodendrocytes and their precursors. J Neurosci 33(36):14288–14300

Benington JH, Heller HC (1994) Does the function of REM sleep concern non-REM sleep or waking? Prog Neurobiol 44(5):433–449

Benington J, Heller HC (1995) Restoration of brain energy metabolism as the function of sleep. Prog Neurobiol 45(4):347–360

Benington JH et al (1995) Stimulation of A1 adenosine receptors mimics the electroencephalographic effects of sleep deprivation. Brain Res 692(1):79–85

Blanco W et al (2015) Synaptic homeostasis and restructuring across the sleep-wake cycle. PLoS Comput Biol 11(5):e1004241

Bobillier P et al (1971) Deprivation of paradoxical sleep and in vitro cerebral protein synthesis in the rat. Life Sci 10(Part II):1349–1357

Bonhoeffer T, Grinvald A (1996) Optical imaging based on intrinsic signal. The methodology. In: Toga AW, Massiotta HC (eds) Brain mapping: the methods. Academic Press, London, pp 55–97

Borbely AA, Achermann P (1992) Concepts and models of sleep regulation: an overview. J Sleep Res 1(2):63–79

Born J, Wilhelm I (2012) System consolidation of memory during sleep. Psychol Res 76 (2):192–203

Cai DJ et al (2009) Sleep selectively enhances hippocampus-dependent memory in mice. Behav Neurosci 123(4):713–719

Campbell IG et al (2002) Sleep deprivation impairs long-term potentiation in the rat hippocampal slices. J Neurophysiol 88:1073–1076

Chen C et al (2006) Altered NMDA receptor trafficking contributes to sleep deprivation-induced hippocampal synaptic and cognitive impairments. Biochem Biophys Res Commun 340 (2):435–440

Cirelli C, Tononi G (2015) Sleep and synaptic homeostasis. Sleep 38(1):161–162

Cirelli C et al (2004) Extensive and divergent effects of sleep and wakefulness on brain gene expression. Neuron 41(1):35–43

Cooke SF, Bear MF (2010) Visual experience induces long-term potentiation in the primary visual cortex. J Neurosci 30(48):16304–16313

Czikk MJ et al (2003) Cerebral leucine uptake and protein synthesis in the near-term ovine fetus: relation to fetal behavioral state. Am J Physiol Regul Integr Comp Physiol 284(1):R200–R207

Davidson TJ et al (2009) Hippocampal replay of extended experience. Neuron 63(4):497–507
Davis CJ et al (2003) REM sleep deprivation-induced deficits in the latency-to-peak induction and maintenance of long-term potentiation within the CA1 region of the hippocampus. Brain Res 973(2):293–297
Davis CJ et al (2006) REM sleep deprivation attenuates actin-binding protein cortactin: a link between sleep and hippocampal plasticity. Neurosci Lett 400(3):191–196
de Sanchez VC et al (1993) Day-night variations of adenosine and its metabolizing enzymes in the brain cortex of the rat – possible physiological significance for the energetic homeostasis and the sleep-wake cycle. Brain Res 612:115–121
de Vivo L et al (2017) Ultrastructural evidence for synaptic scaling across the wake/sleep cycle. Science 355(6324):507
Denin NN et al (1980) Concentration of proteins and RNA in neurons and gliocytes of the rat locus coeruleus during natural sleep and REM-sleep deprivation. Fiziol ZH SSSR Im I Sechnonovia 66(11):1626–1631
Diering GH et al (2017) Homer1a drives homeostatic scaling-down of excitatory synapses during sleep. Science 355(6324):511
Dumoulin Bridi MC et al (2015) Rapid eye movement sleep promotes cortical plasticity in the developing brain. Sci Adv 1(6):e1500105
Dumoulin MC et al (2015) Extracellular signal-regulated kinase (ERK) activity during sleep consolidates cortical plasticity in vivo. Cereb Cortex 25(2):507–515
Durkin J, Aton SJ (2016) Sleep-dependent potentiation in the visual system is at odds with the synaptic homeostasis hypothesis. Sleep 39:155–159
Durkin J et al (2017) Cortically coordinated NREM thalamocortical oscillations play an essential, instructive role in visual system plasticity. Proc Natl Acad Sci 114(39):10485–10490
Ego-Stengel V, Wilson MA (2010) Disruption of ripple-associated hippocampal activity during rest impairs spatial learning in the rat. Hippocampus 20(1):1–10
Endo T et al (1997) Selective and total sleep deprivation: effect on the sleep EEG in the rat. Psychiatry Res 66:97–110
Endo T et al (1998) Selective REM sleep deprivation in humans: effects on sleep and sleep EEG. Am J Phys 274(4 Pt 2):R1186–R1194
Espinosa JS, Stryker MP (2012) Development and plasticity of the primary visual cortex. Neuron 75(2):230–249
Falkowska A et al (2015) Energy metabolism of the brain, including the cooperation between astrocytes and neurons, especially in the context of glycogen metabolism. Int J Mol Sci 16 (11):25959–25981
Faraguna U et al (2008) A causal role for brain-derived neurotrophic factor in the homeostatic regulation of sleep. J Neurosci 28(15):4088–4095
Faraguna U et al (2010) Unilateral cortical spreading depression affects sleep need and induces molecular and electrophysiological signs of synaptic potentiation in vivo. Cereb Cortex 20 (12):2939–2947
Farooqui SM et al (1996) Changes in monoamines and their metabolite concentrations in REM sleep-deprived rat forebrain nuclei. Pharmacol Biochem Behav 54(2):385–391
Florian C et al (2011) Astrocyte-derived adenosine and A1 receptor activity contribute to sleep loss-induced deficits in hippocampal synaptic plasticity and memory in mice. J Neurosci 31 (19):6956–6962
Foster DJ (2017) Replay comes of age. Annu Rev Neurosci 40(1):581–602
Frank MG (2005) Sleep, synaptic plasticity and the developing brain. In: Luppi P-H (ed) Sleep circuits and functions. CRC Press, Boca Raton, pp 177–192
Frank MG (2006) The mystery of sleep function: current perspectives and future directions. Rev Neurosci 17:375–392
Frank MG (2010) The functions of sleep. In: Winkelman JW, Plante DT (eds) Foundations of psychiatric sleep medicine. Cambridge University Press, Cambridge, pp 59–78
Frank MG (2012) Erasing synapses in sleep: is it time to be SHY? Neural Plast 2012:264–378

Frank MG (2013) Why I'm not shy: a reply to Tononi and Cirelli. Neural Plast 2013:394946
Frank M (2015) Sleep and synaptic plasticity in the developing and adult brain. Curr Top Behav Neurosci 25:123–149
Frank MG, Cantera R (2014) Sleep, clocks, and synaptic plasticity. Trends Neurosci 37(9):491–501
Frank M, Issa NP, Stryker MP (2001) Sleep enhances plasticity in the developing visual cortex. Neuron 30:275–287
Franken P et al (2003) Changes in brain glycogen after sleep deprivation vary with genotype. Am J Physiol Regul Integr Comp Physiol 285(2):R413–R419
Frenkel MY et al (2006) Instructive effect of visual experience in mouse visual cortex. Neuron 51 (3):339–349
Fujisawa S, Buzsáki G (2011) A 4 Hz oscillation adaptively synchronizes prefrontal, VTA, and hippocampal activities. Neuron 72(1):153–165
Gip P et al (2002) Sleep deprivation decreases glycogen in the cerebellum but not in the cortex of young rats. Am J Physiol Regul Integr Comp Physiol 283(1):R54–R59
Girardeau G et al (2009) Selective suppression of hippocampal ripples impairs spatial memory. Nat Neurosci 12:1222
Giuditta A et al (1980a) Influence of synchronized sleep on the biosynthesis of RNA in neuronal and mixed fractions isolated from rabbit cerebral cortex. J Neurochem 35(6):1267–1272
Giuditta A et al (1980b) Influence of synchronized sleep on the biosynthesis of RNA in two nuclear classes isolated from rabbit cerebral cortex. J Neurochem 35(6):1259–1266
Giuditta A et al (1995) The sequential hypothesis of the function of sleep. Behav Brain Res 69:157–166
Graves LA et al (2003) Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. Learn Mem 10(3):168–176
Greene RW et al (2017) The adenosine-mediated, neuronal-glial, homeostatic sleep response. Curr Opin Neurobiol 44:236–242
Guzman-Marin R et al (2003) Sleep deprivation reduces proliferation of cells in the dentate gyrus of the hippocampus in rats. J Physiol Lond 549(2):563–571
Guzman-Marin R et al (2005) Sleep deprivation suppresses neurogenesis in the adult hippocampus of rats. Eur J Neurosci 22(8):2111–2116
Guzman-Marin R et al (2006) Suppression of hippocampal plasticity-related gene expression by sleep deprivation. J Physiol Lond 575(Pt 3):807–819
Guzman-Marin R et al (2008) Rapid eye movement sleep deprivation contributes to reduction of neurogenesis in the hippocampal dentate gyrus of the adult rat. Sleep 31(2):167–175
Hagewoud R et al (2009) Sleep deprivation impairs spatial working memory and reduces hippocampal AMPA receptor phosphorylation. J Sleep Res 19(2):280–288
Hairston IS et al (2005) Sleep restriction suppresses neurogenesis induced by hippocampus-dependent learning. J Neurophysiol 94(6):4224–4233
Halassa MM et al (2009) Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. Neuron 61(2):213–219
Hall-Porter JM et al (2014) The effect of two benzodiazepine receptor agonist hypnotics on sleep-dependent memory consolidation. J Clin Sleep Med 10(1):27–34
Havekes R, Abel T (2017) The tired hippocampus: the molecular impact of sleep deprivation on hippocampal function. Curr Opin Neurobiol 44:13–19
Havekes R et al (2007) The tired hippocampus: effects of sleep deprivation on AMPA receptor function and cell proliferation. Sleep Biol Rhythms 5(Supplement 1):A48
Havekes R et al (2016) Sleep deprivation causes memory deficits by negatively impacting neuronal connectivity in hippocampal area CA1. elife 5:e13424
Heller HC et al (2014) Adaptive and pathological inhibition of neuroplasticity associated with circadian rhythms and sleep. Behav Neurosci 128(3):273–282
Hendricks JC et al (2000a) Rest in drosophila is a sleep-like state. Neuron 25(1):129–138
Hendricks JC et al (2000b) The need for a simple animal model to understand sleep. Prog Neurobiol 61(4):339–351

Hengen KB et al (2016) Neuronal firing rate homeostasis is inhibited by sleep and promoted by wake. Cell 165(1):180–191
Herculano-Houzel S (2013) Sleep it out. Science 342(6156):316–317
Hill S et al (2008) Sleep improves the variability of motor performance. Brain Res Bull 76 (6):605–611
Hipolide DC et al (1998) Heterogeneous effects of rapid eye movement sleep deprivation on binding to [alpha]- and [beta]-adrenergic receptor subtypes in rat brain. Neuroscience 86 (3):977–987
Hobson JA (1999) Neural control of sleep. In: Turek FW, Zee PC (eds) Regulation of sleep and circadian rhythms, vol 133. Marcel Dekker, New York, pp 81–110
Holscher C (1999) Synaptic plasticity and learning and memory: LTP and beyond. J Neurosci Res 58:62–75
Hubel DH, Wiesel TN (1970) The period of susceptibility to the physiological effects of unilateral eye closure in kittens. J Physiol 206:419–436
Huber R (2007) TMS-induced cortical potentiation during wakefulness locally increases slow wave activity during sleep. PLoS One 2:e276
Huber R, Ghilardi MF, Massimini M, Tononi G (2004) Local sleep and learning. Nature 430:78–81
Huerta PT, Lisman JE (1996) Low-frequency stimulation at the troughs of theta-oscillation induces long-term depression of previously potentiated CA1 synapses. J Neurophysiol 75(2):877–884
Hulme SR et al (2014) Mechanisms of heterosynaptic metaplasticity. Philos Trans R Soc Lond B Biol Sci 369(1633):20130148
Iliff JJ, Nedergaard M (2013) Is there a cerebral lymphatic system? Stroke 44(6 suppl 1):S93
Inoki K et al (2012) AMPK and mTOR in cellular energy homeostasis and drug targets. Annu Rev Pharmacol Toxicol 52(1):381–400
Ishikawa A et al (2006) Selective rapid eye movement sleep deprivation impairs the maintenance of long-term potentiation in the rat hippocampus. Eur J Neurosci 24(1):243–248
Jha SK et al (2005) Sleep-dependent plasticity requires cortical activity. J Neurosci 25 (40):9266–9274
Ji D, Wilson MA (2007) Coordinated memory replay in the visual cortex and hippocampus during sleep. Nat Neurosci 10(1):100–106
Kaplan ES et al (2016) Contrasting roles for parvalbumin-expressing inhibitory neurons in two forms of adult visual cortical plasticity. elife 5:e11450
Khodagholy D et al (2017) Learning-enhanced coupling between ripple oscillations in association cortices and hippocampus. Science 358(6361):369
Kim E et al (2005) REM sleep deprivation inhibits LTP in vivo in area CA1 of rat hippocampus. Neurosci Lett 388(3):163–167
Kong J et al (2002) Brain glycogen decreases with increased periods of wakefulness: implications for homeostatic drive to sleep. J Neurosci 22(13):5581–5587
Kopp C et al (2006) Insufficient sleep reversibly alters bidirectional synaptic plasticity and NMDA receptor function. J Neurosci 26(48):12456–12465
Lee AK, Wilson MA (2002) Memory of sequential experience in the hippocampus during slow wave sleep. Neuron 36:1183–1194
Li W et al (2017) REM sleep selectively prunes and maintains new synapses in development and learning. Nat Neurosci 20(3):427–437
Liu Z-W et al (2010) Direct evidence for wake-related increases and sleep-related decreases in synaptic strength in rodent cortex. J Neurosci 30(25):8671–8675
Longordo F et al (2009) NR2A at CA1 synapses is obligatory for the susceptibility of hippocampal plasticity to sleep loss. J Neurosci 29(28):9026–9041
Louie K, Wilson MA (2001) Temporally structured replay of awake hippocampal ensemble activity during rapid eye movement sleep. Neuron 29:145–156
Lundgaard I et al (2016) Glymphatic clearance controls state-dependent changes in brain lactate concentration. J Cereb Blood Flow Metab 37(6):2112–2124

Mackiewicz M et al (2007) Macromolecule biosynthesis – a key function of sleep. Physiol Genomics 31:441–457
Majumdar S, Mallick BN (2005) Cytomorphometric changes in rat brain neurons after rapid eye movement sleep deprivation. Neuroscience 135(3):679–690
Maloney KJ et al (2002) c-Fos expression in dopaminergic and GABAergic neurons of the ventral mesencephalic tegmentum after paradoxical sleep deprivation and recovery. Eur J Neurosci 15 (4):774–778
Maret S et al (2011) Sleep and waking modulate spine turnover in the adolescent mouse cortex. Nat Neurosci 14:1418–1420
Marks CA, Wayner MJ (2005) Effects of sleep disruption on rat dentate granule cell LTP in vivo. Brain Res Bull 66(2):114–119
McDermott CM et al (2003) Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. J Neurosci 23(29):9687–9695
McDermott CM et al (2006) Sleep deprivation-induced alterations in excitatory synaptic transmission in the CA1 region of the rat hippocampus. J Physiol Lond 570(3):553–565
Mehta MR (2007) Cortico-hippocampal interaction during up-down states and memory consolidation. Nat Neurosci 10:13
Merchant-Nancy H et al (1992) c-fos proto-oncogene changes in relation to REM sleep duration. Brain Res 579(2):342–346
Molle M et al (2006) Hippocampal sharp wave-ripples linked to slow oscillations in rat slow-wave sleep. J Neurophysiol 96:62–70
Mueller AD et al (2008) Sleep deprivation can inhibit adult hippocampal neurogenesis independent of adrenal stress hormones. Am J Physiol Regul Integr Comp Physiol 294(5):R1693–R1703
Naidoo N et al (2005) Sleep deprivation induces the unfolded protein response in mouse cerebral cortex. J Neurochem 92(5):1150–1157
Nakanishi H et al (1997) Positive correlations between cerebral protein synthesis rates and deep sleep in Macaca mulatta. Eur J Neurosci 9:271–279
Nere AT et al (2013) Sleep dependent synaptic down-selection (I): modeling the benefits of sleep on memory consolidation and integration. Front Neurol 4:143
Norimoto H et al (2018) Hippocampal ripples down-regulate synapses. Science 359:1524–1527
O'Donnell C, Sejnowski TJ (2014) Selective memory generalization by spatial patterning of protein synthesis. Neuron 82(2):398–412
Olcese U et al (2010) Sleep and synaptic renormalization: a computational study. J Neurophysiol 104(6):3476–3493
Oudiette D, Paller KA (2013) Upgrading the sleeping brain with targeted memory reactivation. Trends Cogn Sci 17(3):142–149
Palchykova S et al (2010) Manipulation of adenosine kinase affects sleep regulation in mice. J Neurosci 30(39):13157–13165
Pedrazzoli M, Benedito MAC (2004) Rapid eye movement sleep deprivation-induced down-regulation of beta-adrenergic receptors in the rat brainstem and hippocampus. Pharmacol Biochem Behav 79(1):31–36
Petit J-M et al (2002) Sleep deprivation modulates brain mRNAs encoding genes of glycogen metabolism. Eur J Neurosci 16(6):1163–1167
Petit J-M et al (2015) Glycogen metabolism and the homeostatic regulation of sleep. Metab Brain Dis 30(1):263–279
Poe GR, Nitz DA, McNaughton BL, Barnes DA (2000) Experience-dependent phase-reversal of hippocampal neuron firing during REM sleep. Brain Res 855:176–180
Porkka-Heiskanen T et al (1997) Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness. Science 276(5316):1265–1268
Porrka-Heiskanen T et al (1995) Noradrenergic activity in rat brain during rapid eye movement sleep deprivation and rebound sleep. Am J Physiol 268(37):R1456–R1463
Prichard J et al (1991) Lactate rise detected by 1H NMR in human visual cortex during physiological stimulation. PNAS 88:5829–5831

Puentes-Mestril C, Aton SJ (2017) Linking network activity to synaptic plasticity during sleep: hypotheses and recent data. Front Neural Circuits 11:61
Radulovacki M et al (1984) Adenosine analogs and sleep in rats. J Pharmacol Exp Ther 228 (2):268–274
Ramirez S, Tonegawa D, Liu X (2013) Identification and optogenetic manipulation of memory engrams in the hippocampus. Front Behav Neurosci 7:226
Ramm P, Smith CT (1990) Rates of cerebral protein synthesis are linked to slow-wave sleep in the rat. Physiol Behav 48:749–753
Rasch B, Born J (2007) Maintaining memories by reactivation. Curr Opin Neurobiol 17:698–703
Rasch B, Born J (2013) About sleep's role in memory. Physiol Rev 93(2):681–766
Ravassard P et al (2006) Paradoxical sleep amount modulates neuronal plasticity in adult rat hippocampus. J Sleep Res 15:191–191
Ravassard P et al (2009) Paradoxical (REM) sleep deprivation causes a large and rapidly reversible decrease in long-term potentiation, synaptic transmission, glutamate receptor protein levels, and ERK/MAPK activation in the dorsal hippocampus. Sleep 32(2):227–240
Ravassard P et al (2015) REM sleep-dependent bidirectional regulation of hippocampal-based emotional memory and LTP. Cereb Cortex 26:1488–1500
Rechtschaffen A (1998) Current perspectives on the function of sleep. Perspect Biol Med 41 (3):359–390
Rechtschaffen A et al (2002) Sleep deprivation in the rat: X. Integration and discussion of the findings. 1989. Sleep 25(1):68–87
Ribeiro S (2011) Sleep and plasticity. Pfluegers Arch Eur J Physiol 463:111–120
Rolls A et al (2013) Sleep to forget: interference of fear memories during sleep. Mol Psychiatry 18:1166
Romcy-Pereira R, Pavlides C (2004) Distinct modulatory effects of sleep on the maintenance of hippocampal and medial prefrontal cortex LTP. Eur J Neurosci 20(12):3453–3462
Rudoy JD et al (2009) Strengthening individual memories by reactivating them during sleep. Science 326(5956):1079
Sadowski JH et al (2016) Sharp-wave ripples orchestrate the induction of synaptic plasticity during reactivation of place cell firing patterns in the hippocampus. Cell Rep 14(8):1916–1929
Seibt J et al (2008) The non-benzodiazepine hypnotic Zolpidem impairs sleep-dependent cortical plasticity. Sleep 31(10):1381–1392
Seibt J et al (2012) Protein synthesis during sleep consolidates cortical plasticity *in vivo*. Curr Biol 22(8):676–682
Seibt J et al (2017) Cortical dendritic activity correlates with spindle-rich oscillations during sleep in rodents. Nat Commun 8(1):684
Sengpiel F (2001) Cortical plasticity: learning while you sleep? Curr Biol 11(16):R647–R650
Shapiro C, Girdwood P (1981) Protein synthesis in rat brain during sleep. Neuropharmacology 20:457–460
Shaw PJ et al (2000) Correlates of sleep and waking in Drosophila melanogaster. Science 287 (5459):1834–1837
Siapas AG et al (2005) Prefrontal phase locking to hippocampal theta oscillations. Neuron 46 (1):141–151
Smith C (2001) Sleep states and memory processes in humans: procedural versus declarative memory systems. Sleep Med Rev 5:491–506
Smith GB et al (2009) Bidirectional synaptic mechanisms of ocular dominance plasticity in visual cortex. Philos Trans R Soc Lond B Biol Sci 364(1515):357–367
Soulé J et al (2012) Balancing arc synthesis, mRNA decay, and proteasomal degradation. J Biol Chem 287(26):22354–22366
Spolidoro M et al (2008) Plasticity in the adult brain: lessons from the visual system. Exp Brain Res 192:335–341
Steriade M, Timofeev I (2003) Neuronal plasticity in thalamocortical networks during sleep and waking oscillations. Neuron 37(4):563–576

Stickgold R (2005) Sleep-dependent memory consolidation. Nature 437(7063):1272–1278
Taishi P et al (2001) Conditions that affect sleep alter the expression of molecules associated with synaptic plasticity. Am J Phys 281:R839–R845
Tartar JL et al (2006) Hippocampal synaptic plasticity and spatial learning are impaired in a rat model of sleep fragmentation. Eur J Neurosci 23(10):2739–2748
Terao A et al (2003) Differential increase in the expression of heat shock protein family members during sleep deprivation and during sleep. Neuroscience 116(1):187–200
Timofeev I, Chauvette S (2017) Sleep slow oscillation and plasticity. Curr Opin Neurobiol 44:116–126
Tobler I (2005) Phylogeny of sleep regulation. In: Kryger M, Roth T, Dement WC (eds) Principles and practice of sleep medicine. W. B. Saunders, Philadelphia, pp 72–90
Tononi G, Cirelli C (2003) Sleep and synaptic homeostasis: a hypothesis. Brain Res Bull 62 (2):143–150
Tononi G, Cirelli C (2006) Sleep function and synaptic homeostasis. Sleep Med Rev 10(1):49–62
Tononi G, Cirelli C (2014) Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. Neuron 81(1):12–34
Toppila J et al (1995) REM sleep deprivation induces galanin gene expression in the rat brain. Neurosci Lett 183(3):171–174
Tropea D et al (2009) Molecular mechanisms of experience-dependent plasticity in visual cortex. Philos Trans R Soc Lond B Biol Sci 364(1515):341–355
Tsanov M, Manahan-Vaughan D (2007) The adult visual cortex expresses dynamic synaptic plasticity that is driven by the light/dark cycle. J Neurosci 27(31):8414–8421
Tudor JC et al (2016) Sleep deprivation impairs memory by attenuating mTORC1-dependent protein synthesis. Sci Signal 9(425):ra41
Tung A et al (2005) Effects of sleep deprivation and recovery sleep upon cell proliferation in adult rat dentate gyrus. Neuroscience 134(3):721–723
Turrigiano G (2007) Homeostatic signaling: the positive side of negative feedback. Curr Opin Neurobiol 17:318–324
van Dongen EV et al (2012) Memory stabilization with targeted reactivation during human slow-wave sleep. Proc Natl Acad Sci 109(26):10575–10580
Vazquez J et al (2008) Rapid alterations in cortical protein profiles underlie spontaneous sleep and wake bouts. J Cell Biochem 105:1472–1484
Vecsey CG et al (2009) Sleep deprivation impairs cAMP signalling in the hippocampus. Nature 461 (7267):1122–1125
Vecsey CG et al (2012) Genomic analysis of sleep deprivation reveals translational regulation in the hippocampus. Physiol Genomics 44(20):981–991
Vyazovskiy VV et al (2008) Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. Nat Neurosci 11(2):200–208
Vyazovskiy VV et al (2009) Cortical firing and sleep homeostasis. Neuron 63(6):865–878
Watson BO et al (2016) Network homeostasis and state dynamics of neocortical sleep. Neuron 90 (4):839–852
Wierzynski CM, Lubenov EV, Gu M, Siapas AG (2009) State-dependent spike-timing relationships between hippocampal and prefrontal circuits during sleep. Neuron 61(4):587–596
Wiesel TN, Hubel DH (1963) Single cell responses in striate cortex of kittens deprived of vision in one eye. J Neurophysiol 28:1029–1040
Wilson MA, McNaughton BL (1994) Reactivation of hippocampal ensemble memories during sleep. Science 265:676–682
Xie L et al (2013) Sleep drives metabolite clearance from the adult brain. Science 342 (6156):373–377
Yang G, Gan W-B (2011) Sleep contributes to dendritic spine formation and elimination in the developing mouse somatosensory cortex. Dev Neurobiol 72:1391–1398
Yang G et al (2014) Sleep promotes branch-specific formation of dendritic spines after learning. Science 344(6188):1173–1178
Zimmerman JE et al (2004) Glycogen in the brain of *Drosophila melanogaster*: diurnal rhythm and the effect of rest deprivation. J Neurochem 88(1):32–40

Neuroanatomical and Neurochemical Bases of Vigilance States

Pierre-Hervé Luppi and Patrice Fort

Contents

Abstract

In the present chapter, hypotheses on the mechanisms responsible for the genesis of the three vigilance states, namely, waking, non-rapid eye movement (non-REM) also called slow-wave sleep (SWS), and REM sleep also called paradoxical sleep (PS), are presented. A huge number of studies first indicate that waking is induced by the activation of multiple waking systems, including

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the serotonergic, noradrenergic, cholinergic, and hypocretin systems. At the onset of sleep, the SWS-active neurons would be activated by the circadian clock localized in the suprachiasmatic nucleus and a hypnogenic factor, adenosine, which progressively accumulates in the brain during waking. A number of studies support the hypothesis that SWS results from the activation of GABAergic neurons localized in the ventrolateral preoptic nucleus (VLPO). However, new GABAergic systems recently described localized in the parafacial, accumbens, and reticular thalamic nuclei will be also presented. In addition, we will show that a large body of data strongly suggests that the switch from SWS to PS is due to the interaction of multiple populations of glutamatergic and GABAergic neurons localized in the posterior hypothalamus and the brainstem.

Keywords

Acetylcholine · Adenosine · Brainstem · GABA · Glycine · Histamine · Hypocretin · Melanin-concentrating hormone · Muscle atonia · Norepinephrine · Orexin · Serotonin

1 Introduction

In most mammals, there are three vigilance states characterized by distinct differences in electroencephalogram (EEG), electromyogram (EMG), and electrooculogram (EOG) recordings. The waking state is characterized by high-frequency (40–300 Hz), low-amplitude (desynchronized) activity on the EEG, sustained EMG activity, and ocular movements; non-rapid eye movement (non-REM), also named slow-wave (SWS) sleep (synchronized), is characterized by low-frequency (0.5–4 Hz), high-amplitude delta oscillations on the EEG, low muscular activity on the EMG, and no ocular movement; and rapid eye movement (REM), also called paradoxical sleep (PS), is characterized by a predominant theta (6–9 Hz) and gamma (30–300 Hz) rhythms similar to waking EEG but with complete disappearance of postural muscle tone and the occurrence of rapid eye movements (REMs) and muscle twitches.

Neuropathological evidence from the nineteenth century indicates that altered states of vigilance can be induced by focal brain lesions and that different neurochemical mechanisms are responsible for the succession of the three vigilance states across the 24 h day (Fort et al. 2009). The aim of this review is to examine possible neuronal networks and mechanisms responsible of the switch from waking to SWS and PS.

2 Neuronal Network and Mechanisms Involved in Waking

The activated cortical state during waking is induced by the activity of multiple waking neurochemical systems. Some of these belong to the ascending reticular activating system. These neurochemical systems include the serotonergic neurons mainly localized in the dorsal raphe nucleus, noradrenergic neurons in the locus

coeruleus, cholinergic neurons in the pontine brainstem, glutamatergic neurons of the medial parabrachial nucleus (Anaclet et al. 2014), and dopaminergic neurons of the ventral tegmental area (Eban-Rothschild et al. 2016), while other systems are located more rostrally in the forebrain. These systems include the cholinergic neurons in the basal forebrain, the histaminergic neurons localized in the tuberomammillary nucleus, and GABAergic and orexin (hypocretin) neurons in the lateral hypothalamus (Fort et al. 2009; Herrera et al. 2016).

Altogether, these systems control wakefulness and arousal through their wide projections to the thalamus and/or the neocortex. When these waking systems are inactivated, the thalamocortical network is no longer activated and return to its default mode characterized by oscillations in the delta range (i.e., the slow-wave mode of activity typical of SWS) (Fort et al. 2009).

A number of studies strongly suggest that sleep is induced by neurons inhibiting the waking systems, possibly by gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the brain. It is well known that serotonergic neurons discharge during wakefulness decrease their activity during SWS to become completely silent during sleep (Gervasoni et al. 2000). It has also been shown that the local application during SWS and PS of bicuculline, a competitive antagonist of GABA-A receptors, restores the waking activity of the neurons. Similar results have been obtained for the noradrenergic neurons of the locus coeruleus (Gervasoni et al. 1998) (Figs. 1 and 2).

3 Mechanisms Involved in Non-REM (Slow-Wave Sleep, SWS) Induction and Maintenance

A large number of studies localized the GABAergic neurons in charge of inhibiting the waking systems during sleep in the preoptic area as described below.

3.1 The Forebrain (Preoptic) Sleep Center

Following studies of patients with postinfluenza encephalitis, the neuropathologist von Economo reported that inflammatory lesions of the preoptic area were often associated with insomnia and therefore proposed that the preoptic area was critical for the production of normal sleep (von Economo 1926). This was further confirmed in monkeys, rats, and cats where preoptic area lesions consistently induced a profound and persistent insomnia (Fort et al. 2009). Consistent with these results, preoptic area electrical stimulation induces EEG slow-wave activity and sleep (SWS) (Fort et al. 2009). A recent study using pharmacogenetics in mice to functionally mark neurons activated in the preoptic area during recovery sleep or alpha-2 adrenergic receptor-induced sedation elegantly demonstrated that selective reactivation of these neurons induces SWS (Zhang et al. 2015). Finally, putative sleep-promoting neurons in the preoptic area displaying an elevated discharge rate during SWS were recorded in freely moving cats (Fort et al. 2009). Altogether, these

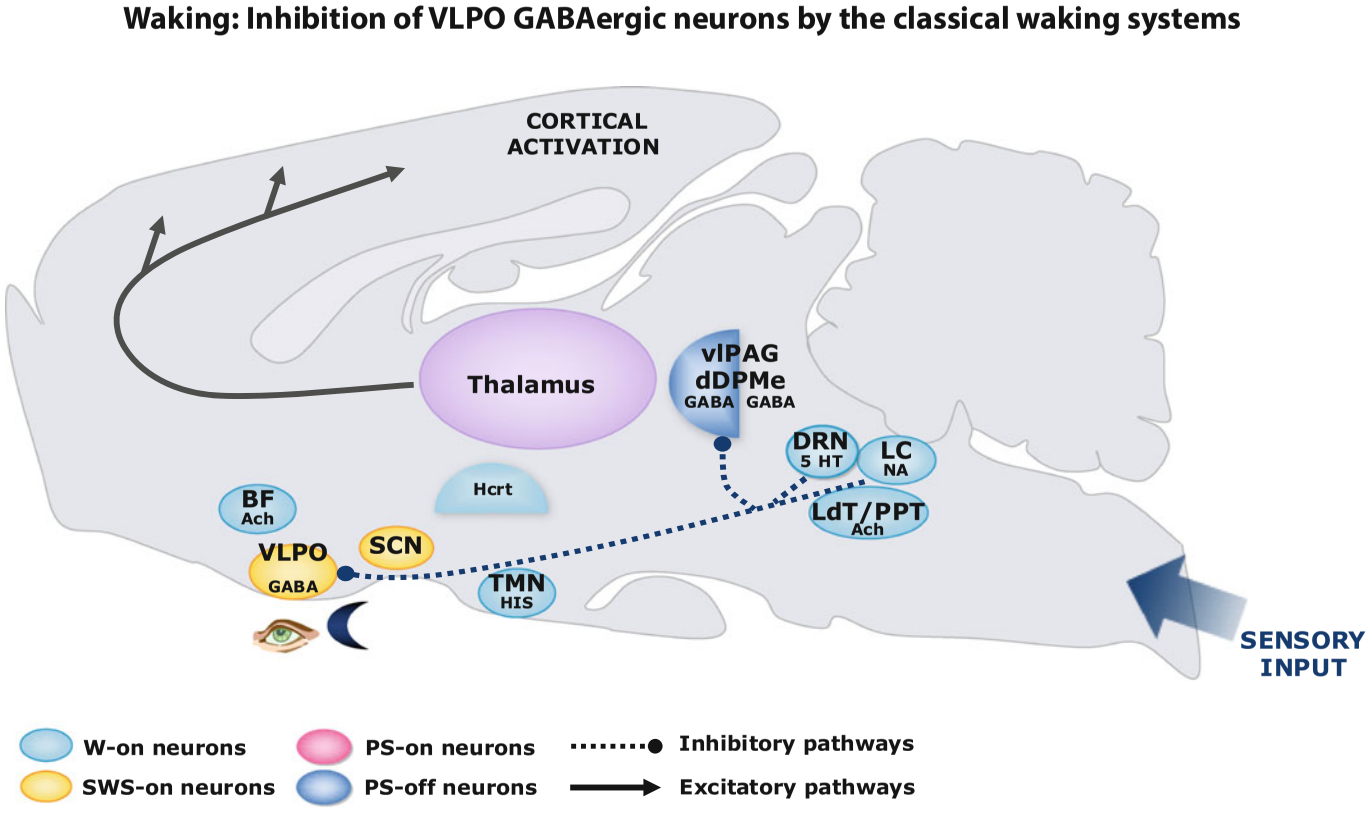

Fig. 1 Classical neuronal networks responsible for waking. At sleep-wake transitions, the hypocretin neurons would be the first to start firing, exciting all the other waking systems (histaminergic, monoaminergic, and cholinergic). In turn, these waking systems activate the thalamus and/or the cortex, leading to cortical

studies support the hypothesis that the preoptic area contains neurons that promote sleep, in particular SWS.

In the following years, to more precisely identify the sleep active neurons, researchers mapped in the preoptic area the localization of the neurons with increased expression of the immediate early gene *c-Fos*, a marker of neuronal activity. They found out that these neurons are distributed in the preoptic area but are more densely packed in the median preoptic nucleus (MnPn) and the ventrolateral preoptic nucleus (VLPO). Interestingly, the number of c-Fos-immunoreactive neurons in the VLPO and MnPn positively correlated with sleep quantity and sleep consolidation during the last hour preceding sacrifice. Numerous c-Fos-positive neurons were also observed in sleep-deprived rats in the MnPn but not in VLPO (Sherin et al. 1996; Gvilia et al. 2006), suggesting that VLPO neurons are responsible for the induction of sleep while MnPn neurons serve a homeostatic role in sleep. It was later demonstrated that VLPO and the suprachiasmatic nucleus (SCN), responsible for the circadian organization of the sleep-waking cycle, have synchronized activity (Fort et al. 2009). Considering that both areas are interconnected and receive inputs from the retinal ganglion cells, it is, thus, possible that circadian- and photic-linked information may be conveyed to modulate VLPO activity (Fort et al. 2009) (Fig. 3).

Electrophysiological experiments in behaving rats have shown that neurons recorded in the VLPO and MnPn are active before and after the onset of SWS and their firing rate is positively correlated with sleep depth and duration, suggesting that they participate to SWS induction and stability. Some of these neurons are also active during PS with a higher firing frequency than during the preceding SWS. In addition, VLPO and MnPn neurons display a firing pattern reciprocal to the wake-active neurons (see below). Retrograde and anterograde tract-tracing studies indicate that VLPO and MnPn neurons are reciprocally connected with wake-active neurons such as those containing histamine in the tuberomammillary nucleus (TMN), hypocretins in the perifornical hypothalamic area (PeF), serotonin in the dorsal raphe nuclei (DRN), noradrenaline in the locus coeruleus (LC), acetylcholine in the pontine (LDT/PPT), and basal forebrain nuclei. In these wake-promoting areas, extracellular levels of GABA increase during SWS compared to W. It has also been shown that c-Fos-positive neurons in the VLPO express galanin mRNA and 80% of

Fig. 1 (continued) activation, and also, importantly, inhibit the GABAergic SWS (non-REM sleep)-active neurons of the VLPO and MnPn. *5HT* 5-hydroxytryptamine (serotonin), *Ach* acetylcholine, *ADA* adenosine, *BF* basal forebrain, *DPGi* dorsal paragigantocellular reticular nucleus, *dDPMe* deep mesencephalic reticular nucleus, *DRN* dorsal raphe nucleus, *GABA* gamma-aminobutyric acid, *GiV* ventral gigantocellular reticular nucleus, *Gly* glycine, *Hcrt* hypocretin (orexin)-containing neurons, *His* histamine, *LC* locus coeruleus, *LdT* laterodorsal tegmental nucleus, *MCH* melanin-concentrating hormone-containing neurons, *NA* noradrenaline, *PH* posterior hypothalamus, *PPT* pedunculopontine tegmental nucleus, *PS* paradoxical sleep, *RT* reticular thalamic neurons, *SCN* suprachiasmatic nucleus, *SLD* sublaterodorsal nucleus, *SWS* slow-wave sleep, *TMN* tuberomamillary nucleus, *vlPAG* ventrolateral periaqueductal gray, *VLPO* ventrolateral preoptic nucleus, *W* waking

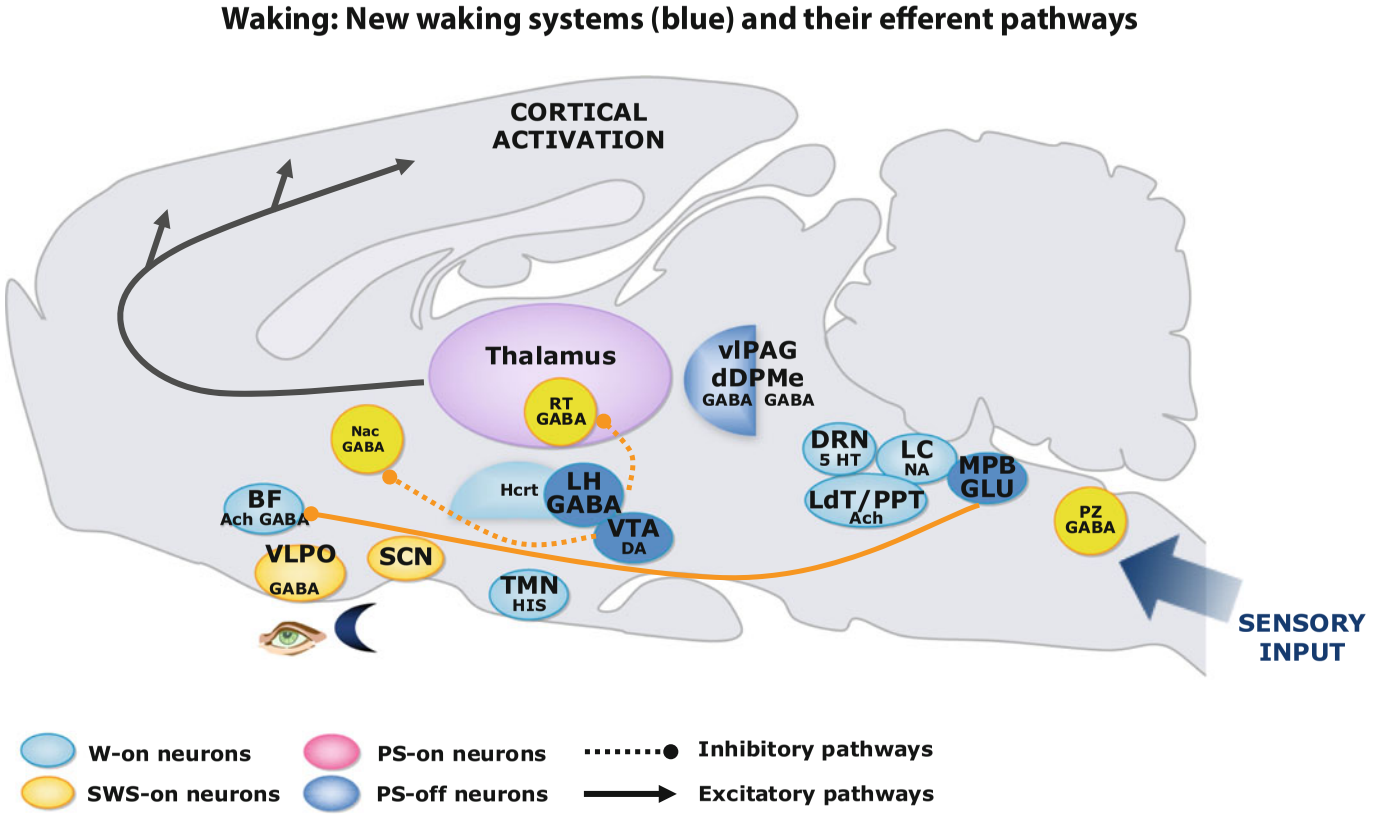

Fig. 2 New neuronal networks responsible for waking. Four new systems have been recently described for inducing waking. The dopaminergic (DA) neurons of the ventral tegmental area (VTA). These neurons would inhibit during waking GABAergic neurons of the nucleus accumbens (Nac). The GABAergic

VLPO neurons projecting to the TMN contain both galanin and glutamic acid decarboxylase (GAD), the GABA-synthesizing enzyme. In addition, electrical stimulation of the VLPO area evokes a GABA-mediated inhibition of TMN neurons, suggesting that VLPO and MnPn efferents to the wake-promoting systems are inhibitory (Fort et al. 2009). Finally, a new study demonstrated that optogenetic activation or inhibition of GABAergic neurons of the VLPO projecting to the TMN enhances or suppresses sleep (Chung et al. 2017). Further, they showed that these neurons are significantly more active during PS and SWS than W and contain cholecystokinin, corticotropin-releasing hormone, and tachykinin 1 (Chung et al. 2017).

3.2 Mechanisms Controlling the Activity of the Preoptic SWS-Inducing Neurons

Electrophysiological whole-cell recordings showed that VLPO contains neuronal groups with specific intrinsic membrane properties and distinct chemo-morphology and that they are inhibited by most of the waking neurotransmitters (Gallopin et al. 2000). Sleep-active neurons are GABAergic and galaninergic in nature and multipolar triangular shaped and exhibit a potent low-threshold calcium potential. These neurons are always inhibited by noradrenaline (NA), via postsynaptic alpha2-adrenoceptors. Interestingly, NA-inhibited neurons are also inhibited by acetylcholine, through muscarinic postsynaptic and nicotinic presynaptic actions on noradrenergic terminals. In contrast, histamine and hypocretin did not modulate the activity of the sleep-active neurons (Gallopin et al. 2000). Finally, serotonin induced either excitation (50%, Type 2) or inhibition (50%, Type 1) of VLPO neurons (Fort et al. 2009).

Homeostatic regulators, involving natural sleep-promoting factors accumulating during waking, play a crucial role in triggering sleep. Among these factors, prostaglandin D2 and adenosine have been functionally implicated in sleep, although their neuronal targets and mechanisms of action remain largely unknown. In this context, it has been shown that application of an adenosine A_{2A} receptor ($A_{2A}R$) agonist evoked direct excitatory effects specifically in 50% of the sleep-active neurons that are also activated by serotonin (Fort et al. 2009). Additional results further suggested that adenosine might directly activate VLPO neurons at sleep onset via an action on postsynaptic $A_{2A}R$. Indeed, pharmacological infusion of an $A_{2A}R$ agonist into the subarachnoid space rostral to the VLPO increases SWS and induces c-Fos

Fig. 2 (continued) neurons of the lateral hypothalamic area (LH) would inhibit during waking the GABAergic neurons of the reticular thalamic nucleus (RT). Finally, the glutamatergic (GLU) neurons of the medial parabrachial nucleus (MPB) would induce waking by their excitatory projection to the basal forebrain (BF) cholinergic and GABAergic neurons. The relationship between these systems as well as between them and the VLPO GABAergic neurons remains to be identified

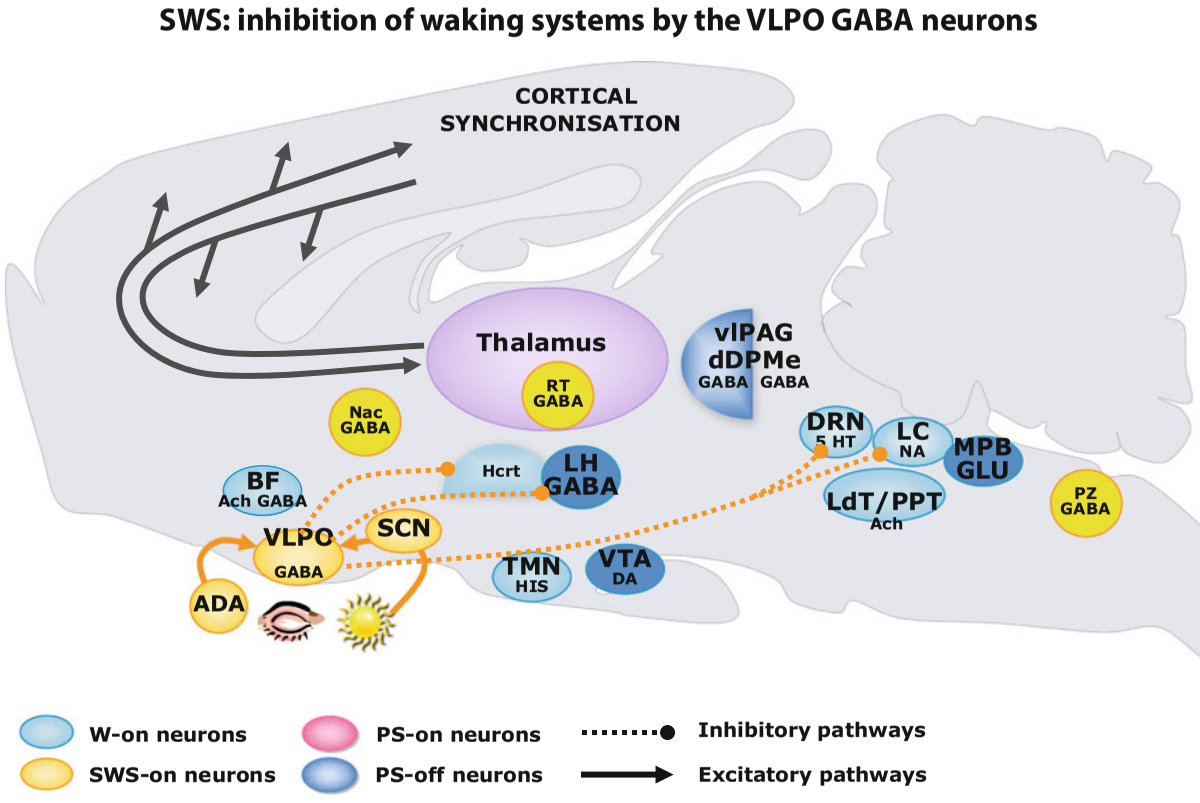

Fig. 3 Classical neuronal networks responsible for slow-wave (non-rapid eye movement [REM]) sleep. VLPO and MnPo GABAergic neurons would be inhibited by noradrenergic and cholinergic inputs during waking. The majority of them would start firing at sleep onset (drowsiness) in response to excitatory,

expression in VLPO neurons (Lazarus et al. 2011) (Fig. 3). It has also recently been shown that VLPO GABAergic neurons are excited by glucose (Varin et al. 2015). Further, injection of glucose in the VLPO promotes SWS and selectively increases the density of c-Fos+ neurons in the VLPO (Varin et al. 2015). These results suggest that glucose likely contributes to sleep onset facilitation by increasing the excitability of sleep-promoting GABAergic neurons in the VLPO.

Besides, a number of studies showed that adenosine A_1 receptors (A_1R) promote sleep through inhibition of the wake-promoting neurons, in particular cholinergic and hypocretin neurons (Porkka-Heiskanen et al. 2000). However, transgenic mice that lack A_1R exhibit normal homeostatic regulation of sleep. In contrast, the lack of $A_{2A}R$ prevents normal sleep regulation and blocks the wake-inducing effect of caffeine, suggesting that the activation of $A_{2A}R$ is crucial in SWS (Huang et al. 2005; Lazarus et al. 2017).

3.3 The Sleep GABAergic Neurons of the Nucleus Accumbens

Rather than through the VLPO, a recent study provides convincing evidence that the waking effect induced by caffeine is mediated by GABAergic neurons located in the nucleus accumbens and expressing adenosine $A_{2A}R$ and dopamine D2 receptors. Indeed, it has been shown that the induction of waking by caffeine was abolished in rats in which the expression of the $A_{2A}R$ mRNAs was removed from the shell of the nucleus accumbens (Lazarus et al. 2011). Further, chemogenetic or optogenetic activation of adenosine A2A receptor-expressing GABAergic neurons in the nucleus accumbens induces SWS, while their chemogenetic inhibition prevents sleep induction (Oishi et al. 2017). GABAergic neurons of the nucleus accumbens might induce sleep via their inhibitory projections to the waking systems such as the hypothalamic hypocretin and histaminergic neurons and the ventral tegmental dopaminergic and the noradrenergic locus coeruleus neurons (Lazarus et al. 2013). These observations are of great interest. Unit recording experiments are nevertheless necessary to demonstrate that adenosine A2A receptor-expressing GABAergic neurons of the nucleus accumbens are specifically active during sleep. Further, the effect on sleep of their optogenetic or pharmacogenetic manipulation needs to be determined.

Fig. 3 (continued) homeostatic (adenosine), and circadian drives (suprachiasmatic input). These activated neurons, through the reciprocal GABAergic inhibition of all wake-promoting systems, would be in a position to suddenly unbalance the "flip-flop" network, as required for switching from W (drowsiness) to a consolidation of SWS sleep. Conversely, the slow removal of excitatory influences would result in a progressive firing decrease in VLPO neurons and therefore an activation of wake-promoting systems leading to the awakening event

3.4 The Sleep GABAergic Neurons of the Thalamic Reticular Nucleus

It is well accepted that GABAergic reticular thalamic (RT) neurons are responsible for spindle generation during NREM sleep (Huguenard and McCormick 2007). Their repetitive spike bursts cause rhythmic inhibitory postsynaptic potentials in thalamocortical neurons. In turn, spike-burst activity in thalamocortical neurons due to a post-inhibitory rebound generates excitatory postsynaptic potentials in cortical cells (Steriade 1999). It has recently been shown that strong phasic optogenetic stimulation of RT neurons during SWS, unlike in the awake state, induces spindles (Halassa et al. 2011, 2014). In contrast, tonic and low-power activation of the RT induces slow waves and sleep in awake mice without affecting power in the spindle band (Lewis et al. 2015). In addition, RT inhibition reduces slow waves in mice during SWS (Lewis et al. 2015). Further, it has also recently been shown that silencing of RT GABAergic neurons using optogenetics during SWS induces rapid sleep-to-wake transitions (Herrera et al. 2016). Altogether, these results indicate that RT GABAergic neurons play a key role in sleep induction and maintenance, as well as the genesis of spindles and delta activity. It has been shown recently that during waking RT GABAergic neurons are inhibited by GABAergic neurons located in the lateral hypothalamic area, in addition to cholinergic, serotoninergic, noradrenergic, and histaminergic inhibitory inputs (Herrera et al. 2016). Optogenetic and/or pharmacogenetic experiments are still needed to determine the respective roles of each of the inhibitory inputs from the multiple waking systems, as well as the pathways and mechanisms responsible for the activation of RT GABAergic neurons during sleep.

3.5 The Sleep Nitric Oxide Synthase/GABAergic Neurons of the Cortex

Interestingly, a subpopulation of GABAergic cortical interneurons, which express the enzyme neuronal nitric oxide synthase (nNOS) and the neurokinin-1 receptor, has been found to be activated during SWS using c-Fos. The extent of activation of these nNOS neurons has been shown to be proportional to SWS sleep time, SWS bout duration, and EEG delta power during SWS, an index of homeostatic sleep drive (Gerashchenko et al. 2008; Morairty et al. 2013). Transgenic mice knockout for the nNOS gene shows reduced SWS time, shorter SWS bouts, and decreased power in the low delta range during SWS. These observations strongly suggest a role for nNos/GABAergic cortical interneurons in SWS regulation. Unit recording and specific optogenetic or pharmacogenetic manipulations of these neurons are now needed to confirm that they play a role in homeostatic sleep regulation and delta oscillations. Furthermore, the specific role of GABA in these interneurons still needs to be assessed using genetic inactivation.

3.6 The Sleep GABAergic Neurons of the Medullary Parafacial Zone

It has been recently shown that GABA/glycinergic neurons located lateral and dorsal to the descending branch of the facial nerve, a region termed the parafacial zone (PZ), could play a role in sleep induction and maintenance (Anaclet et al. 2012). Indeed, these inhibitory PZ neurons express c-Fos after sleep but not after waking, and lesions of these neurons induce an increase in waking (Anaclet et al. 2012). Their pharmacogenetic activation promotes SWS and inhibits both waking and PS for 3 h, whereas their inhibition strongly decreases SWS and PS (Anaclet et al. 2014). GABAergic PZ neurons directly inhibit glutamatergic neurons within the pontine medial parabrachial nucleus. Further, the glutamatergic medial parabrachial neurons project to and excite cortically projecting magnocellular basal forebrain neurons. Since lesions of the latter two structures induce a coma state, it has been proposed that GABA/glycinergic neurons of the parafacial zone would induce sleep by inhibiting this pontine-basal forebrain-cortical pathway (Anaclet et al. 2014). Altogether, these results are strongly in favor of a role for the GABA/glycinergic neurons located in the parafacial zone in the induction and maintenance of SWS. However, unit recordings of PZ neurons recently showed that they are not selectively active during SWS (Sakai 2017).

In summary, a number of new sleep-inducing systems have been recently identified in addition to the VLPO. It is of great importance to confirm that they are really involved in sleep control and to determine their specific roles in comparison with the classical preoptic SWS-inducing neurons.

3.7 Overview of the Neuronal Network Responsible for SWS (Non-REM) Sleep

A large body of evidence suggested that both the VLPO and the MnPn contain neurons responsible for sleep onset and maintenance. It has been proposed that during wakefulness, VLPO GABAergic neurons are inhibited by inputs from waking systems such as the noradrenergic and cholinergic inputs. Conversely, emergence from sleep would result from a rapid reactivation of arousal circuits, concomitant with the inhibition of VLPO neurons (Fort et al. 2009). However, the recent years have seen the discovery of new structures and pathways involved in sleep and waking such as the parafacial zone, reticular thalamic nucleus, nNOS GABAergic cortical interneurons, or the nucleus accumbens. We illustrate all pathways in Fig. 2. Further work is now necessary to find out the role of each of the pathways identified and the relationships among them (Fig. 4).

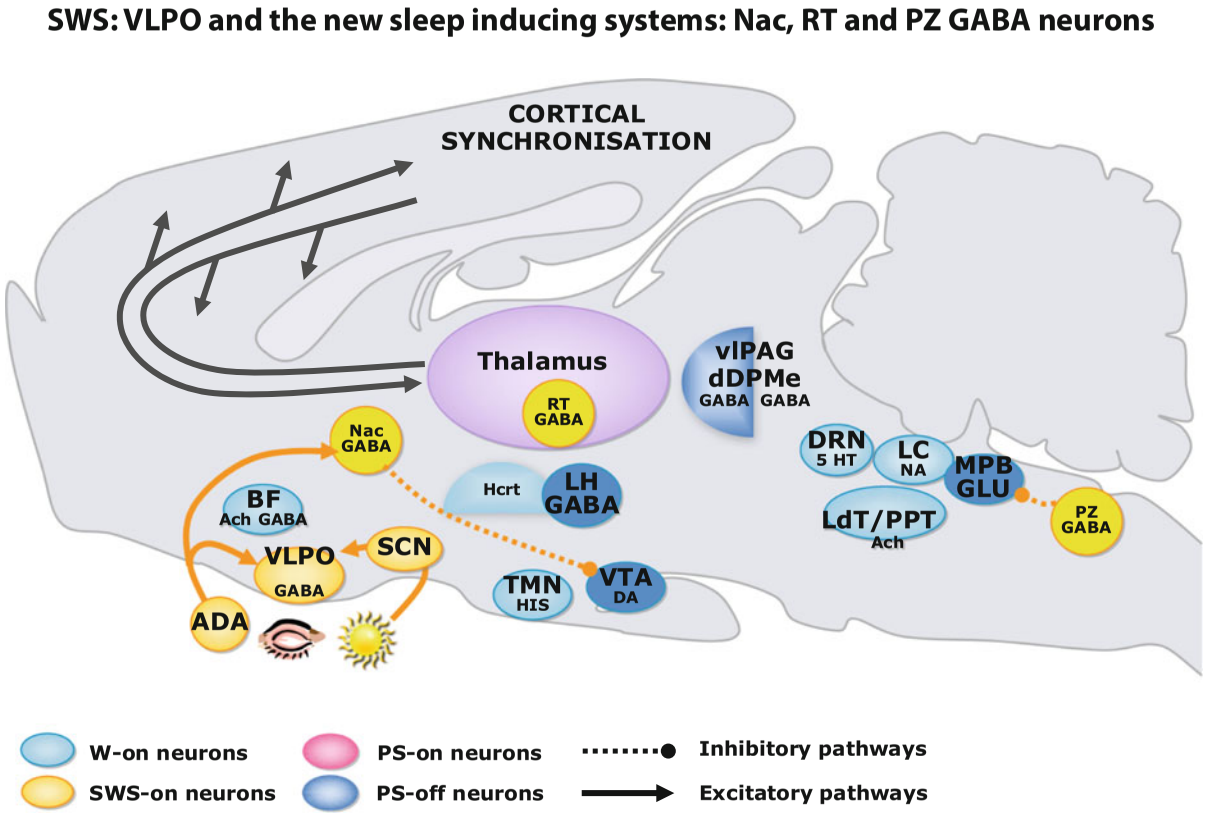

Fig. 4 New neuronal networks responsible for slow-wave (non-REM) sleep. Three new sleep-inducing systems have been identified. The GABAergic neurons of the nucleus accumbens (Nac) would induce sleep by means of their inhibitory projection to the dopaminergic neurons of the ventral tegmental area (VTA).

4 Mechanisms Involved in Paradoxical (REM) Sleep (PS) Genesis

4.1 The Neurons Generating PS Are Localized in the Pontine Reticular Formation

It was first shown that a state characterized by muscle atonia and rapid eye movements persists following decortication, cerebellar ablation, or brainstem transections rostral to the pons and in the "pontine cat," a preparation in which all the structures rostral to the pons have been removed (Jouvet 1962). These results suggested that brainstem structures are necessary and sufficient to trigger and maintain the state of PS. Electrolytic and chemical lesions showed that the dorsal part of pontis oralis (PnO) and caudalis (PnC) nuclei also named peri-locus coeruleus α (peri-LCα), pontine inhibitory area (PIA), and subcoeruleus nucleus (SubC) contains the neurons responsible for PS onset (Jouvet 1962). More recently, a corresponding area has been identified in rats and named the sublaterodorsal tegmental nucleus (SLD) (Boissard et al. 2002). It was also shown that bilateral injection in cats of a cholinergic agonist, carbachol, into the SLD dramatically increases PS quantities in cats (Vanni-Mercier et al. 1989). In addition, the SLD and the adjacent laterodorsal (LDT) and pedunculopontine tegmental (PPT) cholinergic nuclei contain many neurons that show a tonic firing selective to PS state (called "PS-on" neurons) (Sakai and Koyama 1996). From these early 1970s to 1980s studies, it was hypothesized that the PS-on neurons generating PS were cholinoceptive and cholinergic.

4.2 Paradoxical (REM) Sleep-Generating Neurons: The Switch from Acetylcholine to Glutamate

However, in contrast to cats, carbachol iontophoresis into the rat SLD failed to induce a significant increase in PS quantities (Boissard et al. 2002). Further, only a few cholinergic neurons were immunostained for c-Fos in the LDT, PPT, and SLD after PS hypersomnia (Verret et al. 2005). Finally, neurochemical lesions in rats of both the LDT and PPT had no effect on PS and cortical activation (Lu et al. 2006). It has been recently further demonstrated that most of the c-Fos-positive neurons localized in the SLD after PS recovery express vGlut2 (Clement et al. 2011),

Fig. 4 (continued) They would be driven by the increase of adenosine excitation through A2a adenosine receptors. The GABAergic neurons of the reticular thalamic nucleus would also be activated when the wake-active GABAergic neurons of the lateral hypothalamic area stop to inhibit them. They would induce SWS by means of their inhibitory projections to the other thalamic nuclei. The parafacial (PZ) GABAergic neurons would induce sleep by means of their inhibition of the glutamatergic neurons of the medial parabrachial nucleus (MPB). The mechanisms responsible for their activation remain to be identified

suggesting that the PS-on SLD neurons triggering PS are glutamatergic. Confirming this hypothesis, we recently showed that genetic inactivation of glutamatergic transmission in SLD neurons induces a 30% decrease of PS quantities and the abolition of muscle atonia and the occurrence of violent movements (Valencia Garcia et al. 2017).

A number of recent results further suggest that PS-on glutamatergic neurons located in the SLD generate muscle atonia via descending projections to PS-on GABA/glycinergic premotoneurons located at medullary level rather than directly in the spinal cord. First, using intracellular recordings during PS, it has been shown that trigeminal, hypoglossal, and spinal motoneurons are tonically hyperpolarized by large inhibitory postsynaptic potentials (IPSPs) during PS (Chase et al. 1989). Furthermore, local iontophoretic application of strychnine (a specific antagonist of the inhibitory neurotransmitter, glycine) decreased the hyperpolarization of motoneurons, suggesting that they are tonically inhibited by glycinergic neurons during PS (Chase et al. 1989). Interestingly, it has then been shown that the levels of glycine but also that of GABA increase within hypoglossal and spinal motor pools during PS-like atonia suggesting that GABA, in addition to glycine, might contribute to motoneurons hyperpolarization during PS (Kodama et al. 2003). Finally, it has been recently shown that combined microdialysis of bicuculline, strychnine, and phaclophen (a GABA-B antagonist) in the trigeminal nucleus is necessary to restore jaw muscle tone during PS (Brooks and Peever 2012) and that mice with impaired glycinergic and GABAergic transmissions display PS without muscle atonia (Brooks and Peever 2011).

In addition, SLD neurons send direct efferent projections to GABA/glycinergic medullary neurons located in the nucleus raphe magnus (RMg) and the ventral (GiV), alpha (Gia) gigantocellular, and lateral paragigantocellular (LPGi) reticular nuclei (Boissard et al. 2002; Valencia Garcia et al. 2017). Besides, GABA/glycinergic neurons of the Gia, GiV, LPGi, and RMg express c-Fos after induction of PS by bicuculline (Bic, a GABA-A antagonist) injection in the SLD (Boissard et al. 2002). In addition, nearly all c-Fos-labeled neurons localized in these nuclei after 3 h of PS hypersomnia following 72 h of PS deprivation express GAD67 mRNA (Sapin et al. 2009).

In view of all these results, it can be proposed that the SLD glutamatergic PS-on neurons induce muscle atonia during PS by means of direct projections to medullary RMg/GiA/GiV/LPGi GABA/glycine neurons. These neurons hyperpolarize motoneurons mainly using glycine but also to a minor extent GABA acting on GABA-A and GABA-B receptors.

It has finally also been shown that cholinergic and non-cholinergic neurons located in the SLD and pedunculopontine and laterodorsal tegmental nuclei and glutamatergic neurons located in the reticular formation are active both during waking and PS and project rostrally to the thalamus and hypothalamus and play a role in cortical activation during PS (Boissard et al. 2002; Fort et al. 2009). In addition to these systems, we recently demonstrated that only a few limbic cortical structures including the dentate gyrus, the anterior cingulate, and retrosplenial (Koike et al. 2017) and medial entorhinal cortices are activated during PS (Renouard

et al. 2015). We further showed that the lateral part of the supramammillary nucleus activates the dentate granule cells during PS, while the claustrum activates the other cortical structures (Renouard et al. 2015; Billwiller et al. 2016). The link between these structures and the other nuclei generating PS remains to be identified.

4.3 Mechanisms Responsible for the Activation of SLD PS-on Neurons During PS

In cats and rats, microdialysis administration in the SLD of kainic acid, a glutamate agonist, induces a PS-like state (Boissard et al. 2002). A long-lasting PS-like hypersomnia can also be pharmacologically induced with a short latency in head-restrained unanesthetized rats by iontophoretic application into the SLD of bicuculline or gabazine, two GABA-A receptor antagonists (Boissard et al. 2002). Further, application of kynurenate, a glutamate antagonist, reverses the PS-like state induced by bicuculline (Boissard et al. 2002). In the head-restrained rat, neurons within the SLD specifically active during PS and excited following bicuculline or gabazine iontophoresis have been recorded. Taken together, these data indicate that the activation of SLD PS-on neurons is mainly due to the removal during PS of a tonic GABAergic tone present during W and SWS combined with the continuous presence of a glutamatergic input. Combining retrograde tracing, c-Fos, and glutamate decarboxylase 67 (GAD67) staining, it was recently demonstrated that the ventrolateral part of the periaqueductal gray (vlPAG) and the adjacent dorsal part of the deep mesencephalic nucleus (dDPMe) are the only ponto-medullary structures containing a large number of GABAergic neurons activated during PS deprivation and projecting to the SLD (Sapin et al. 2009). Further, injection of a GABAa agonist (muscimol) in the vlPAG and/or the dDpMe induces strong increases in PS quantities in cats (Sastre et al. 1996) and rats (Sapin et al. 2009). In addition, neurochemical lesion of these two structures induces profound increases in PS quantities (Lu et al. 2006). Further, optogenetic stimulation of PS-off GABAergic neurons within the vlPAG and the dDpMe almost completely suppresses PS onset and shortens the duration of PS episodes (Weber et al. 2015). DREADD activation of vlPAG/dDPMe GABAergic neurons reduces PS and increases SWS (Hayashi et al. 2015). Conversely, their pharmacogenetic inhibition increases PS, mainly by increasing the number of PS episodes (Hayashi et al. 2015). These congruent experimental studies strongly support our hypothesis that PS-off GABAergic neurons located in the vlPAG and the dDpMe gate PS by tonically inhibiting PS-on neurons of the SLD during W and SWS. Our results indicate that these GABAergic neurons are crucial to gate PS although they do not rule out a secondary role for monoaminergic neurons since increases in monoaminergic transmission either by reuptake blockers or agonists is well known to inhibit PS (Luppi et al. 2011). Monoamines are known to project to most of brain structures including those controlling PS (Dahlström 1964). It is therefore challenging to identify the targets of monoaminergic neurons responsible for their inhibitory effects on PS. They can either excite PS-off neurons or inhibit PS-on neurons. One possibility is that the

monoaminergic neurons are exciting the GABAergic vlPAG and dDpMe PS-off neurons during waking to preclude PS onset.

4.4 Neurons Inhibiting the GABAergic and Monoaminergic PS-off Neurons at the Onset and During PS

It has been previously reported that bicuculline application on serotonergic and noradrenergic neurons during SWS or PS restores a tonic firing pattern in both types of neurons (Gervasoni et al. 1998, 2000). These results strongly suggest that an increased GABA release is responsible for the PS-selective inactivation of monoaminergic neurons. This hypothesis is well supported by microdialysis experiments in cats, which measured a significant increase in GABA release in the dorsal raphe (DRN) and locus coeruleus (LC) during PS as compared to W and SWS but no detectable changes in glycine concentration (Nitz and Siegel 1997a, b).

By combining retrograde tracing with CTb and GAD immunohistochemistry in rats, it has been found that the vlPAG and the dorsal and lateral paragigantocellular nuclei (DPGi and LPGi) (Gervasoni et al. 2000) contained numerous GABAergic neurons projecting both to the DRN and LC. It has then demonstrated by combining c-Fos and retrograde labeling that these nuclei contain numerous LC-projecting neurons selectively activated during PS rebound following PS deprivation (Verret et al. 2006). Further, it has been found that the DPGi contains numerous PS-on neurons that are increasing their activity specifically during PS (Luppi et al. 2011). Taken together, these data strongly suggest that the DPGi and the LPGi contains the neurons responsible for the inactivation of LC noradrenergic neurons during PS (Luppi et al. 2011). A contribution from the vlPAG in the inhibition during PS of LC noradrenergic and dorsal raphe serotonergic neurons is also likely. Indeed, an increase in the number of c-Fos/GAD immunoreactive neurons has been reported in the vlPAG after a PS rebound following deprivation in rats (Sapin et al. 2009). In summary, a large body of data indicates that GABAergic PS-on neurons localized in the vlPAG and the LPGi/DPGi hyperpolarize the monoaminergic neurons during PS.

It has been first proposed that these neurons might also be responsible of the inhibition of the dDPMe/vlPAG PS-off GABAergic neurons during PS. To test this hypothesis, neurons active during PS hypersomnia projecting to the dDPMe/vlPAG PS-off GABAergic neurons were localized (Clement et al. 2012). It has been found out that the vlPAG and the DPGi, respectively, contained a substantial and a small number of CTb/c-Fos double-labeled neurons in PS hypersomniac rats. Although, the GABAergic nature of these neurons remains to be demonstrated, our results indicate that the vlPAG and the DPGi might contain PS-on GABAergic neurons inhibiting the vlPAG/dDPMe PS-off GABAergic neurons at the onset and during PS. At variance with our hypothesis, it has recently been proposed that ascending

PS-on neurons located in the LPGi play a key role in that matter. Indeed, optogenetic stimulation during SWS of ascending fibers of GABAergic LPGi neurons located in the vlPAG/dDPMe induced PS (Weber et al. 2015). However, we did not find in the LPGi neurons activated during PS hypersomnia and projecting to the vlPAG/dDPMe (Clement et al. 2012). One possibility is that the fibers projecting to the LC were stimulated in Weber's experiments. We further demonstrated that the lateral hypothalamic area (LH) is the only brain structure containing a very large number of neurons activated during PS hypersomnia and projecting to the VLPAG/dDpMe (Clement et al. 2012). We further demonstrated that 44% of these neurons express the neuropeptide melanin-concentrating hormone (MCH). These results indicate that LH hypothalamic neurons might play a crucial role in PS onset and maintenance by means of descending projections to the vlPAG/dDPMe PS-off GABAergic neurons (Clement et al. 2012). They confirmed previous data discussed below indicating that the posterior hypothalamus contains neurons implicated in PS control.

4.5 Role of the MCH/GABAergic Neurons of the Lateral Hypothalamic Area in PS Control

To localize all the brain areas activated during PS, an extensive mapping of the distribution of c-Fos-positive neurons in control rats, rats selectively deprived of PS for 72 h and rats allowed to recover from such deprivation, has been done (Verret et al. 2003). Surprisingly, a very large number of c-Fos-positive cells were observed in the posterior hypothalamus (PH), including zona incerta (ZI), perifornical area (PeF), and the lateral hypothalamic area (LH). This result is in agreement with the fact that (1) bilateral injection of muscimol in the cat mammillary and tuberal hypothalamus induce a drastic inhibition of PS (Lin et al. 1989) and (2) neurons specifically active during PS were recorded in the posterior hypothalamus of cats or head-restrained rats (Goutagny et al. 2005). By using double immunostaining, it has been further shown that around 75% of posterior hypothalamus cells labeled for c-Fos after PS rebound express GAD67 mRNA and are therefore GABAergic (Sapin et al. 2010). One third of these GABAergic neurons were also immunoreactive for the neuropeptide, MCH. Almost 60% of all the MCH-immunoreactive neurons counted in PeF, ZI, and LHA were c-Fos-positive (Verret et al. 2003). It has been recently demonstrated that these neurons co-express the peptide nesfatin (Jego et al. 2012). In support of our c-Fos data, it has then been shown in head-restrained rats that MCH neurons fire exclusively during PS (Hassani et al. 2009). Importantly, MCH neurons start to fire at the onset of PS and, therefore, can play a role in PS maintenance but not in PS induction. Nevertheless, rats receiving intracerebroventricular (icv) administration of MCH showed a strong dose-dependent increase in PS and, to a minor extent, SWS quantities, possibly due to an increased number of PS bouts (Verret et al. 2003). Further, subcutaneous injection of an MCH antagonist

decreases SWS and PS quantities (Ahnaou et al. 2008). The increase in sleep induced by MCH system activation was recently confirmed using chronic (24 h) optogenetic activation (Konadhode et al. 2013). Of interest, mice with genetically inactivated MCH signaling exhibit altered vigilance state architecture and sleep homeostasis (Jego et al. 2013). In addition, disruption of nesfatin-1 signaling by icv administration of nesfatin-1 antiserum or antisense against the nucleobindin2 (NUCB2) prohormone suppressed PS, whereas the infusion of nesfatin-1 antiserum after a selective PS deprivation precluded PS recovery (Jego et al. 2012). Finally, it has been recently shown that optogenetic activation of MCH neurons specifically during SWS episodes did not increase SWS duration but increase the probability of SWS-to-PS transitions PS. Further, optogenetic stimulation of MCH neurons during PS significantly prolonged the duration of PS episodes (Jego et al. 2013). In agreement with our results showing that MCH neurons constitute only one third of the GABAergic neurons activated during PS hypersomnia, it has been recently shown that a large population of GABAergic neurons, but not expressing MCH, are localized in the LH area discharge maximally during PS (Hassani et al. 2010). These neurons are mostly silent during active W with high muscle tone and progressively increase their discharge from quiet W through SWS to be maximally active during PS. Since these neurons anticipate PS onset, they could play a role in triggering PS state. Supporting this hypothesis, it has recently been shown that a genetic activation and inhibition of a subset of the GABAergic neurons of the zona incerta which express the LIM homeodomain factor Lhx6 of Lhx6-positive bidirectionally regulate PS in adult mice (Liu et al. 2017).

To determine the function of the MCH+/GABA+ and MCH−/GABA+ neurons in PS control, all lateral hypothalamic area neurons were inactivated with muscimol (a GABA-A agonist) or only those bearing alpha-2 adrenergic receptors using clonidine. Such inactivation induced an inhibition of PS with or without an increase in SWS quantities, respectively. It has been further shown that after muscimol injection in the LH, the vlPAG/dDpMe region contains a large number of c-FOS/GAD67+ and of c-FOS/CTb+ neurons in animals with a CTb injection in the SLD. These results indicate that the activation of PS-on MCH/GABAergic neurons localized in the LH is a necessary step for PS to occur. They further suggest that MCH/GABAergic PS-on neurons localized in the LH control PS onset and maintenance by means of a direct inhibitory projection to vlPAG/dDpMe PS-off GABAergic neurons. From these results, it can be proposed that MCH/GABAergic neurons of the LH constitute a “master” generator of PS that controls a “slave” generator located in the brainstem. To reconcile the Jouvet hypothesis (i.e., brainstem is necessary and sufficient to generate a state characterized by muscle atonia and REM, Jouvet 1962) with these new results, it can be proposed that after removal of the forebrain, the brainstem generator is sufficient to induce a state with muscle atonia and REM by means of a reorganization of the brainstem systems generating PS. However, the brainstem generator would be under control of the LH generator in intact animals.

In addition to the descending pathway to the PS-off GABAergic neurons, the MCH/GABAergic PS-on neurons might also promote PS by means of other pathways to the histaminergic neurons, the monoaminergic PS-off neurons, and the hypocretinergic neurons (Boissard et al. 2002; Jego et al. 2013; Liu et al. 2017). Indeed, Jego et al. (2013) recently showed that optogenetic activation of MCH neurons inhibit postsynaptic targets, including histaminergic cells in the TMN, through activation of GABAA receptor.

The mechanisms at the origin of the activation of the MCH/GABAergic neurons of the LH at the entrance into PS remain to be identified. A large number of studies indicate that MCH neurons also play a key role in metabolism control. Therefore, the activation of these neurons at the onset and during PS could be influenced by the metabolic state. In addition, it is likely that yet undiscovered endogenous cellular or molecular clocklike mechanisms may play a role in their activation.

The cessation of activity of the MCH/GABAergic PS-on neurons and more largely of all the PS-on neurons at the end of PS episodes may be due to a different mechanism than the entrance into the state, possibly the reactivation of the waking systems, which are known to silence MCH neurons in vitro (van den Pol et al. 2004). Indeed, animals are entering PS slowly from SWS, while in contrast they exit from it abruptly by a micro-arousal. This indicates that the end of PS episodes is induced by the activation of the W systems like the monoaminergic, hypocretin, or the histaminergic neurons. However, the precise mechanisms responsible for their activation remain to be identified.

4.6 A Network Model for PS Onset and Maintenance (Fig. 5)

The onset of PS would be due to the activation of PS-on MCH/GABAergic neurons localized in the lateral hypothalamic area (LH) by intrinsic and extrinsic factors. At PS onset, these neurons would inhibit the PS-off GABAergic neurons localized in the vlPAG and the dDpMe tonically inhibiting during W and SWS the glutamatergic PS-on neurons from the SLD. The disinhibited descending glutamatergic PS-on SLD neurons would induce muscle atonia via their excitatory projections to GABA/glycinergic premotoneurons localized in the raphe magnus, alpha, and ventral gigantocellular reticular nuclei. PS-on GABAergic neurons localized in the LH (including the MCH neurons), DPGi, and vlPAG would also inactivate the PS-off orexin (hypocretin) and aminergic neurons during PS. The exit from PS would be due to the activation of waking/arousal systems since PS episodes are almost always terminated by a transition to wakefulness state. The waking systems would reciprocally inhibit the GABAergic PS-on neurons localized in the LH, vlPAG, and DPGi. Since the duration of PS is negatively coupled with the metabolic rate, it can be proposed that the activity of the waking systems is triggered to end PS to restore crucial physiological parameters like thermoregulation.

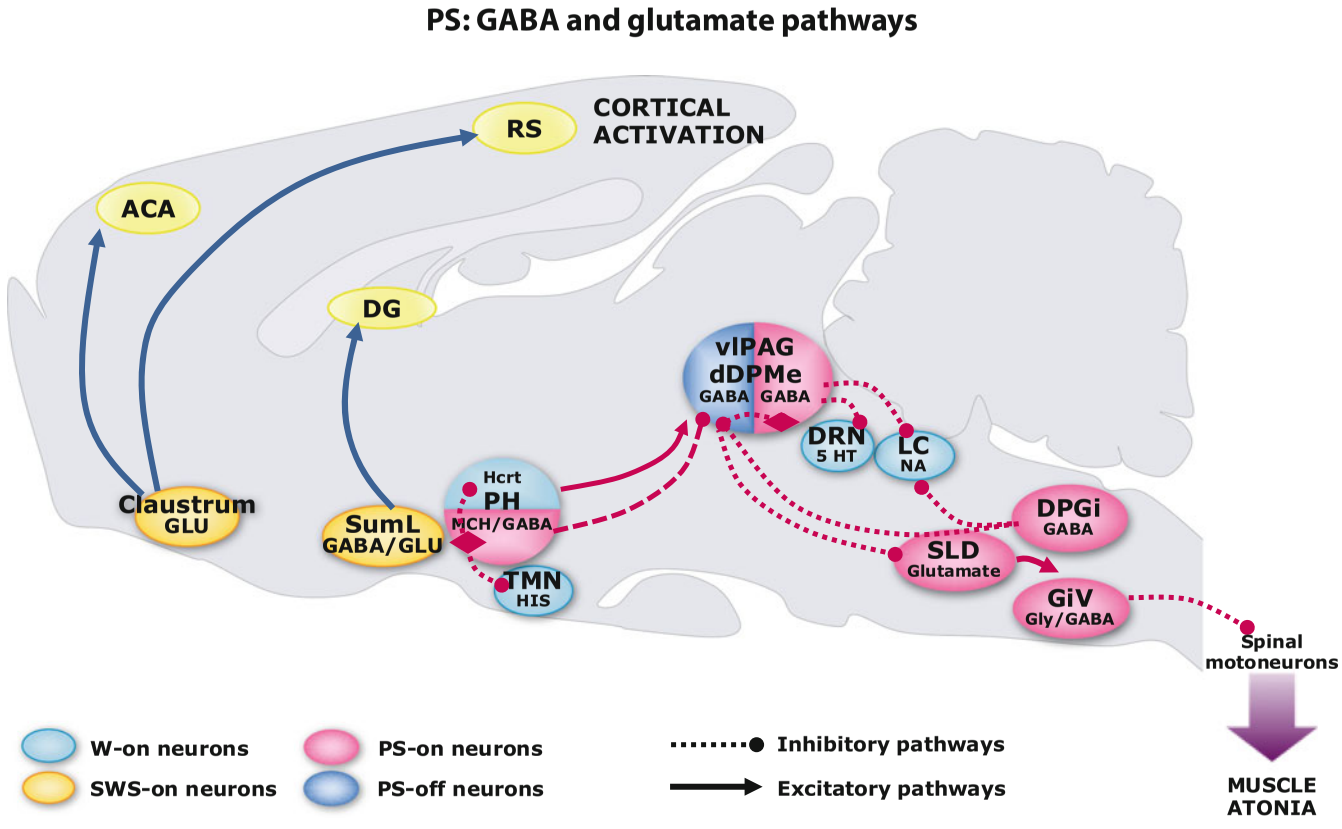

Fig. 5 Neuronal networks responsible for paradoxical (REM) sleep (PS). PS onset would be due to the activation of glutamatergic PS-on neurons localized in the sublaterodorsal tegmental nucleus (SLD). During waking (W) and SWS (non-REM) sleep, these PS-on neurons would be inhibited by a tonic inhibitory

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References

Ahnaou A, Drinkenburg WH, Bouwknecht JA, Alcazar J, Steckler T, Dautzenberg FM (2008) Blocking melanin-concentrating hormone MCH1 receptor affects rat sleep-wake architecture. Eur J Pharmacol 579:177–188

Anaclet C, Lin JS, Vetrivelan R, Krenzer M, Vong L, Fuller PM, Lu J (2012) Identification and characterization of a sleep-active cell group in the rostral medullary brainstem. J Neurosci 32:17970–17976

Anaclet C, Ferrari L, Arrigoni E, Bass CE, Saper CB, Lu J, Fuller PM (2014) The GABAergic parafacial zone is a medullary slow wave sleep-promoting center. Nat Neurosci 17:1217–1224

Billwiller F, Renouard L, Clement O, Fort P, Luppi PH (2016) Differential origin of the activation of dorsal and ventral dentate gyrus granule cells during paradoxical (REM) sleep in the rat. Brain Struct Funct 222(3):1495–1507

Boissard R, Gervasoni D, Schmidt MH, Barbagli B, Fort P, Luppi PH (2002) The rat ponto-medullary network responsible for paradoxical sleep onset and maintenance: a combined microinjection and functional neuroanatomical study. Eur J Neurosci 16:1959–1973

Brooks PL, Peever JH (2011) Impaired GABA and glycine transmission triggers cardinal features of rapid eye movement sleep behavior disorder in mice. J Neurosci 31:7111–7121

Brooks PL, Peever JH (2012) Identification of the transmitter and receptor mechanisms responsible for REM sleep paralysis. J Neurosci 32:9785–9795

Chase MH, Soja PJ, Morales FR (1989) Evidence that glycine mediates the postsynaptic potentials that inhibit lumbar motoneurons during the atonia of active sleep. J Neurosci 9:743–751

Chung S, Weber F, Zhong P, Tan CL, Nguyen TN, Beier KT, Hormann N, Chang WC, Zhang Z, Do JP, Yao S, Krashes MJ, Tasic B, Cetin A, Zeng H, Knight ZA, Luo L, Dan Y (2017) Identification of preoptic sleep neurons using retrograde labelling and gene profiling. Nature 545:477–481

Fig. 5 (continued) GABAergic tone originating from PS-off neurons localized in the ventrolateral periaqueductal gray (vlPAG) and the dorsal part of the deep mesencephalic nucleus (dDpMe). These neurons would be activated during W by the hypocretin (Hcrt) neurons and the monoaminergic neurons. The onset of PS would be due to the activation by intrinsic mechanisms of PS-on GABAergic neurons localized in the posterior lateral hypothalamic area, the dorsal paragigantocellular reticular nucleus (DPGi), and the vlPAG. These neurons would also inactivate the PS-off monoaminergic neurons during PS. The disinhibited descending SLD PS-on neurons would in turn induce muscle atonia via their excitatory projections to glycinergic premotoneurons localized in the alpha and ventral gigantocellular reticular nuclei (Gia and GiV). The cortical activation during PS would be restricted to a few limbic structures including the retrosplenial (RS) and anterior cingulate (ACA) cortices and the dentate gyrus (DG). The cortices would be activated by glutamatergic neurons of the claustrum (CLA). The dentate granule cells would be activated by GABA/glutamate neurons localized in the lateral part of the supramammillary nucleus (SumL). The exit from PS would be due to the activation of waking systems since PS episodes are almost always terminated by an arousal. The waking systems would inhibit the GABAergic PS-on neurons localized in the DPGi and vlPAG. Since the duration of PS is negatively coupled with the metabolic rate, it can be proposed that the activity of the waking systems is triggered to end PS to restore competing physiological parameters like thermoregulation

Clement O, Sapin E, Berod A, Fort P, Luppi PH (2011) Evidence that neurons of the sublaterodorsal tegmental nucleus triggering paradoxical (REM) sleep are glutamatergic. Sleep 34:419–423

Clement O, Sapin E, Libourel PA, Arthaud S, Brischoux F, Fort P, Luppi PH (2012) The lateral hypothalamic area controls paradoxical (REM) sleep by means of descending projections to brainstem GABAergic neurons. J Neurosci 32:16763–16774

Dahlström AFK (1964) Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta Physiol Scand 232:1–55

Eban-Rothschild A, Rothschild G, Giardino WJ, Jones JR, de Lecea L (2016) VTA dopaminergic neurons regulate ethologically relevant sleep-wake behaviors. Nat Neurosci 19:1356–1366

Fort P, Bassetti CL, Luppi PH (2009) Alternating vigilance states: new insights regarding neuronal networks and mechanisms. Eur J Neurosci 29:1741–1753

Gallopin T, Fort P, Eggermann E, Cauli B, Luppi PH, Rossier J, Audinat E, Muhlethaler M, Serafin M (2000) Identification of sleep-promoting neurons in vitro. Nature 404:992–995

Gerashchenko D, Wisor JP, Burns D, Reh RK, Shiromani PJ, Sakurai T, de la Iglesia HO, Kilduff TS (2008) Identification of a population of sleep-active cerebral cortex neurons. Proc Natl Acad Sci U S A 105:10227–10232

Gervasoni D, Darracq L, Fort P, Souliere F, Chouvet G, Luppi PH (1998) Electrophysiological evidence that noradrenergic neurons of the rat locus coeruleus are tonically inhibited by GABA during sleep. Eur J Neurosci 10:964–970

Gervasoni D, Peyron C, Rampon C, Barbagli B, Chouvet G, Urbain N, Fort P, Luppi PH (2000) Role and origin of the GABAergic innervation of dorsal raphe serotonergic neurons. J Neurosci 20:4217–4225

Goutagny R, Luppi PH, Salvert D, Gervasoni D, Fort P (2005) GABAergic control of hypothalamic melanin-concentrating hormone-containing neurons across the sleep-waking cycle. Neuroreport 16:1069–1073

Gvilia I, Turner A, McGinty D, Szymusiak R (2006) Preoptic area neurons and the homeostatic regulation of rapid eye movement sleep. J Neurosci 26:3037–3044

Halassa MM, Siegle JH, Ritt JT, Ting JT, Feng G, Moore CI (2011) Selective optical drive of thalamic reticular nucleus generates thalamic bursts and cortical spindles. Nat Neurosci 14:1118–1120

Halassa MM, Chen Z, Wimmer RD, Brunetti PM, Zhao S, Zikopoulos B, Wang F, Brown EN, Wilson MA (2014) State-dependent architecture of thalamic reticular subnetworks. Cell 158:808–821

Hassani OK, Lee MG, Jones BE (2009) Melanin-concentrating hormone neurons discharge in a reciprocal manner to orexin neurons across the sleep-wake cycle. Proc Natl Acad Sci U S A 106:2418–2422

Hassani OK, Henny P, Lee MG, Jones BE (2010) GABAergic neurons intermingled with orexin and MCH neurons in the lateral hypothalamus discharge maximally during sleep. Eur J Neurosci 32(3):448–457

Hayashi Y, Kashiwagi M, Yasuda K, Ando R, Kanuka M, Sakai K, Itohara S (2015) Cells of a common developmental origin regulate REM/non-REM sleep and wakefulness in mice. Science 350:957–961

Herrera CG, Cadavieco MC, Jego S, Ponomarenko A, Korotkova T, Adamantidis A (2016) Hypothalamic feedforward inhibition of thalamocortical network controls arousal and consciousness. Nat Neurosci 19:290–298

Huang ZL, Qu WM, Eguchi N, Chen JF, Schwarzschild MA, Fredholm BB, Urade Y, Hayaishi O (2005) Adenosine A2A, but not A1, receptors mediate the arousal effect of caffeine. Nat Neurosci 8:858–859

Huguenard JR, McCormick DA (2007) Thalamic synchrony and dynamic regulation of global forebrain oscillations. Trends Neurosci 30:350–356

Jego S, Salvert D, Renouard L, Mori M, Goutagny R, Luppi PH, Fort P (2012) Tuberal hypothalamic neurons secreting the satiety molecule Nesfatin-1 are critically involved in paradoxical (REM) sleep homeostasis. PLoS One 7:e52525

Jego S, Glasgow SD, Herrera CG, Ekstrand M, Reed SJ, Boyce R, Friedman J, Burdakov D, Adamantidis AR (2013) Optogenetic identification of a rapid eye movement sleep modulatory circuit in the hypothalamus. Nat Neurosci 16(11):1637–1643

Jouvet M (1962) Recherches sur les structures nerveuses et les mécanismes responsables des différentes phases du sommeil physiologique. Arch Ital Biol 100:125–206

Kodama T, Lai YY, Siegel JM (2003) Changes in inhibitory amino acid release linked to pontine-induced atonia: an in vivo microdialysis study. J Neurosci 23:1548–1554

Koike BDV, Farias KS, Billwiller F, Almeida-Filho D, Libourel PA, Tiran-Cappello A, Parmentier R, Blanco W, Ribeiro S, Luppi PH, Queiroz CM (2017) Electrophysiological evidence that the retrosplenial cortex displays a strong and specific activation phased with hippocampal theta during paradoxical (REM) sleep. J Neurosci 37:8003–8013

Konadhode RR, Pelluru D, Blanco-Centurion C, Zayachkivsky A, Liu M, Uhde T, Glen WB Jr, van den Pol AN, Mulholland PJ, Shiromani PJ (2013) Optogenetic stimulation of MCH neurons increases sleep. J Neurosci 33:10257–10263

Lazarus M, Shen HY, Cherasse Y, Qu WM, Huang ZL, Bass CE, Winsky-Sommerer R, Semba K, Fredholm BB, Boison D, Hayaishi O, Urade Y, Chen JF (2011) Arousal effect of caffeine depends on adenosine A2A receptors in the shell of the nucleus accumbens. J Neurosci 31:10067–10075

Lazarus M, Chen JF, Urade Y, Huang ZL (2013) Role of the basal ganglia in the control of sleep and wakefulness. Curr Opin Neurobiol 23:780–785

Lazarus M, Chen JF, Huang ZL, Urade Y, Fredholm BB (2017) Adenosine and sleep. Handb Exp Pharmacol. https://doi.org/10.1007/164_2017_36

Lewis LD, Voigts J, Flores FJ, Schmitt LI, Wilson MA, Halassa MM, Brown EN (2015) Thalamic reticular nucleus induces fast and local modulation of arousal state. elife 4:e08760

Lin JS, Sakai K, Vanni-Mercier G, Jouvet M (1989) A critical role of the posterior hypothalamus in the mechanisms of wakefulness determined by microinjection of muscimol in freely moving cats. Brain Res 479:225–240

Liu K, Kim J, Kim DW, Zhang YS, Bao H, Denaxa M, Lim SA, Kim E, Liu C, Wickersham IR, Pachinis V, Hattar S, Song J, Brown SP, Blackshaw S (2017) Lhx6-positive GABA-releasing neurons of the zona incerta promote sleep. Nature 548:582–587

Lu J, Sherman D, Devor M, Saper CB (2006) A putative flip-flop switch for control of REM sleep. Nature 441:589–594

Luppi PH, Clement O, Sapin E, Gervasoni D, Peyron C, Leger L, Salvert D, Fort P (2011) The neuronal network responsible for paradoxical sleep and its dysfunctions causing narcolepsy and rapid eye movement (REM) behavior disorder. Sleep Med Rev 15:153–163

Morairty SR, Dittrich L, Pasumarthi RK, Valladao D, Heiss JE, Gerashchenko D, Kilduff TS (2013) A role for cortical nNOS/NK1 neurons in coupling homeostatic sleep drive to EEG slow wave activity. Proc Natl Acad Sci U S A 110:20272–20277

Nitz D, Siegel J (1997a) GABA release in the dorsal raphe nucleus: role in the control of REM sleep. Am J Phys 273:R451–R455

Nitz D, Siegel JM (1997b) GABA release in the locus coeruleus as a function of sleep/wake state. Neuroscience 78:795–801

Oishi Y, Xu Q, Wang L, Zhang BJ, Takahashi K, Takata Y, Luo YJ, Cherasse Y, Schiffmann SN, de Kerchove d'Exaerde A, Urade Y, Qu WM, Huang ZL, Lazarus M (2017) Slow-wave sleep is controlled by a subset of nucleus accumbens core neurons in mice. Nat Commun 8:734

Porkka-Heiskanen T, Strecker RE, McCarley RW (2000) Brain site-specificity of extracellular adenosine concentration changes during sleep deprivation and spontaneous sleep: an in vivo microdialysis study. Neuroscience 99:507–517

Renouard L, Billwiller F, Ogawa K, Clément O, Camargo N, Abdelkarim M, Gay N, Scoté-Blachon C, Touré R, Libourel P-A, Ravassard P, Salvert D, Peyron C, Claustrat B,

Léger L, Salin P, Malleret G, Fort P, Luppi P-H (2015) The supramammillary nucleus and the claustrum activate the cortex during REM sleep. Sci Adv 1:e1400177

Sakai K (2017) Are there sleep-promoting neurons in the mouse parafacial zone? Neuroscience 367:98–109

Sakai K, Koyama Y (1996) Are there cholinergic and non-cholinergic paradoxical sleep-on neurones in the pons? Neuroreport 7:2449–2453

Sapin E, Lapray D, Berod A, Goutagny R, Leger L, Ravassard P, Clement O, Hanriot L, Fort P, Luppi PH (2009) Localization of the brainstem GABAergic neurons controlling paradoxical (REM) sleep. PLoS One 4:e4272

Sapin E, Berod A, Leger L, Herman PA, Luppi PH, Peyron C (2010) A very large number of GABAergic neurons are activated in the tuberal hypothalamus during paradoxical (REM) sleep hypersomnia. PLoS One 5:e11766

Sastre JP, Buda C, Kitahama K, Jouvet M (1996) Importance of the ventrolateral region of the periaqueductal gray and adjacent tegmentum in the control of paradoxical sleep as studied by muscimol microinjections in the cat. Neuroscience 74:415–426

Sherin JE, Shiromani PJ, McCarley RW, Saper CB (1996) Activation of ventrolateral preoptic neurons during sleep. Science 271:216–219

Steriade M (1999) Cellular substrates of oscillations in corticothalamic systems during states of vigilance. In: Lydic R, Baghdoyan HA (eds) Handbook of behavioral state control. Cellular and molecular mechanisms. CRC Press, New York, pp 327–347

Valencia Garcia S, Libourel PA, Lazarus M, Grassi D, Luppi PH, Fort P (2017) Genetic inactivation of glutamate neurons in the rat sublaterodorsal tegmental nucleus recapitulates REM sleep behaviour disorder. Brain 140:414–428

van den Pol AN, Acuna-Goycolea C, Clark KR, Ghosh PK (2004) Physiological properties of hypothalamic MCH neurons identified with selective expression of reporter gene after recombinant virus infection. Neuron 42:635–652

Vanni-Mercier G, Sakai K, Lin JS, Jouvet M (1989) Mapping of cholinoceptive brainstem structures responsible for the generation of paradoxical sleep in the cat. Arch Ital Biol 127:133–164

Varin C, Rancillac A, Geoffroy H, Arthaud S, Fort P, Gallopin T (2015) Glucose induces slow-wave sleep by exciting the sleep-promoting neurons in the ventrolateral preoptic nucleus: a new link between sleep and metabolism. J Neurosci 35:9900–9911

Verret L, Goutagny R, Fort P, Cagnon L, Salvert D, Leger L, Boissard R, Salin P, Peyron C, Luppi PH (2003) A role of melanin-concentrating hormone producing neurons in the central regulation of paradoxical sleep. BMC Neurosci 4:19

Verret L, Leger L, Fort P, Luppi PH (2005) Cholinergic and noncholinergic brainstem neurons expressing Fos after paradoxical (REM) sleep deprivation and recovery. Eur J Neurosci 21:2488–2504

Verret L, Fort P, Gervasoni D, Leger L, Luppi PH (2006) Localization of the neurons active during paradoxical (REM) sleep and projecting to the locus coeruleus noradrenergic neurons in the rat. J Comp Neurol 495:573–586

von Economo C (1926) Die pathologie des schlafes. In: Von Bethe A, Bergman GV, Embden G, Ellinger UA (eds) Handbuch des normalen und pathologischen physiologie. Springer, Berlin, pp 591–610

Weber F, Chung S, Beier KT, Xu M, Luo L, Dan Y (2015) Control of REM sleep by ventral medulla GABAergic neurons. Nature 526:435–438

Zhang Z, Ferretti V, Guntan I, Moro A, Steinberg EA, Ye Z, Zecharia AY, Yu X, Vyssotski AL, Brickley SG, Yustos R, Pillidge ZE, Harding EC, Wisden W, Franks NP (2015) Neuronal ensembles sufficient for recovery sleep and the sedative actions of alpha adrenergic agonists. Nat Neurosci 18:553–561

Omics Approaches in Sleep-Wake Regulation

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Contents

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Abstract

Although sleep seems an obvious and simple behaviour, it is extremely complex involving numerous interactions both at the neuronal and the molecular levels. While we have gained detailed insight into the molecules and neuronal networks responsible for the circadian organization of sleep and wakefulness, the molecular underpinnings of the homeostatic aspect of sleep regulation are still unknown and the focus of a considerable research effort. In the last 20 years, the development of techniques allowing the simultaneous measurement of hundreds to thousands of molecular targets (i.e. 'omics' approaches) has enabled the unbiased study of the molecular pathways regulated by and regulating sleep. In this chapter, we will review how the different omics approaches, including transcriptomics, epigenomics, proteomics, and metabolomics, have advanced sleep research. We present relevant data in the framework of the two-process model in which circadian and homeostatic processes interact to regulate sleep. The integration of the different omics levels, known as 'systems genetics', will eventually lead to a better understanding of how information flows from the genome, to molecules, to networks, and finally to sleep both in health and disease.

Keywords

Circadian timing system · Epigenomics · Metabolomics · Proteomics · Sleep homeostasis · Transcriptomics

1 Introduction

Sleep plays a multitude of roles in physiology. In the past two decades, it has become possible to study the molecular and cellular consequences of sleep and sleep loss at a much deeper and broader level with the advent of so-called 'omics' technologies. In essence, 'omics' refers to the entirety of technologies used to characterize and quantify, in an unbiased manner, the various types of molecules and their interactions at the 'genome-wide' level which hitherto was impossible. Yet, translating this new wealth of data into useful knowledge about the structure, function, and dynamics of a cell, a system, or a whole organism remains a challenge. In this chapter, we will review the contribution these technologies have made to the understanding of the regulation of sleep at the molecular level, considering the factors and genes that regulate the many aspects of sleep, including the molecular consequences of sleep deprivation. Identifying these factors represents a pivotal step in developing interventions to target the adverse consequences of sleep loss that are so prevalent in today's '24/7' society or to alleviate sleep-wake disturbances in the context of disease.

The study of genome sequence – genomics – was the first 'omics' technology to be developed. Genomic analyses yielded a wealth of data on sequence variants among individuals that could be directly exploited to infer causal relationships with sleep traits and sleep disorders. Such studies include genome-wide association

studies (GWAS) and quantitative trait locus (QTL) analysis, which have been applied to diverse sleep phenotypes and served to elucidate some of the genetic architecture underpinning the sleep machinery (Nicod et al. 2016; Gottlieb et al. 2015; Ollila et al. 2014; Winrow et al. 2009; Franken et al. 2001; Tafti et al. 1999; Toth and Williams 1999). GWAS have also contributed to the understanding of genetic variations associated with sleep disorders (Faraco et al. 2013; Hor et al. 2010). Although relevant to understand the molecular wiring of sleep regulation, the genetics of sleep disorders will be covered elsewhere (i.e. Part III. Sleep-Wake Pathologies), and this chapter will specifically focus on data obtained from 'healthy' sleepers and directed towards the understanding of the physiological sleep-wake regulation.

GWAS and QTL analysis interrogate the full genome. However, these approaches mostly yield very few and often single targets that typically explain a minor portion of the phenotypic variance when considered individually, which is in line with the fact that sleep and its regulation are complex, involving multiple interacting networks in a variety of brain regions and cellular compartments. Moreover, although linkage and association studies can establish gene variant-to-phenotype correlations, the flow of information from the genome to transcriptome, proteome, and metabolome, and finally phenotype, cannot be assessed. To capture the complexity of sleep, systems genetics should be favoured to quantify, at a genome-wide level, gene expression (transcriptomics), epigenetic marks (epigenomics), proteins (proteomics), and metabolites (metabolomics), in a genetically well-characterized population as pictured in Fig. 1. The main techniques used

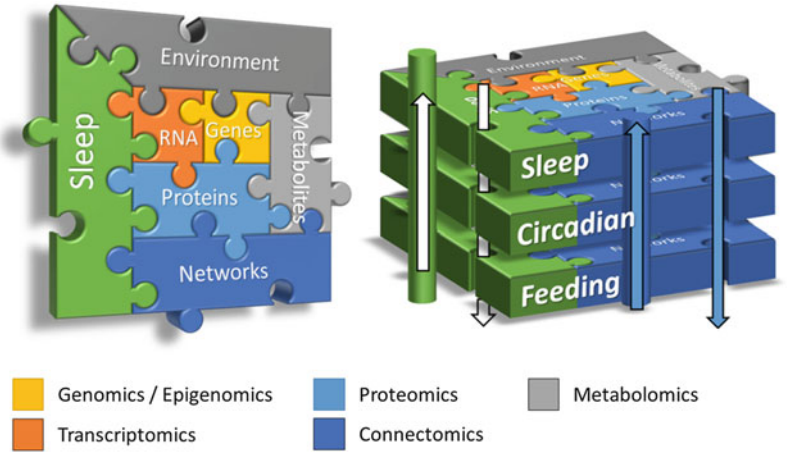

Fig. 1 Intricately interacting networks underlying sleep. Left: Epigenetic modifications of the genome lead to changes in transcription, which associate with changes in the levels of proteins and metabolites, which feed back into further changes in gene expression. Sleep, loss of sleep, and the environment can influence these molecular interactions that, in turn, affect sleep. Right: Networks underlying a particular behaviour interact with each other through shared network components to produce an integrative behavioural pattern

and the overall contributions to knowledge on sleep-wake regulation of these main layers of omics approaches will be emphasized in the following sections.

The contributions of transcriptomics, epigenomics, and proteomics/metabolomics to knowledge on sleep will more precisely be described in the context of models by which wakefulness and sleep duration and quality are regulated by the interactions between a circadian timing system and a sleep homeostat (Daan et al. 1984). To identify molecules impacted by or impacting the circadian timing system, measurements are usually performed at different circadian times under constant environmental conditions, whereas sleep deprivation with sampling at the same 'time' as undisturbed controls is used to identify molecular components linked to sleep homeostasis. However, because the distribution of wakefulness and sleep varies simultaneously with endogenous circadian time, disentangling circadian determinants and sleep-wake-driven ('homeostatic') components can only be achieved by specific experimental designs such as the forced desynchrony protocol or by combining circadian sampling and sleep deprivation, which are only rarely performed (Archer et al. 2014; Maret et al. 2007). Data concerning both the independent and combined strategies will be highlighted in the present chapter.

2 Transcriptomics

To understand the complex interplay between circadian time and homeostatic sleep drive, researchers have studied the dynamic response of an organism to these two factors by quantifying the ensemble of RNA transcripts. The transcriptome represents the levels of all RNAs (including protein-coding messenger RNAs [mRNAs] and non-coding RNAs such as microRNAs) at a particular time (Wang et al. 2009). Although techniques can be adapted to quantify nascent transcripts, usually steady-state RNA levels are measured in transcriptomic studies, including both actively transcribed genes and stored RNA pools. While the relationship between a gene's transcription and its protein levels is often not linear, a transcriptomic analysis can nevertheless be seen as the genome's *active components* (to contrast *functional components* covered in the proteomics section below). Transcriptomic studies have contributed to elucidate the role of specific genes and intracellular signalling pathways in regulating sleep and in mediating the consequences of sleep loss and thereby have identified potential therapeutic targets for sleep and associated brain disorders. Quantifying the transcriptome of a sample has been achieved using two main technologies that will first be summarized below: hybridization-based and, more recently, sequencing-based technologies.

2.1 Microarrays and RNA Sequencing

In hybridization-based technologies, a high-density array of oligonucleotides (i.e. microarray) is incubated with fluorescently labelled complementary DNA

(cDNA) synthetized from an RNA sample. The fluorescence quantifying the amount of cDNA binding each oligo spot (probe set) on the array is then read. Although it is possible to design custom arrays for organisms lacking a reference genome, commercial microarrays rely on high-density synthesis of oligonucleotides of defined sequences onto the slide, allowing inferences to be made as to the expression of various predicted gene isoforms (Wang et al. 2009; Okoniewski and Miller 2006). Thus, a key shortcoming of this technology is its dependence on high-quality genome annotation to optimally design oligonucleotide probes, which also limits this technology to the identification of known rather than novel transcripts. Despite these shortcomings, most of our understanding of the molecular underpinnings of sleep regulation comes from microarray studies performed in a number of organisms, including the fruit fly (Zimmerman et al. 2006; Cirelli et al. 2005), birds (Jones et al. 2008), rodents (Mackiewicz et al. 2007; Cirelli et al. 2004), as well as humans (Archer et al. 2014; Möller-Levet et al. 2013; Pellegrino et al. 2012).

The recent development of high-throughput DNA sequencing methods has provided novel techniques for quantifying transcriptomes. In this technique, the expressed RNA is reversed transcribed to form cDNA, which is used to make a platform-specific DNA library ready for sequencing (commonly involving DNA fragmentation and adapter ligation) (Syed et al. 2009). As next-generation sequencing generates short read lengths that must be assembled, this is sometimes called whole transcriptome shotgun sequencing, or more commonly RNA sequencing (RNA-Seq). Compared to microarray techniques, this technology is both more sensitive and accurate due to its higher dynamic range, with little or no background noise that otherwise would limit the detection of rare transcripts, and less signal saturation for highly expressed transcripts (Darlington et al. 2013; Marguerat and Bähler 2010; Wang et al. 2009). RNA-Seq is also more informative in that it can detect previously unannotated splice variants and single-nucleotide polymorphisms. Nevertheless, the use of this technology has been limited by the significant bioinformatic skills required to analyse the data. In addition, RNA-Seq also depends on high-quality genome annotation to perform genomic alignment. To date, RNA-Seq has been used in few sleep studies (Sabir et al. 2015; Darlington et al. 2013), but given its advantages and the high-quality information it outputs, it is evident that future sleep research will increasingly utilize this method. In planning future research using transcriptomics (as well as other omics), we wish to direct readers to recently published methodological and analytical guidelines for genome-wide circadian-related studies (Hughes et al. 2017).

2.2 The Circadian Transcriptome

Many microarray studies have reported on the extent of 24-h variations in the transcriptome in numerous organisms and tissues (Keegan et al. 2007; Storch et al. 2007; Akhtar et al. 2002). We will focus here on mammalian datasets only. In the mouse forebrain, more than 2,000 rhythmic probe sets have been reported under normal light-dark (LD) conditions (Maret et al. 2007), while in the pineal gland, the

brain structure responsible for melatonin secretion, more than 600 genes showed differential day-night expression in the rat (Bailey et al. 2009). However, many genes that show a cycling pattern of expression in the brain will directly or indirectly respond to the light-dark cycle (or the feeding schedule). Accordingly, genes directly regulated by circadian time need to be distinguished by performing experiments in constant darkness (DD), here referred to as circadian studies. For instance, in an organ specialized to respond to light, the eye, only about 10% of the transcripts that were rhythmic under 24-h LD conditions remained rhythmic under DD conditions, which represented 120 genes (Storch et al. 2007). Circadian studies have notably revealed a high tissue specificity of the circadian transcriptome (Miller et al. 2007; Duffield 2003), and the dependency of the peripheral circadian transcriptome on the integrity of the brain, or in particular of the main mammalian clock located in the hypothalamic suprachiasmatic nuclei (SCN) (Kolbe et al. 2016; Hughes et al. 2012; Akhtar et al. 2002).

Overall, circadian studies have described hundreds of genes cycling with internal (circadian) time. However, as highlighted in the introduction, among the strongest confounding factors in these studies is the distribution of wakefulness and sleep that covaries with circadian time. A consensus is even emerging that the sleep-wake-driven transcriptome is larger than the ensemble of circadian transcripts. For instance, of the >2,000 rhythmic probes in the mouse forebrain under baseline conditions, fewer than 400 retained a rhythmic expression profile when the sleep-wake influence was accounted for by submitting animals to a 6-h sleep deprivation prior to around-the-clock tissue sampling (Maret et al. 2007). A similar >80% reduction of the number of rhythmic transcripts was observed in the human blood transcriptome: of the ~1,400 genes that showed circadian rhythmicity, the expression of only 230 genes remained rhythmic when sleep no longer occurred at the correct circadian phase (Archer et al. 2014). This underscores the predominance of the sleep-wake distribution in driving cycling gene expression. Nevertheless, a more recent study by the same group using 19 transcriptome datasets available from humans and mice identified circadian transcripts that were not influenced by the sleep-wake cycle (Laing et al. 2015). The genes identified were related to processes involved in lipid metabolism, glucocorticoid signalling, and immune function. Given the complexity of adequately quantifying the circadian transcriptome, the circadian genome-wide gene expression pattern that is independent of the sleep-wake cycle remains to be defined for most brain structures regulating sleep.

2.3 Brain Transcriptomic Response to Sleep Loss

The quantification of the transcriptome after sleep deprivation has been used to find molecular correlates of sleep need as well as to identify molecular pathways associated with functions of sleep. Early studies examined rat cerebral cortex transcript expression and found that during spontaneous wakefulness or enforced wakefulness (i.e. sleep deprivation), various genes were upregulated relative to their levels during sleep. These included immediate early genes, genes involved in

metabolism, neurotransmission and synaptic function, and genes coding for heat shock proteins or chaperones (Cirelli and Tononi 2000). Additional studies have corroborated the impact of sleep deprivation on these gene categories, showing thousands of genes being up- and downregulated (Mongrain et al. 2010; Maret et al. 2007; Mackiewicz et al. 2007; Terao et al. 2003). Some additional genes identified by these studies included genes involved in intracellular calcium homeostasis and glutamate signalling suggesting a role for sleep in neuroprotection (Mongrain et al. 2010; Maret et al. 2007).

Some of the sleep deprivation-driven changes in the transcriptome are observed at the level of the whole brain in mammals (Mongrain et al. 2010; Maret et al. 2007). However, sleep transcriptomic studies have also characterized alterations in the cerebral cortex as indicated above (Mackiewicz et al. 2007; Terao et al. 2003; Cirelli and Tononi 2000) and also in the hypothalamus and hippocampus (Porter et al. 2012; Vecsey et al. 2012; Mackiewicz et al. 2007). The hippocampus is a brain region particularly sensitive to the effect of sleep loss (Havekes and Abel 2017), and its transcriptomic signature after sleep deprivation is indicative of impaired protein synthesis (Vecsey et al. 2012). Such studies may shed light on the molecular origin of sleep loss-induced alterations in memory processes.

Collectively, these studies and those in non-mammalian models contributed to hypotheses regarding the functions of sleep, which include to respond to cellular stress and to maintain metabolism and synaptic plasticity (Mackiewicz et al. 2009). One meta-analysis identified immediate early gene families (*Egr*, *Nr4a*) as conserved sleep deprivation-affected genes in insects, birds, and mammals and showed the cAMP-responsive element (CRE) to be one of the primary *cis*-regulatory elements in the enhancers of those genes (Wang et al. 2010). However, one limitation of sleep deprivation studies is the observation that many transcripts previously thought to be affected by sleep loss are actually responding to the corticosterone surge induced by sleep deprivation (Mongrain et al. 2010). Removal of the corticosterone surge using adrenalectomy resulted in the identification of protein synthesis, RNA post-transcriptional modification, and neuroprotection pathways as likely to be more directly linked to sleep need (Mongrain et al. 2010). MicroRNAs, small non-coding RNAs controlling mRNA expression, also featured in the list of potential direct correlates of the sleep homeostat, which is reminiscent of other studies linking microRNAs to sleep regulation (Davis et al. 2007, 2012).

2.4 Sleep Loss Affects the Peripheral Transcriptome

In mice, microarray studies have shown that sleep loss affects the transcriptome in the heart and lung similar to the brain (Anafi et al. 2013). In the liver, however, a higher number of transcripts showed sleep deprivation-induced alterations than in the brain (Maret et al. 2007). Such findings challenge the historical view that sleep is solely required for the brain (Hobson 2005). Peripheral organs, and in particular blood, are better suited for human studies because of easier accessibility and the possibility to assay *biomarkers* for sleep need. A comprehensive review outlined the

state of the literature in humans (Archer and Oster 2015). In the human blood, prolonging wakefulness to 60 h changed the expression of over 500 genes, including genes involved in the immune system, stress response, and DNA damage repair, with many downregulated following sleep loss and subsequently showing a recovery with sleep (Pellegrino et al. 2012). More recently, an attempt was made to identify molecular correlates of sleep loss-sensitive and sleep loss-resistant phenotypes in humans using transcriptomics (Arnardottir et al. 2014). This microarray study described that subjects resistant to sleep loss showed a reduction in the amplitude of expression of rhythmic transcripts in comparison to sleep loss-sensitive subjects. Such findings may eventually allow predicting whether an individual will be able to perform under high sleep pressure conditions.

2.5 Future of Sleep Transcriptomics and Limitations

Besides assessing the sleep/wake-dependent transcriptome of discrete brain regions, research has now come to the study of specific cell types (Yelin-Bekerman et al. 2015; Bellesi et al. 2013, 2015). Worth mentioning are studies of the oligodendrocyte and astrocyte transcriptomes in the cerebral cortex assessed using the combination of microarray and translating ribosome affinity purification (Bellesi et al. 2013, 2015), which captures mRNAs that are actively being translated by ribosomes. During wakefulness, genes translated in oligodendrocytes included genes involved in apoptosis, cellular stress response, and cell differentiation, whereas astrocyte-translated genes were involved in metabolism, extracellular matrix, and elongation of peripheral astrocytic processes. In both cell types, the team also described pathways linked to genes that are transcribed specifically during sleep.

Although we now have a clear portrait of how gene expression changes with the distribution of sleep and wakefulness, most of these transcriptomic studies remain limited by the fact that they cannot establish if alterations in gene expression profiles are merely a consequence of the behavioural state or if the genes are causally involved in regulating the behavioural state. To overcome this limitation, application of transcriptomics to inbred strains or genetically modified animals with alterations in sleep seems a promising avenue to identify networks of genes specifically involved in sleep regulation (Freyburger et al. 2016; Hasan et al. 2014; Massart et al. 2014; Darlington et al. 2013; Maret et al. 2007). Studies using mutant mice with deficits in neural development and plasticity highlighted that the transcriptome response to sleep deprivation is only slightly impacted by these mutations and is thus extremely robust (Freyburger et al. 2016; Massart et al. 2014). Yet, *Fgf1* (fibroblast growth factor 1) was identified as a potential cell-specific element contributing to recovery sleep using the combination of transcriptomics with genetic engineering (Massart et al. 2014).

Another area of transcriptomic research with important clinical relevance concerns the consequence of chronic sleep loss. In fact, the gene expression response to multiple days of sleep restriction was shown to differ from the acute effects of sleep loss measured at the end of the prolonged wakefulness episode (Cirelli et al.

2006). In humans, sleep restriction was shown to disturb the circadian regulation of the blood transcriptome and to impact on biological processes like gene-expression regulation, metabolism, and inflammatory and stress responses (Aho et al. 2016; Möller-Levet et al. 2013). More research is clearly needed to understand how the impact of acute total sleep loss on the transcriptome relates to that of chronic sleep restriction better mimicking clinical scenarios seen in patients with sleep, psychiatric, or neurological disorders. Lastly, a noticed limitation of transcriptomic studies is that changes in mRNA expression may not be reflected in protein expression and therefore might not be indicative of functional observations. This will partly be addressed in the following sections.

3 Epigenomics

In the previous section, we have emphasized that the transcriptome of both the brain and the periphery changes over the sleep/wake cycle and is altered following sleep deprivation. Mechanisms underlying these changes in gene expression are inevitably involving epigenetic modifications, which are required for modifying the state of a gene (e.g. silent vs. primed vs. actively transcribed). The epigenome refers to the genome-wide pattern of specific marks found on DNA and histones, which determine the state of chromatin (heterochromatin vs. euchromatin), and thus DNA accessibility and gene expression (McGowan and Szyf 2010; Wolffe and Kurumizaka 1998). In general, epigenetic modifications comprise all changes to DNA (chemical or physical due to histone modifications) that do not alter the DNA sequence.

Epigenetic modification of gene expression operates across very different timescales. For instance, epigenetic mechanisms play important roles in determining cell fate and behaviour during development (Khalyfa et al. 2014; Koh and Rao 2013; McGowan and Szyf 2010), which implicates stable modifications that will last throughout the existence of an organism. However, epigenetic changes can be much more dynamic (Qureshi and Mehler 2014) and respond to changes in environmental conditions lasting a few weeks or even minutes (Azzi et al. 2014; Zhao et al. 2010). In addition, the fact that epigenetic mechanisms are profoundly linked to the establishment of brain circuitry and neuronal plasticity (Bale 2015; Miller and Sweatt 2007) places the epigenome at a central position to regulate wakefulness and sleep. We will here present specific epigenetic modifications and methodologies for their genome-wide quantification and, in addition, review findings linking epigenomics to the circadian timing system and the sleep homeostat.

3.1 Epigenetic Marks and Techniques

We will first provide a brief description of DNA methylation and derivatives and of histone post-translational modifications. DNA methylation consists of the addition of a methyl group to the 5′ position of cytosine (5-methylcytosine or 5mC). In

general, 5mC correlates with gene silencing when occurring in the promoter of genes because the methyl group prevents the binding of transcription factors required for gene expression and attracts methylated DNA-binding proteins that recruit corepressors, histone methyltransferases, and histone deacetylases promoting a closed chromatin configuration (McGowan and Szyf 2010). This of course applies to the mammalian brain (Massart et al. 2017). DNA methyltransferases (DNMTs) catalyse the 5mC reaction and have thus themselves been implicated in all functions attributed to 5mC, including in plasticity of the central nervous system (Miller and Sweatt 2007).

The methylated cytosine can undergo further chemical modifications that have also been shown to regulate gene expression and cellular functions, including hydroxymethylation (5hmC), formylation (5fC), and carboxylation (5caC). These modifications are mediated by DNA hydroxylases (i.e. ten-eleven translocation methylcytosine dioxygenase or TETs) (Koh and Rao 2013). The exact roles of these additional modifications remain to be determined, which is a particular challenge for 5fC and 5caC because of the difficulty to precisely quantify these marks (Koh and Rao 2013). The 5hmC mark has been proposed to serve as an intermediate to demethylation or as a mark diversifying 5mC (Koh and Rao 2013; Wu et al. 2011) and is highly abundant in the adult brain, particularly in the cerebral cortex (Münzel et al. 2010). Importantly, TETs have been involved in neurogenesis and brain function (Zhang et al. 2013).

Histone post-translational modifications also represent a major category of epigenetic marks. These include phosphorylation, ubiquitination, acetylation, and methylation, the last two of which are controlled by histone acetyltransferases and deacetylases and histone methyltransferases and demethylases, respectively (Qureshi and Mehler 2014). Histone modifications exert their effects on gene expression by directly influencing chromatin structure via shaping chromatin compaction and regulating the binding of effector molecules (Bannister and Kouzarides 2011). An extensive literature exists on the role of histone modifications in brain development and plasticity, and some specific examples relevant to the regulation of sleep and wakefulness will be highlighted hereafter.

A standard technique to study the role of histones, DNMTs, and TETs is chromatin immunoprecipitation (ChIP) that allows enrichment of DNA associated with these specific epigenetic elements. To achieve an omic (genome-wide) resolution, the DNA captured by ChIP can be assayed using microarrays (ChIP-chip) or high-throughput sequencing (ChIP-Seq) (Takahashi et al. 2015). Similarly, genome-wide quantification of 5mC is generally achieved using methylated DNA immunoprecipitation (MeDIP) (Massart et al. 2014). These techniques all depend on effective antibodies that will specifically recognize the targeted epigenetic elements. Sequencing can also be combined with bisulfite treatment (Bis-Seq) to measure the genome-wide methylation landscape (Massart et al. 2017), but this technique does not allow to distinguish between 5mC and 5hmC (Koh and Rao 2013), which requires the use of Tet-assisted bisulfite sequencing (Tab-Seq) (Yu et al. 2012). Future studies on the regulation of sleep will also feature data from ATAC-Seq that assesses genome-wide chromatin accessibility (Buenrostro et al. 2015).

3.2 The Circadian Epigenome

The circadian field has a considerable advance compared to the sleep field with regard to understanding the function of epigenetic mechanisms in physiology (Sahar and Sassone-Corsi 2013; Takahashi et al. 2015). In particular, genome-wide quantifications of the circadian epigenetic landscape have portrayed the profound implication of epigenetic mechanisms in the circadian timing system (Valekunja et al. 2013; Koike et al. 2012; Le Martelot et al. 2012). Most, if not all, histone modifications play roles in circadian transcription. For example, the circadian expression and activity pattern of histone acetyltransferase p300 and histone methyltransferases MLL1 and MLL3 are essential in generating circadian rhythms (Valekunja et al. 2013; Katada and Sassone-Corsi 2010; Etchegaray et al. 2003). An important discovery was that the core circadian protein CLOCK is itself a histone acetyltransferase that binds other chromatin modifiers such as MLL1 and the histone deacetylase SIRT1 to regulate circadian gene expression (Sahar and Sassone-Corsi 2013). Although most currently available datasets have been obtained from the mouse liver, these observations suggest that similar mechanisms will govern circadian timekeeping in the brain, implying this might also regulate sleep.

With regard to 5mC mechanisms, DNMTs were shown to both be regulated by molecular clock mechanisms and to play roles in clock outputs. In fact, on the one hand, the mRNA expression of DNMTs was shown to cycle with time-of-day in the mouse liver (Xia et al. 2015; Maekawa et al. 2012). On the other hand, DNA methylation regulates the expression of core circadian clock components in the mouse SCN (Azzi et al. 2014; Ji et al. 2010). Importantly, genome-wide reprogramming of 5mC in the SCN was shown to change the rhythmic locomotor activity pattern, which was prevented when a methyltransferase inhibitor was administered (Azzi et al. 2014). This indicated that epigenetic mechanisms coordinate plasticity of the main circadian clock. Another important study using a genome-wide design reported on rhythmic 5mC in the human prefrontal cortex and on its relationship with the transcriptome (Lim et al. 2014). Moreover, the authors observed that the amplitude of the DNA methylome rhythm was positively linked to the amplitude of activity rhythms before death, suggesting a functional role for 5mC rhythm in the human brain. Nonetheless, similar to the transcriptome, further experiments will be required to understand the contribution of the wake/sleep distribution to the circadian epigenome.

3.3 The Epigenome Responds to Sleep Loss

Transcriptomic studies suggest that epigenetic mechanisms and the sleep homeostat are also fundamentally linked, as sleep deprivation and mistimed sleep extensively modify gene expression including expression of genes coding for histones, histone-modifying enzymes, and DNMTs (Archer et al. 2014; Massart et al. 2014; Möller-Levet et al. 2013; Mongrain et al. 2010). Furthermore, sleep deprivation impacts genome-wide 5mC and 5hmC patterns with, respectively, hundreds and

thousands of genes being affected (Massart et al. 2014). More precisely, 5mC modifications were found in genes involved in neuritogenesis and synaptic plasticity, whereas 5hmC alterations were found in genes involved in cytoskeleton organization, signalling, and neurotransmission. These biological functions are in line with the results of transcriptomic studies (previous section). In addition, a significant proportion of 5hmC changes observed in this study correlated with changes in gene expression assessed using RNA-Seq (Fig. 2a). In a follow-up experiment, our group has also observed a significant association between sleep deprivation-driven changes in 5mC located at enhancers (i.e. bound to H3K4me1) and gene expression (Fig. 2b), supporting a role for 5mC in regulating gene expression under high sleep need conditions. A recent study making use of omics approaches in human blood samples (Nilsson et al. 2016) and studies looking at individual targets (Ventskovska et al. 2015) also support this notion.

The relationship between sleep regulation and the epigenome is further supported by studies showing that modifications of epigenetic elements alter markers of the sleep homeostat in rodents (Wither et al. 2012) and sleep in flies and humans (Pirooznia et al. 2012; Winkelmann et al. 2012). Moreover, epigenetic mechanisms have been shown to be involved in the impact of sleep loss on progeny in mice (Khalyfa et al. 2014). This study showed that 5mC and 5hmC, the expression of DNMTs and TETs, and histone acetyltransferase activity are all altered in visceral adipose tissue of the offspring of pregnant mice exposed to sleep fragmentation. Some of these changes applied to adiponectin, which is involved in insulin resistance and obesity, and were associated with metabolic dysfunction observed in the offspring during adulthood. This dataset highlights a mechanism by which sleep disruption can impact physiology on the long term, namely, through epigenetic modifications.

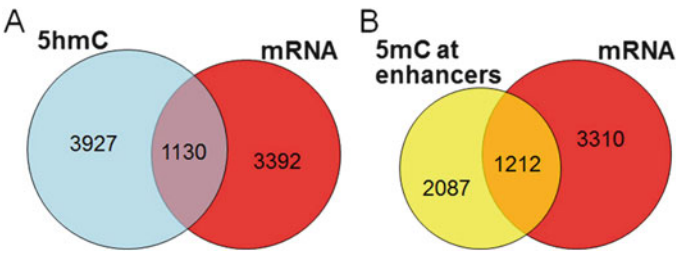

Fig. 2 (**a**) Venn diagram showing the overlap between genes differentially hydroxymethylated (5hmC) after a 6-h sleep deprivation (SD) in comparison to control condition (false discovery rate [FDR] < 0.15) and genes differentially expressed (mRNA) after SD (FDR < 0.05) in the mouse cerebral cortex. DNA 5hmC data are from Massart et al. (2014). Gene expression was quantified by high-throughput sequencing (RNA-Seq) using three pools of three control and three SD mice. 22.3% of changes in DNA 5hmC overlap with changes in gene expression. (**b**) Overlap between genes differentially expressed (mRNA) after SD (see also **a**) and genes showing significant changes in methylation (5mC) at H3K4me1 enhancers measured by combining chromatin immunoprecipitation, bisulfite treatment, and high-throughput sequencing (ChIP-Bis-Seq). 36.7% of changes in 5mC at enhancers overlap with changes in gene expression (unpublished data)

3.4 Future of Sleep Epigenomics

To understand how sleep impacts brain function and health via epigenetic mechanisms is an exciting and promising area of future sleep research. In blood, the analysis of the genome-wide methylation landscape in shift-workers revealed epigenetic modifications, which included genes with roles in circadian physiology as well as genes belonging to cancer-associated pathways (Shi et al. 2013; Zhu et al. 2011). Alterations in blood 5mC are also seen in obstructive sleep apnoea patients (Kim et al. 2012). Various epigenetic changes are also expected to occur in the central nervous system in response to the above perturbations of sleep and to many others, including insomnia, and to underlie, at least in part, the relationships between sleep and psychiatric or neurological disorders. The potential for epigenetic marks to serve as biomarkers for diseases showing sleep and circadian disturbances is clear, and identifying relevant molecular targets could facilitate the development of novel interventions that will allow targeting networks of genes. Nevertheless, much remains to be done before we understand how the epigenome, and particularly the brain epigenome, regulates the normal wakefulness and sleep cycle.

Non-coding RNAs represent another class of molecules playing epigenetic roles in regulating gene expression. Non-coding RNAs are divided on the basis of their size and of particular interest are long non-coding RNAs (lncRNAs, >200 nucleotides) and piwi-associated non-coding RNAs (piRNAs, 26–32 nucleotides) because they play roles in 5mC and chromatin remodelling (Gomes et al. 2013; Rajasethupathy et al. 2012). One lncRNA was specifically shown to regulate sleep in flies (Soshnev et al. 2011). Their role in sleep regulation remains to be explored in mammals, in which investigation is supported by observation that sleep deprivation alters the expression of some lncRNAs in the mouse hypothalamus (Davis et al. 2016).

4 Proteomics and Metabolomics

The last category of omics approaches that will be covered in this chapter could be seen as functional outputs from transcriptomics and epigenomics. However, proteomics and metabolomics can reveal additional/different networks of components not necessarily identifiable in transcriptomic/epigenomic studies because there is not always a direct correlation between the expression of a gene and of the protein product that it produces. Indeed, in circadian rhythm research, a rhythmic protein can be associated with a nonrhythmic mRNA expression, and the opposite has also been observed (Chiang et al. 2014; Mauvoisin et al. 2014; Reddy et al. 2006). Similarly, an impact of sleep or sleep loss can be observed only at the level of protein amount and not for mRNA expression or vice versa (Tudor et al. 2016; Seibt et al. 2012). In addition, post-translational modifications shaping the activity of functional components can only be captured at the proteome level (Tudor et al. 2016; Lück et al. 2014; Seibt et al. 2012). This emphasizes the need for the different measurements to be performed in parallel (i.e. systems genetics).

Although metabolites clearly differ from proteins, methodologies used for the quantification of the metabolome are nevertheless very similar to those for the proteome except that they are applied to physiological fluids containing metabolites such as blood and urine. To simultaneously quantify the expression of hundreds to thousands of proteins or metabolites, two-dimensional difference gel electrophoresis (2D-DiGE) is generally used with the gold standard technique of liquid chromatography combined with mass spectrometry (LC-MS) (Kim et al. 2014; Ang et al. 2012; Deery et al. 2009; Reddy et al. 2006), of which different technologies exist such as high-performance liquid chromatography-electrospray tandem mass spectrometry (Chiang et al. 2014).

Assessment of the metabolome is particularly suitable for human studies because metabolites measured in the blood can be sampled easily, are a reliable index of the physiology, and can be directly compared to research in other mammals (Weljie et al. 2015). In addition, metabolites might eventually serve as accessible biomarkers. Studies have unequivocally reported that the brain proteome, the peripheral proteome, and the metabolome change with circadian time and sleep need, as will be summarized below.

4.1 The Circadian Proteome/Metabolome

The circadian proteome has been assayed in both the central and peripheral clocks, with the liver being particularly well-studied (Mauvoisin et al. 2014; Masri et al. 2013; Reddy et al. 2006). Pioneer work by Reddy et al. (2006) identified 60 proteins with a strong circadian variation among the 642 proteins reliably measured. The rhythmic hits included proteins involved in fundamental liver functions like urea formation and sugar metabolism, which highlighted that the liver is a functionally different organ at different endogenous times (Reddy et al. 2006). More recent data emphasized the contribution of the feeding schedule to these 24-h variations of the liver proteome (Mauvoisin et al. 2014). Post-translational modification can also be targeted in proteomic analyses. In particular, the rhythmic acetylated proteome (acetylome) differs in animals lacking a functional molecular clock, with differences including proteins linked to amino acid metabolism, nitrogen metabolism, and glycolysis/gluconeogenesis (Masri et al. 2013).

In the murine SCN, about 20% of the ~2,100 proteins identified showed a time-of-day abundancy pattern (Chiang et al. 2014). These were linked to neurotransmitter release, neural plasticity, and mitochondrial function. Among these, 48 proteins specifically showed a circadian (LD cycle-independent) rhythm (Chiang et al. 2014). These observations mostly corroborated earlier findings concerning synaptic vesicle cycling as a major biological pathway showing circadian rhythmicity in the mouse SCN proteome (Deery et al. 2009). A recent observation also indicates that the level of many amino acids shows a circadian rhythm in the mouse SCN (Fustin et al. 2017). Day-night variations in the proteome have also been reported for the pineal gland (Møller et al. 2007). Together, these studies underline the important

contribution of proteomics to the current understanding of roles of circadian processes in different tissues.

In the human blood, the level of 15–19% of metabolites reliably detected was observed to vary with time-of-day (Ang et al. 2012; Dallmann et al. 2012), similar in extent to circadian results obtained using saliva (Dallmann et al. 2012). Notably, such studies allowed for the identification of novel rhythmic metabolites such as acylcarnitines and phospholipids (Ang et al. 2012). In a follow-up study utilizing human urine, authors have reported that the sleep-wake cycle impacts on circadian variations of several metabolites by measuring 24-h levels under undisturbed sleep-wake cycle and during total sleep deprivation (Giskeødegård et al. 2015). This observation is not surprising given data showing the impact of sleep loss on the proteome and metabolome that will be presented in the next section.

4.2 The Proteomic/Metabolomic Response to Sleep Deprivation

A number of proteomic studies have reported on the impact of sleep loss in several brain areas, including the cerebral cortex, the hypothalamus, and the basal forebrain. In the basal forebrain, an area particularly involved in sleep regulation (sleep homeostasis), 89 proteins were shown to have a twofold or greater difference after an acute 6-h sleep deprivation in rats, including many synaptic and cytoskeleton proteins (Basheer et al. 2005). In the mouse cerebral cortex, sleep deprivation was shown to specifically affect the level of three proteins: alpha tubulin, heat shock cognate 71, and lactate dehydrogenase (Pawlyk et al. 2007). These two studies identified elements in line with neurotransmission, cytoskeleton, metabolism, and heat shock pathways identified in microarray studies. In rats, sleep was shown to be associated with higher level of the C-terminal fragment of histone 4 in the cerebral cortex (Cirelli et al. 2009), again emphasizing the link between the epigenome and sleep deprivation. It thus seems that there is convergence of transcriptomics and proteomics data on the impact of sleep deprivation even if, in general, fewer proteins are significantly changed by sleep deprivation compared to reported changes in gene expression presented earlier (unit to dozens vs. hundreds to thousands, respectively). Technical reasons likely explain such a difference, which may also be due to mixing distinct cell types. For instance, a more recent study examining only hypothalamic astrocytes has identified as many as 139 proteins with significantly altered levels after chronic sleep restriction that were related to glial activation and gliotransmission (Kim et al. 2014).

The blood metabolome was also shown to be altered by sleep loss. Indeed, recent studies indicate that the level of many lipids, in particular phospholipids, is modified by both acute and chronic sleep loss (Weljie et al. 2015; Davies et al. 2014). Interestingly, metabolomics have identified potential biomarkers of chronic sleep loss encompassing different mammalian species, namely, oxalic acid and diacylglycerol 36:3 (Weljie et al. 2015). Equally interesting is the observation that acute sleep deprivation increases human plasma levels of serotonin, tryptophan, and taurine, potentially relevant to the antidepressive effect of acute sleep deprivation

(Davies et al. 2014). Similar to transcriptomic studies, these studies emphasized the fact that sleep loss not only impacts the brain but also importantly affects peripheral organs. Future research should determine whether and how these changes participate in regulating sleep and how this information can contribute to establish sleep's function(s).

4.3 Future of Sleep Proteomics/Metabolomics

As mentioned for transcriptomic studies, most of these studies are solely descriptive, cataloging the changes in protein and metabolite levels across circadian time and sleep need. How changes in protein amount underlie functions of sleep such as neuronal plasticity is beginning to be addressed at the level of individual targets (Tudor et al. 2016; Seibt et al. 2012), but the use of strategies targeting networks of elements should eventually be favoured. In addition, to fully delineate the complexity of sleep/wake regulation, combinations of omics approaches (i.e. systems genetics) are increasingly being used such as transcriptomics with proteomics or metabolomics (Aho et al. 2016; Lück et al. 2014; Eckel-Mahan et al. 2012) and integrating the acetylome with the transcriptome, proteome, and metabolome (Masri et al. 2013). New related technologies including breathomics and lipidomics (Martinez-Lozano Sinues et al. 2014; Thimgan et al. 2015) may also shed light on sleep regulation.

5 Conclusions and Perspectives

Overall, this chapter has highlighted the power of specific omics approaches to identify molecular networks associated with circadian and homeostatic sleep regulation. Yet, the exact contribution of sleep-wake-driven changes to 'circadian' genome-wide oscillations still remains to be determined for distinct brain areas and peripheral tissues using adequate study design. It is evident that these technologies offer the potential to comprehensively investigate impacts of pharmacological agents and evaluate their suitability to mimic or restore a normal or pathological molecular 'landscape'. Such studies have only begun to be available, and a good example is found in the description of the genome-wide signature of different wake-promoting pharmacotherapies (i.e. pharmacogenomics) (Hasan et al. 2009). This study even used the more promising approach of systems genetics by studying different inbred strains and thus combining transcriptomics with genetics. In order to adapt treatment in diseases, assessing circadian/sleep-dependent genome-wide effects of the numerous drugs reported to affect sleep variables (e.g. Monti et al. 2017; Schwartz et al. 2016; Arbon et al. 2015; Bettica et al. 2012) seems required. In addition, the combination of pharmacogenomics with pharmacogenetics (i.e. considering genetic variations in pharmacological interventions) is of importance for personalized treatment of sleep disorders as highlighted recently (Holst et al. 2016). In the long term, omics will without doubt permit to develop

therapeutics that simultaneously target networks of relevant pathways, which seems particularly promising with regard to epigenomics.

The present work has focused on transcriptomics, epigenomics, and proteomics/metabolomics approaches and their contributions to wakefulness and sleep regulation. Nevertheless, some omics approaches that have not been specifically discussed in the present chapter and that are equally relevant to the regulation of sleep include microbiomics and connectomics. Microbiomics generally consists in sequencing the bacterial flora of different tissues, most often the gut. The use of such approach is exponentially increasing and has revealed that the deregulation of the gut microbiota due to circadian disruption and consequent abnormal feeding schedule crucially impacts metabolism in both humans and mice (Thaiss et al. 2014). Considering the importance of the gut-brain axis in brain functions (Montiel-Castro et al. 2013), this type of research will certainly reveal additional determinants of the normal regulation of wakefulness and sleep, in addition to increase the understanding of diseases of sleep such as sleep apnoea (Durgan et al. 2016).

At probably the most macroscopic level of omics approaches stands connectomics, which is applied to the mammalian brain. The brain connectome is defined as the global connectivity between all brain areas and is generally measured with imaging techniques such as functional magnetic resonance imaging, but also using electrophysiological methods (Liu et al. 2015). This approach has notably revealed that functional connectivity during sleep in children seems less complex than in adults (Manning et al. 2013) and that, not surprisingly, sleep deprivation radically alters the connectivity of a number of brain networks including hippocampal networks (Kaufmann et al. 2016). The application of these other omics methodologies to sleep research and their use in combination with genomics and pharmacological testing will also allow the development of more efficient strategies to regulate wakefulness and sleep. Overall, we have definitely entered a 'sleepomics' era, which will contribute tremendously to understanding the determinants of brain functions and health.

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References

Aho V, Ollila HM, Kronholm E, Bondia-Pons I, Soininen P et al (2016) Prolonged sleep restriction induces changes in pathways involved in cholesterol metabolism and inflammatory responses. Sci Rep 6:24828

Akhtar RA, Reddy AB, Maywood ES, Clayton JD, King VM et al (2002) Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. Curr Biol 12(7):540–550

Anafi RC, Pellegrino R, Shockley KR, Romer M, Tufik S, Pack AI (2013) Sleep is not just for the brain: transcriptional responses to sleep in peripheral tissues. BMC Genomics 14:362

Ang JE, Revell V, Mann A, Mäntele S, Otway DT et al (2012) Identification of human plasma metabolites exhibiting time-of-day variation using an untargeted liquid chromatography-mass spectrometry metabolomic approach. Chronobiol Int 29(7):868–881

Arbon EL, Knurowska M, Dijk DJ (2015) Randomised clinical trial of the effects of prolonged-release melatonin, temazepam and zolpidem on slow-wave activity during sleep in healthy people. J Psychopharmacol 29(7):764–776

Archer SN, Oster H (2015) How sleep and wakefulness influence circadian rhythmicity: effects of insufficient and mistimed sleep on the animal and human transcriptome. J Sleep Res 24 (5):476–493

Archer SN, Laing EE, Möller-Levet CS, van der Veen DR, Bucca G et al (2014) Mistimed sleep disrupts circadian regulation of the human transcriptome. Proc Natl Acad Sci U S A 111(6): E682–E691

Arnardottir ES, Nikonova EV, Shockley KR, Podtelezhnikov AA, Anafi RC et al (2014) Blood-gene expression reveals reduced circadian rhythmicity in individuals resistant to sleep deprivation. Sleep 37(10):1589–1600

Azzi A, Dallmann R, Casserly A, Rehrauer H, Patrignani A et al (2014) Circadian behavior is light-reprogrammed by plastic DNA methylation. Nat Neurosci 17(3):377–382

Bailey MJ, Coon SL, Carter DA, Humphries A, Kim JS et al (2009) Night/day changes in pineal expression of >600 genes: central role of adrenergic/cAMP signaling. J Biol Chem 284 (12):7606–7622

Bale TL (2015) Epigenetic and transgenerational reprogramming of brain development. Nat Rev Neurosci 16(6):332–344

Bannister AJ, Kouzarides T (2011) Regulation of chromatin by histone modifications. Cell Res 21 (3):381–395

Basheer R, Brown R, Ramesh V, Begum S, McCarley RW (2005) Sleep deprivation-induced protein changes in basal forebrain: implications for synaptic plasticity. J Neurosci Res 82 (5):650–658

Bellesi M, Pfister-Genskow M, Maret S, Keles S, Tononi G, Cirelli C (2013) Effects of sleep and wake on oligodendrocytes and their precursors. J Neurosci 33(36):14288–14300

Bellesi M, de Vivo L, Tononi G, Cirelli C (2015) Effects of sleep and wake on astrocytes: clues from molecular and ultrastructural studies. BMC Biol 13:66

Bettica P, Squassante L, Groeger JA, Gennery B, Winsky-Sommerer R, Dijk DJ (2012) Differential effects of a dual orexin receptor antagonist (SB-649868) and zolpidem on sleep initiation and consolidation, SWS, REM sleep, and EEG power spectra in a model of situational insomnia. Neuropsychopharmacology 37(5):1224–1233

Buenrostro JD, Wu B, Chang HY, Greenleaf WJ (2015) ATAC-seq: a method for assaying chromatin accessibility genome-wide. Curr Protoc Mol Biol 109:21.29.1–21.29.9

Chiang CK, Mehta N, Patel A, Zhang P, Ning Z et al (2014) The proteomic landscape of the suprachiasmatic nucleus clock reveals large-scale coordination of key biological processes. PLoS Genet 10(10):e1004695

Cirelli C, Tononi G (2000) Gene expression in the brain across the sleep-waking cycle. Brain Res 885(2):303–321

Cirelli C, Gutierrez CM, Tononi G (2004) Extensive and divergent effects of sleep and wakefulness on brain gene expression. Neuron 41(1):35–43

Cirelli C, LaVaute TM, Tononi G (2005) Sleep and wakefulness modulate gene expression in Drosophila. J Neurochem 94(5):1411–1419

Cirelli C, Faraguna U, Tononi G (2006) Changes in brain gene expression after long-term sleep deprivation. J Neurochem 98(5):1632–1645

Cirelli C, Pfister-Genskow M, McCarthy D, Woodbury R, Tononi G (2009) Proteomic profiling of the rat cerebral cortex in sleep and waking. Arch Ital Biol 147(3):59–68

Daan S, Beersma DG, Borbély AA (1984) Timing of human sleep: recovery process gated by a circadian pacemaker. Am J Phys 246(2 Pt 2):R161–R183

Dallmann R, Viola AU, Tarokh L, Cajochen C, Brown SA (2012) The human circadian metabolome. Proc Natl Acad Sci U S A 109(7):2625–2629

Darlington TM, Ehringer MA, Larson C, Phang TL, Radcliffe RA (2013) Transcriptome analysis of inbred long sleep and inbred short sleep mice. Genes Brain Behav 12(2):263–274

Davies SK, Ang JE, Revell VL, Holmes B, Mann A et al (2014) Effect of sleep deprivation on the human metabolome. Proc Natl Acad Sci U S A 111(29):10761–10766
Davis CJ, Bohnet SG, Meyerson JM, Krueger JM (2007) Sleep loss changes microRNA levels in the brain: a possible mechanism for state-dependent translational regulation. Neurosci Lett 422 (1):68–73
Davis CJ, Clinton JM, Krueger JM (2012) MicroRNA 138, let-7b, and 125a inhibitors differentially alter sleep and EEG delta-wave activity in rats. J Appl Physiol 113(11):1756–1762
Davis CJ, Taishi P, Honn KA, Koberstein JN, Krueger JM (2016) P2X7 receptors in body temperature, locomotor activity, and brain mRNA and lncRNA responses to sleep deprivation. Am J Physiol Regul Integr Comp Physiol 311(6):R1004–R1012
Deery MJ, Maywood ES, Chesham JE, Sládek M, Karp NA et al (2009) Proteomic analysis reveals the role of synaptic vesicle cycling in sustaining the suprachiasmatic circadian clock. Curr Biol 19(23):2031–2036
Duffield GE (2003) DNA microarray analyses of circadian timing: the genomic basis of biological time. J Neuroendocrinol 15(10):991–1002
Durgan DJ, Ganesh BP, Cope JL, Ajami NJ, Phillips SC et al (2016) Role of the gut microbiome in obstructive sleep apnea-induced hypertension. Hypertension 67(2):469–474
Eckel-Mahan KL, Patel VR, Mohney RP, Vignola KS, Baldi P, Sassone-Corsi P (2012) Coordination of the transcriptome and metabolome by the circadian clock. Proc Natl Acad Sci U S A 109 (14):5541–5546
Etchegaray JP, Lee C, Wade PA, Reppert SM (2003) Rhythmic histone acetylation underlies transcription in the mammalian circadian clock. Nature 421(6919):177–182
Faraco J, Lin L, Kornum BR, Kenny EE, Trynka G et al (2013) ImmunoChip study implicates antigen presentation to T cells in narcolepsy. PLoS Genet 9(2):e1003270
Franken P, Chollet D, Tafti M (2001) The homeostatic regulation of sleep need is under genetic control. J Neurosci 21(8):2610–2621
Freyburger M, Pierre A, Paquette G, Bélanger-Nelson E, Bedont J et al (2016) EphA4 is involved in sleep regulation but not in the electrophysiological response to sleep deprivation. Sleep 39 (3):613–624
Fustin JM, Karakawa S, Okamura H (2017) Circadian profiling of amino acids in the SCN and cerebral cortex by laser capture microdissection-mass spectrometry. J Biol Rhythms 32 (6):609–620
Giskeødegård GF, Davies SK, Revell VL, Keun H, Skene DJ (2015) Diurnal rhythms in the human urine metabolome during sleep and total sleep deprivation. Sci Rep 5:14843
Gomes AQ, Nolasco S, Soares H (2013) Non-coding RNAs: multi-tasking molecules in the cell. Int J Mol Sci 14(8):16010–16039
Gottlieb DJ, Hek K, Chen TH, Watson NF, Eiriksdottir G et al (2015) Novel loci associated with usual sleep duration: the CHARGE Consortium Genome-Wide Association Study. Mol Psychiatry 20(10):1232–1239
Hasan S, Pradervand S, Ahnaou A, Drinkenburg W, Tafti M, Franken P (2009) How to keep the brain awake? The complex molecular pharmacogenetics of wake promotion. Neuropsychopharmacology 34(7):1625–1640
Hasan S, van der Veen DR, Winsky-Sommerer R, Hogben A, Laing EE et al (2014) A human sleep homeostasis phenotype in mice expressing a primate-specific PER3 variable-number tandem-repeat coding-region polymorphism. FASEB J 28(6):2441–2454
Havekes R, Abel T (2017) The tired hippocampus: the molecular impact of sleep deprivation on hippocampal function. Curr Opin Neurobiol 44:13–19
Hobson JA (2005) Sleep is of the brain, by the brain and for the brain. Nature 437(7063):1254–1256
Holst SC, Valomon A, Landolt HP (2016) Sleep pharmacogenetics: personalized sleep-wake therapy. Annu Rev Pharmacol Toxicol 56:577–603
Hor H, Kutalik Z, Dauvilliers Y, Valsesia A, Lammers GJ et al (2010) Genome-wide association study identifies new HLA class II haplotypes strongly protective against narcolepsy. Nat Genet 42(9):786–789

Hughes ME, Hong HK, Chong JL, Indacochea AA, Lee SS et al (2012) Brain-specific rescue of clock reveals system-driven transcriptional rhythms in peripheral tissue. PLoS Genet 8(7): e1002835

Hughes ME, Abruzzi KC, Allada R, Anafi R, Arpat AB et al (2017) Guidelines for genome-scale analysis of biological rhythms. J Biol Rhythms 32(5):380–393

Ji Y, Qin Y, Shu H, Li X (2010) Methylation analyses on promoters of mPer1, mPer2, and mCry1 during perinatal development. Biochem Biophys Res Commun 391(4):1742–1747

Jones S, Pfister-Genskow M, Benca RM, Cirelli C (2008) Molecular correlates of sleep and wakefulness in the brain of the white-crowned sparrow. J Neurochem 105(1):46–62

Katada S, Sassone-Corsi P (2010) The histone methyltransferase MLL1 permits the oscillation of circadian gene expression. Nat Struct Mol Biol 17(12):1414–1421

Kaufmann T, Elvsåshagen T, Alnæs D, Zak N, Pedersen PØ et al (2016) The brain functional connectome is robustly altered by lack of sleep. NeuroImage 127:324–332

Keegan KP, Pradhan S, Wang JP, Allada R (2007) Meta-analysis of Drosophila circadian microarray studies identifies a novel set of rhythmically expressed genes. PLoS Comput Biol 3(11):e208

Khalyfa A, Mutskov V, Carreras A, Khalyfa AA, Hakim F, Gozal D (2014) Sleep fragmentation during late gestation induces metabolic perturbations and epigenetic changes in adiponectin gene expression in male adult offspring mice. Diabetes 63(10):3230–3241

Kim J, Bhattacharjee R, Khalyfa A, Kheirandish-Gozal L, Capdevila OS et al (2012) DNA methylation in inflammatory genes among children with obstructive sleep apnea. Am J Respir Crit Care Med 185(3):330–338

Kim JH, Kim JH, Cho YE, Baek MC, Jung JY et al (2014) Chronic sleep deprivation-induced proteome changes in astrocytes of the rat hypothalamus. J Proteome Res 13(9):4047–4061

Koh KP, Rao A (2013) DNA methylation and methylcytosine oxidation in cell fate decisions. Curr Opin Cell Biol 25(2):152–161

Koike N, Yoo SH, Huang HC, Kumar V, Lee C et al (2012) Transcriptional architecture and chromatin landscape of the core circadian clock in mammals. Science 338(6105):349–354

Kolbe I, Husse J, Salinas G, Lingner T, Astiz M, Oster H (2016) The SCN clock governs circadian transcription rhythms in murine epididymal white adipose tissue. J Biol Rhythms 31(6):577–587

Laing EE, Johnston JD, Möller-Levet CS, Bucca G, Smith CP et al (2015) Exploiting human and mouse transcriptomic data: identification of circadian genes and pathways influencing health. BioEssays 37(5):544–556

Le Martelot G, Canella D, Symul L, Migliavacca E, Gilardi F et al (2012) Genome-wide RNA polymerase II profiles and RNA accumulation reveal kinetics of transcription and associated epigenetic changes during diurnal cycles. PLoS Biol 10(11):e1001442

Lim AS, Srivastava GP, Yu L, Chibnik LB, Xu J et al (2014) 24-hour rhythms of DNA methylation and their relation with rhythms of RNA expression in the human dorsolateral prefrontal cortex. PLoS Genet 10(11):e1004792

Liu X, Yanagawa T, Leopold DA, Fujii N, Duyn JH (2015) Robust long-range coordination of spontaneous neural activity in waking, sleep and anesthesia. Cereb Cortex 25(9):2929–2938

Lück S, Thurley K, Thaben PF, Westermark PO (2014) Rhythmic degradation explains and unifies circadian transcriptome and proteome data. Cell Rep 9(2):741–751

Mackiewicz M, Shockley KR, Romer MA, Galante RJ, Zimmerman JE et al (2007) Macromolecule biosynthesis: a key function of sleep. Physiol Genomics 31(3):441–457

Mackiewicz M, Zimmerman JE, Shockley KR, Churchill GA, Pack AI (2009) What are microarrays teaching us about sleep? Trends Mol Med 15(2):79–87

Maekawa F, Shimba S, Takumi S, Sano T, Suzuki T et al (2012) Diurnal expression of Dnmt3b mRNA in mouse liver is regulated by feeding and hepatic clockwork. Epigenetics 7 (9):1046–1056

Manning JH, Courchesne E, Fox PT (2013) Intrinsic connectivity network mapping in young children during natural sleep. NeuroImage 83:288–293

Maret S, Dorsaz S, Gurcel L, Pradervand S, Petit B et al (2007) Homer1a is a core brain molecular correlate of sleep loss. Proc Natl Acad Sci U S A 104(50):20090–20095

Marguerat S, Bähler J (2010) RNA-seq: from technology to biology. Cell Mol Life Sci 67 (4):569–579

Martinez-Lozano Sinues P, Tarokh L, Li X, Kohler M, Brown SA et al (2014) Circadian variation of the human metabolome captured by real-time breath analysis. PLoS One 9(12):e114422

Masri S, Patel VR, Eckel-Mahan KL, Peleg S, Forne I et al (2013) Circadian acetylome reveals regulation of mitochondrial metabolic pathways. Proc Natl Acad Sci U S A 110(9):3339–3344

Massart R, Freyburger M, Suderman M, Paquet J, El Helou J et al (2014) The genome-wide landscape of DNA methylation and hydroxymethylation in response to sleep deprivation impacts on synaptic plasticity genes. Transl Psychiatry 4:e347

Massart R, Suderman M, Mongrain V, Szyf M (2017) DNA methylation and transcription onset in the brain. Epigenomics 9(6):797–809

Mauvoisin D, Wang J, Jouffe C, Martin E, Atger F et al (2014) Circadian clock-dependent and -independent rhythmic proteomes implement distinct diurnal functions in mouse liver. Proc Natl Acad Sci U S A 111(1):167–172

McGowan PO, Szyf M (2010) The epigenetics of social adversity in early life: implications for mental health outcomes. Neurobiol Dis 39(1):66–72

Miller CA, Sweatt JD (2007) Covalent modification of DNA regulates memory formation. Neuron 53(6):857–869

Miller BH, McDearmon EL, Panda S, Hayes KR, Zhang J et al (2007) Circadian and CLOCK-controlled regulation of the mouse transcriptome and cell proliferation. Proc Natl Acad Sci U S A 104(9):3342–3347

Møller M, Sparre T, Bache N, Roepstorff P, Vorum H (2007) Proteomic analysis of day-night variations in protein levels in the rat pineal gland. Proteomics 7(12):2009–2018

Möller-Levet CS, Archer SN, Bucca G, Laing EE, Slak A et al (2013) Effects of insufficient sleep on circadian rhythmicity and expression amplitude of the human blood transcriptome. Proc Natl Acad Sci U S A 110(12):E1132–E1141

Mongrain V, Hernandez SA, Pradervand S, Dorsaz S, Curie T et al (2010) Separating the contribution of glucocorticoids and wakefulness to the molecular and electrophysiological correlates of sleep homeostasis. Sleep 33(9):1147–1157

Monti JM, Torterolo P, Pandi Perumal SR (2017) The effects of second generation antipsychotic drugs on sleep variables in healthy subjects and patients with schizophrenia. Sleep Med Rev 33:51–57

Montiel-Castro AJ, González-Cervantes RM, Bravo-Ruiseco G, Pacheco-López G (2013) The microbiota-gut-brain axis: neurobehavioral correlates, health and sociality. Front Integr Neurosci 7:70

Münzel M, Globisch D, Bruckl T, Wagner M, Welzmiller V et al (2010) Quantification of the sixth DNA base hydroxymethylcytosine in the brain. Angew Chem Int Ed Engl 49(31):5375–5377

Nicod J, Davies RW, Cai N, Hassett C, Goodstadt L et al (2016) Genome-wide association of multiple complex traits in outbred mice by ultra-low-coverage sequencing. Nat Genet 48 (8):912–918

Nilsson EK, Boström AE, Mwinyi J, Schiöth HB (2016) Epigenomics of total acute sleep deprivation in relation to genome-wide DNA methylation profiles and RNA expression. OMICS 20 (6):334–342

Okoniewski MJ, Miller CJ (2006) Hybridization interactions between probesets in short oligo microarrays lead to spurious correlations. BMC Bioinformatics 7:276

Ollila HM, Kettunen J, Pietiläinen O, Aho V, Silander K et al (2014) Genome-wide association study of sleep duration in the Finnish population. J Sleep Res 23(6):609–618

Pawlyk AC, Ferber M, Shah A, Pack AI, Naidoo N (2007) Proteomic analysis of the effects and interactions of sleep deprivation and aging in mouse cerebral cortex. J Neurochem 103 (6):2301–2313

Pellegrino R, Sunaga DY, Guindalini C, Martins RC, Mazzotti DR et al (2012) Whole blood genome-wide gene expression profile in males after prolonged wakefulness and sleep recovery. Physiol Genomics 44(21):1003–1012

Pirooznia SK, Chiu K, Chan MT, Zimmerman JE, Elefant F (2012) Epigenetic regulation of axonal growth of Drosophila pacemaker cells by histone acetyltransferase tip60 controls sleep. Genetics 192(4):1327–1345

Porter NM, Bohannon JH, Curran-Rauhut M, Buechel HM, Dowling AL et al (2012) Hippocampal CA1 transcriptional profile of sleep deprivation: relation to aging and stress. PLoS One 7(7): e40128

Qureshi IA, Mehler MF (2014) An evolving view of epigenetic complexity in the brain. Philos Trans R Soc Lond B Biol Sci 369(1652):20130506

Rajasethupathy P, Antonov I, Sheridan R, Frey S, Sander C et al (2012) A role for neuronal piRNAs in the epigenetic control of memory-related synaptic plasticity. Cell 149(3):693–707

Reddy AB, Karp NA, Maywood ES, Sage EA, Deery M et al (2006) Circadian orchestration of the hepatic proteome. Curr Biol 16(11):1107–1115

Sabir M, Gaudreault PO, Freyburger M, Massart R, Blanchet-Cohen A et al (2015) Impact of traumatic brain injury on sleep structure, electrocorticographic activity and transcriptome in mice. Brain Behav Immun 47:118–130

Sahar S, Sassone-Corsi P (2013) The epigenetic language of circadian clocks. Handb Exp Pharmacol 217:29–44

Schwartz MD, Nguyen AT, Warrier DR, Palmerston JB, Thomas AM, et al (2016) Locus coeruleus and tuberomammillary nuclei ablations attenuate hypocretin/orexin antagonist-mediated REM sleep. eNeuro 3(2):ENEURO.0018-16.2016

Seibt J, Dumoulin MC, Aton SJ, Coleman T, Watson A et al (2012) Protein synthesis during sleep consolidates cortical plasticity in vivo. Curr Biol 22(8):676–682

Shi F, Chen X, Fu A, Hansen J, Stevens R et al (2013) Aberrant DNA methylation of miR-219 promoter in long-term night shiftworkers. Environ Mol Mutagen 54(6):406–413

Soshnev AA, Ishimoto H, McAllister BF, Li X, Wehling MD et al (2011) A conserved long noncoding RNA affects sleep behavior in Drosophila. Genetics 189(2):455–468

Storch KF, Paz C, Signorovitch J, Raviola E, Pawlyk B et al (2007) Intrinsic circadian clock of the mammalian retina: importance for retinal processing of visual information. Cell 130(4):730–741

Syed F, Grunenwald H, Caruccio N (2009) Next-generation sequencing library preparation: simultaneous fragmentation and tagging using in vitro transposition. Nat Methods 6:i–ii

Tafti M, Chollet D, Valatx JL, Franken P (1999) Quantitative trait loci approach to the genetics of sleep in recombinant inbred mice. J Sleep Res 8(Suppl 1):37–43

Takahashi JS, Kumar V, Nakashe P, Koike N, Huang HC et al (2015) ChIP-seq and RNA-seq methods to study circadian control of transcription in mammals. Methods Enzymol 551:285–321

Terao A, Steininger TL, Hyder K, Apte-Deshpande A, Ding J et al (2003) Differential increase in the expression of heat shock protein family members during sleep deprivation and during sleep. Neuroscience 116(1):187–200

Thaiss CA, Zeevi D, Levy M, Zilberman-Schapira G, Suez J et al (2014) Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. Cell 159(3):514–529

Thimgan MS, Seugnet L, Turk J, Shaw PJ (2015) Identification of genes associated with resilience/vulnerability to sleep deprivation and starvation in Drosophila. Sleep 38(5):801–814

Toth LA, Williams RW (1999) A quantitative genetic analysis of slow-wave sleep and rapid-eye movement sleep in CXB recombinant inbred mice. Behav Genet 29(5):329–337

Tudor JC, Davis EJ, Peixoto L, Wimmer ME, van Tilborg E et al (2016) Sleep deprivation impairs memory by attenuating mTORC1-dependent protein synthesis. Sci Signal 9(425):ra41

Valekunja UK, Edgar RS, Oklejewicz M, van der Horst GT, O'Neill JS et al (2013) Histone methyltransferase MLL3 contributes to genome-scale circadian transcription. Proc Natl Acad Sci U S A 110(4):1554–1559

Vecsey CG, Peixoto L, Choi JH, Wimmer M, Jaganath D et al (2012) Genomic analysis of sleep deprivation reveals translational regulation in the hippocampus. Physiol Genomics 44 (20):981–991

Ventskovska O, Porkka-Heiskanen T, Karpova NN (2015) Spontaneous sleep-wake cycle and sleep deprivation differently induce Bdnf1, Bdnf4 and Bdnf9a DNA methylation and transcripts levels in the basal forebrain and frontal cortex in rats. J Sleep Res 24(2):124–130

Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 10(1):57–63

Wang H, Liu Y, Briesemann M, Yan J (2010) Computational analysis of gene regulation in animal sleep deprivation. Physiol Genomics 42(3):427–436

Weljie AM, Meerlo P, Goel N, Sengupta A, Kayser MS et al (2015) Oxalic acid and diacylglycerol 36:3 are cross-species markers of sleep debt. Proc Natl Acad Sci U S A 112(8):2569–2574

Winkelmann J, Lin L, Schormair B, Kornum BR, Faraco J et al (2012) Mutations in DNMT1 cause autosomal dominant cerebellar ataxia, deafness and narcolepsy. Hum Mol Genet 21 (10):2205–2210

Winrow CJ, Williams DL, Kasarskis A, Millstein J, Laposky AD et al (2009) Uncovering the genetic landscape for multiple sleep-wake traits. PLoS One 4(4):e5161

Wither RG, Colic S, Wu C, Bardakjian BL, Zhang L, Eubanks JH (2012) Daily rhythmic behaviors and thermoregulatory patterns are disrupted in adult female MeCP2-deficient mice. PLoS One 7 (4):e35396

Wolffe AP, Kurumizaka H (1998) The nucleosome: a powerful regulator of transcription. Prog Nucleic Acid Res Mol Biol 61:379–422

Wu H, D'Alessio AC, Ito S, Wang Z, Cui K, Zhao K et al (2011) Genome-wide analysis of 5-hydroxymethylcytosine distribution reveals its dual function in transcriptional regulation in mouse embryonic stem cells. Genes Dev 25(7):679–684

Xia L, Ma S, Zhang Y, Wang T, Zhou M et al (2015) Daily variation in global and local DNA methylation in mouse livers. PLoS One 10(2):e0118101

Yelin-Bekerman L, Elbaz I, Diber A, Dahary D, Gibbs-Bar L et al (2015) Hypocretin neuron-specific transcriptome profiling identifies the sleep modulator Kcnh4a. elife 4:e08638

Yu M, Hon GC, Szulwach KE, Song CX, Jin P et al (2012) Tet-assisted bisulfite sequencing of 5-hydroxymethylcytosine. Nat Protoc 7(12):2159–2170

Zhang RR, Cui QY, Murai K, Lim YC, Smith ZD et al (2013) Tet1 regulates adult hippocampal neurogenesis and cognition. Cell Stem Cell 13(2):237–245

Zhao Z, Fan L, Frick KM (2010) Epigenetic alterations regulate estradiol-induced enhancement of memory consolidation. Proc Natl Acad Sci U S A 107(12):5605–5610

Zhu Y, Stevens RG, Hoffman AE, Tjonneland A, Vogel UB et al (2011) Epigenetic impact of long-term shiftwork: pilot evidence from circadian genes and whole-genome methylation analysis. Chronobiol Int 28(10):852–861

Zimmerman JE, Rizzo W, Shockley KR, Raizen DM, Naidoo N et al (2006) Multiple mechanisms limit the duration of wakefulness in Drosophila brain. Physiol Genomics 27(3):337–350

The Role of Glia in Sleep Regulation and Function

Marcos G. Frank

Contents

Abstract

The cellular mechanisms governing the expression, regulation, and function of sleep are not entirely understood. The traditional view is that these mechanisms are neuronal. An alternative view is that glial brain cells may play important roles in these processes. Their ubiquity in the central nervous system makes them well positioned to modulate neuronal circuits that gate sleep and wake. Their ability to respond to chemical neuronal signals suggests that they form feedback loops with neurons that may globally regulate neuronal activity. Their potential role in detoxifying the brain, regulating neuronal metabolism, and promoting synaptic plasticity raises the intriguing possibility that glia mediate important functions ascribed to sleep.

Keywords

Astrocyte · Glia · Homeostasis · Neural · Sleep

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1 Introduction

Glia refers to nonneuronal cells of the nervous system. The three main classes of glia in the mammalian brain include astrocytes, microglia, and oligodendrocytes. Astrocytes perform a number of "housekeeping" functions in the brain, including buffering ions, recycling neurotransmitter, and regulating metabolism. Microglia are the immune cells of the central nervous system and play critical roles in the response to neural injury and cellular stress. Oligodendrocytes produce myelin and enwrap axons and dysfunction in these cells leads to demyelination and disease. They are considered non-excitable, as they do not produce action potentials and respond linearly to current injections. For these reasons, they have historically been viewed as supportive brain cells with no special or direct roles in brain activity or behavior. However, through several different mechanisms, including neurotransmitter uptake, ion transport, and direct chemical signaling (i.e., gliotransmission), glial cells can alter the activity of surrounding neurons. This provides a means of influencing behavior and brain activity (Fiacco et al. 2009; Halassa and Haydon 2010; Hamilton and Atwell 2010). Therefore, glia are well positioned to control not only the expression and regulation of sleep but also sleep functions (Fig. 1).

2 Glial Cells and Sleep Homeostasis

Sleep homeostasis refers to a regulatory mechanism that increases sleep drive, sleep amounts, and sleep intensity as a function of prior time awake (Borbély and Achermann 2000; Dijk and Lockley 2002). In contrast to the circadian clock, which is well characterized anatomically and molecularly, the biological substrates of sleep homeostasis are poorly understood. Several sets of findings support a role for glia in this type of sleep regulation.

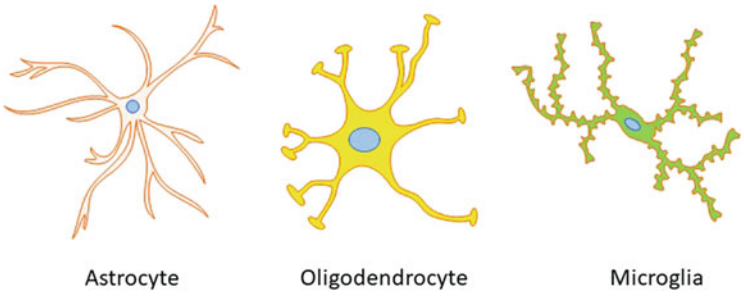

Fig. 1 Glial cells involved in sleep expression, regulation, and function. The three main classes of glia in the mammalian brain include astrocytes, microglia, and oligodendrocytes. All three types of glia may play important roles in mammalian sleep, either by the secretion of somnogenic substances, uptake of neurotransmitter, detoxification of metabolic wastes, or providing energy substrates to neurons

2.1 Exocytosis of Somnogenic Substances

Glial cells in vitro release a variety of molecules that when injected either systemically or into the brain can increase sleep time or non-rapid eye movement (NREM) slow-wave activity (SWA). For example, the cytokine IL-1 derived from cultured mouse astrocytes increases NREM sleep in rats when administered into the ventricles (Tobler et al. 1984). Cultured astrocytes also secrete neurotrophins (e.g., brain-derived neurotrophin factor (BDNF)), prostaglandins (PGD2), and the cytokine tumor necrosis factor (TNFα) that increase sleep time or intensity (e.g., NREM SWA) when injected intraventricularly and/or infused applied to the neocortex (Kushikata et al. 1999; Hayaishi 2002; Huang et al. 2007; Faraguna et al. 2008; Krueger 2008). Astrocytes exocytose additional chemical transmitters that modulate neuronal excitability, including adenosine triphosphate (ATP) which is hydrolyzed to adenosine in the extracellular space (Pascual et al. 2005; Halassa et al. 2009a). This latter observation is particularly important because adenosine is considered a key mediator of mammalian sleep homeostasis.

Although these findings are intriguing, astrocytes in vitro often show very different properties than cells in vivo (Yamamoto et al. 1988; Inagaki and Wada 1994). In addition, although mutant mice lacking the TNFα, interleukin (IL)-1, and purinergic receptors have sleep phenotypes consistent with this general hypothesis (i.e., reduced NREM sleep amounts and intensity), it is not known if this reflects neuronal or astrocytic influences, as neurons release and respond to many cytokines as well. Therefore, determining the precise role of astrocyte cytokine signaling in sleep requires selective manipulations of these signaling pathways in vivo.

This was achieved by manipulating one mechanism of astrocytic exocytosis in vivo. Gliotransmission involves the vesicular release of substances such as ATP via proteins similar to those involved in synaptic release of neurotransmitters (Pascual et al. 2005; Halassa et al. 2009a). The precise mechanisms of gliotransmission are debated (Parpura and Zorec 2010; Nedergaard and Verkhratsky 2012), but for some gliotransmitters, exocytosis may depend on the formation of a SNARE complex between vesicles and the target membrane (Scales et al. 2000). Conditional astrocyte-selective expression of a soluble SNARE domain of the protein synaptobrevin II prevents both tonic and activity-dependent extracellular accumulation of adenosine that acts on A1 receptors in situ (Pascual et al. 2005). The expression of this mutation acts as a dominant/negative (dn)protein which inhibits the normal function of the endogenous SNARE.

The role of SNARE-dependent gliotransmission in sleep homeostasis was investigated in vivo using the tet-off system (Morozov et al. 2003). This allows for conditional expression of a dnSNARE transgene selectively in astrocytes in adult mice (Pascual et al. 2005). Suppressing gliotransmission in vivo had surprisingly little effect on baseline sleep-wake architecture (Halassa et al. 2009b), but behavioral and EEG indices of sleep homeostasis (NREM SWA) were attenuated. More specifically, inhibiting gliotransmission significantly reduced compensatory increases in NREM SWA, sleep time, and sleep continuity. These results are best interpreted as a reduction in adenosine. For example, the dnSNARE phenotype was

"phenocopied" in wt mice via antagonism of the A1 receptor (Halassa et al. 2009b). The dnSNARE mutation reduces the normal accumulation of adenosine during sleep deprivation (Schmitt et al. 2012) and produces a phenotype similar to that in mice with abnormal purinergic signaling (Huang et al. 2005; Krueger et al. 2010). A role for gliotransmission in sleep homeostasis is further supported by studies using optogenetic activation of astrocytes in vivo. This technique increases gliotransmission of ATP (Losi et al. 2017), and optogenetic activation of astrocytes surrounding waking circuits (i.e., histaminergic neurons) increases sleep time and intensity (Pelluru et al. 2016). Gliotransmission of other molecules (D-serine and glutamate) may also play a key role in producing cortical oscillations typical of NREM sleep (Fellin et al. 2009; Poskanzer and Yuste 2016) (Fig. 2).

Cerebral microglia and oligodendrocyte cells also secrete a number of substances in vitro known to influence sleep or brain activity in sleep (e.g., cytokines, prostaglandins, and nitric oxide) (Matsui et al. 2010). Because sleep deprivation is associated with an increase in markers of cellular stress, it has been proposed that substances secreted by microglia may play a central role in sleep regulation (Wisor et al. 2011a, b). For example, attenuation of microglia reactivity with minocycline reduces the normal compensatory increases in NREM SWA in mice following sleep deprivation (Wisor and Clegern 2011). Interestingly, microglia contain membrane-bound purinergic receptors, providing a means of interaction with astrocyte-derived ATP and adenosine (Haynes et al. 2006; Gyoneva et al. 2009). On the other hand,

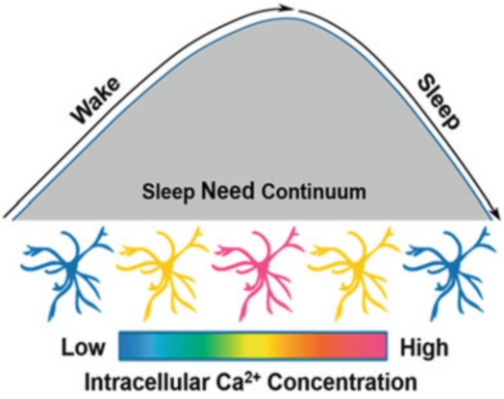

Fig. 2 Astrocytes as integrators of sleep need. Astrocytes respond to neurotransmitters and neuromodulators with slow changes in intracellular calcium concentrations. Consequently, astrocytes may use intracellular calcium oscillations to register and integrate surrounding neuronal activity during wakefulness. This may lead to progressively higher rates of negative feedback (e.g., via gliotransmission) that dampen the "waking" signal and promote sleep. One prediction of this hypothesized relationship is that the amplitude or period of intracellular calcium oscillations should be proportional to sleep drive. Therefore, intracellular calcium concentrations (or period frequency) are predicted to be low at the end of the sleep period and maximal at the end of the active period. Alternatively (or in conjunction), astrocytes may respond to waking signals by expanding and then contracting their distal processes. This can bring glial neurotransmitter uptake or gliotransmitter release mechanisms in closer proximity to synapses as a function of synaptic activity (adapted)

a putative transducer of microglial-mediated effects (the toll-like receptor 4 (TLR4)) does not appear to play a central role in sleep brain activity. Constitutive deletion of TLR4 minimally impacts NREM SWA under baseline conditions or after sleep deprivation (Wisor et al. 2011a). Oligodendrocytes are a source of prostaglandin D2 in the mature brain, which has been shown to be a potent sleep-inducing substance when applied exogenously (Urade and Hayaishi 2011). In addition to increasing behavioral indices of sleep, stimulation of D2 receptors increases NREM SWA in a physiological manner (Urade and Hayaishi 2011). However, in contrast to astrocytes, much less is known about how microglia and oligodendrocytes exocytose somnogenic substances. It is also unknown what signals normally trigger the release of these substances across the sleep-wake cycle.

2.2 Uptake and Transport

Glia can also modulate synaptic activity by passive and active transport of ions, neurotransmitters/neuromodulators, and movement of molecules via gap junctions. In addition to gliotransmission of ATP, astrocytes can control extracellular adenosine concentrations via adenosine kinase (ADK). Deletion of ADK in astrocytes results in an accumulation of adenosine in the extracellular space and an attenuation of the normal discharge of sleep need (as measured by NREM SWA). Whether this reflects direct effects on sleep homeostasis is unclear, as this also results in a general "leakage" of SWA into all brain states (Greene et al. 2017). The movement of small molecules between glial cells may also promote SWA. Blockade of cortical astrocytic gap junctions in vivo reduces anesthesia-induced neuronal synchronization, which is similar (though not identical) to the synchronization underlying NREM SWA (Szabó et al. 2017).

Astrocytes buffer ions which may further facilitate synchronized neuronal activity necessary for NREM SWA (Crunelli et al. 2002). For example, astrocytes in thalamic slices (in situ) exhibit spontaneous intracellular calcium oscillations that fall within "slow" EEG bands (<0.1 Hz). These oscillations can propagate within the slice and elicit NMDA currents in neighboring neurons. This particular coupling between neurons and astrocytes may not be related to the EEG rhythms of sleep, as it predominates at ages when thalamocortical and intracortical EEG activities typical of adult sleep are absent (Crunelli and Hughes 2010). The thalamus also does not appear to be required for NREM cortical slow-wave activity (SWA) (Fuller et al. 2011; but see Crunelli et al. 2011). More compelling evidence that astrocytes contribute to slow, EEG rhythms of sleep via ion buffering comes from studies using dual intracellular recording in cortical astrocytes and neurons (Amzica and Neckelmann 1999; Amzica 2002; Amzica and Massimini 2002). These investigators showed that astrocyte membrane polarization and capacitance oscillate in phase with NREM SWA, suggesting that the cation buffering by these cells may also contribute to cortical oscillations in sleep.

2.3 Morphological Changes in Glia

Glia can be quite dynamic either by proliferating rapidly or extending and retracting their processes in response to changes in the neural environment. This can bring their membranes and the embedded transporters, uptake mechanisms, and vesicle release apparatus closer or further to synapses. Therefore, as originally suggested by Ramon y Cajal (reviewed in García-Marín et al. 2007), morphological changes in glia across the sleep-wake cycle may be a key step in glial control of sleep. Morphological changes in astrocytes have been reported following acute and chronic sleep deprivation in mice. In these studies, serial block face scanning electron microscopy was used to reconstruct astrocytic processes that surround synapses (peri-synaptic processes: PAPs). It was found that sleep deprivation caused the PAPs to move closer to synapses in layer 2 of the cortex. Molecular analyses of astrocytes further revealed that a number of genes implicated in PAP extension were also upregulated by waking (Bellesi et al. 2015). In addition, an important molecule involved in PAP morphology (Fabp7) was shown to cycle in tandem with the sleep-wake cycle in rodents (Gerstner et al. 2012), and mutations in the Fabp7 gene reduced sleep bout length in flies, man, and mice (Gerstner et al. 2017). Interestingly, oligodendrocytes were shown to proliferate during sleep (with positive correlations with REM sleep). This was accompanied by an increase in the cortical expression of oligodendrocyte genes implicated in phospholipid synthesis and myelination (Bellesi et al. 2013).

3 Glial Cells and Sleep Function

Glia mediate several neural processes that have historically been theorized to be important functions of sleep. These include brain metabolism, neural detoxification and repair, and synaptic plasticity. Therefore in addition to potentially playing central roles in sleep regulation, glia may also govern critical functions ascribed to sleep.

3.1 Metabolism

Sleep is thought to be a restorative brain state that relieves the metabolic cost of wakefulness (Frank 2010). How sleep achieves this putative function (and what is restored) is unknown, but this idea is supported by several findings. For example, brain metabolism is reduced (overall) during sleep compared to wakefulness (Hyder et al. 2013; but see Shannon et al. 2012). Sleep is also accompanied by increased synthesis of proteins and neural energy substrates (Ramm and Smith 1990; Kong et al. 2002; Dworak et al. 2010; Clegern et al. 2012; Seibt et al. 2012). Astrocytes are well positioned to influence these processes. In many parts of the brain, astrocytic processes not only surround synapses but are in close approximation to blood vessels (via “end feet”). This provides for nutrient uptake and the regulation of blood flow via constriction and dilation of vessels (Takano et al. 2006; Kreft et al. 2012).

Astrocytes also contain energy substrates than can be rapidly mobilized and used by neurons (lactate and glycogen) (Magistretti 2011).

Early and suggestive findings that support a role for glia in sleep-mediated metabolism were reported by Hyden and Lange. They found sleep-wake rhythms in succinooxidase (a key enzymatic step in the Krebs cycle) activity in neurons and glia (type not specified). Glia showed reduced succinooxidase activity and neurons heightened activity during sleep (Hyden and Lange 1965). The authors proposed that "neuron and glia form a functional unit" and that an exchange of signaling molecules between these cell types might influence sleep.

According to the Benington-Heller hypothesis, the transferred molecules included glucose. It was proposed that astrocytic glycogen – which acts as a reserve glucose store for neurons – is depleted during wakefulness and restored during NREM sleep. The depletion of glycogen was mediated by the heightened release of excitatory neurotransmitters during wake which through enzymatic mechanisms convert astrocytic glycogen into glucose. The utilization of this glucose by neurons (and the subsequent hydrolysis of ATP to adenosine monophosphate) leads to an increase in neuronal adenosine production – which diffuses across the cell membrane. Adenosine, acting on adenosine1 receptors, then reduces neuronal excitability. The restoration of glycogen is favored by states with reduced release of excitatory neurotransmitters, such as NREM sleep (Benington and Heller 1995). In support of the theory, sleep deprivation increases the concentration of mRNA transcripts involved in glycogen metabolism and glycogen synthase activity (Petit et al. 2002, 2010). However, studies of cerebral glycogen across sleep and wakefulness generally do not support this theory. While one study showed decreases in brain glycogen content following sleep deprivation and increases following recovery sleep (Kong et al. 2002), these findings were not replicated by other labs (Gip et al. 2002, 2004; Franken et al. 2003; Zimmerman et al. 2004; Petit et al. 2010).

One alternative mechanism is the neuronal-glial "lactate shuttle," according to which synaptic glutamate release triggers lactate production and release in astrocytes (reviewed in Magistretti 2011). In support of this idea, brain lactate levels increase during wakefulness and decrease during NREM sleep in parallel with NREM SWA (Dash et al. 2012; Naylor et al. 2012; Wisor et al. 2012). A number of genes important in cerebral metabolism (Bellesi et al. 2015) and the lactate shuttle are specifically upregulated in cortical astrocytes after sleep deprivation (Petit et al. 2013). This is consistent with observations that astrocytic metabolic pathways are activated to a greater degree during wakefulness. Astrocytes may also provide energetic support specifically to neurons involved in wakefulness. Astrocytes provide energetic support (via lactate) to hypocretin neurons involved in wakefulness and state stability. Mice with a mutation in this process are unable to sustain normal amounts of wakefulness (Clasadonte et al. 2017).

3.2 Detoxification and Repair

As originally proposed at the beginning of the twentieth century, the "hypnotoxin" theory of sleep function posited that sleep removes a noxious by-product of the waking brain (Ishimori 1909). Modern versions of this theory propose that sleep reverses or protects against neuronal damage caused by prolonged glutamate release or oxidative processes that occur during wakefulness (Reimund 1994; Inoue et al. 1995; Schulze 2004). The evidence in support of the hypnotoxin theory has been rather mixed. For example, there is no compelling evidence that sleep loss produces neural damage as might be expected by unchecked oxidative processes or the accumulation of a toxin (reviewed in Frank 2006). However, more subtle but important detoxification/repair processes may occur during sleep due to astrocytic and microglia activation. It was shown that during sleep (or anesthesia) there is an increased clearance of extracellular proteins from the brain interstitial fluid. This involves a filtration system provided by astrocytes (the "glymphatic" system) (Xie et al. 2013). The bulk flow/convective flow of brain interstitial fluid is greatly attenuated by sleep deprivation (Achariyar et al. 2016), which may lead to the accumulation of proteins such as beta-amyloid and apolipoprotein E, isoforms of which are linked to neurodegeneration (Yulug et al. 2017). Sleep deprivation also produces microglial activation and astrocytic phagocytosis. These latter changes may play an important role in the removal of metabolic waste products and/or synaptic proteins and membranes that accumulate during waking (Bellesi et al. 2017).

3.3 Synaptic Plasticity

Synaptic plasticity is defined as changes in the strength of existing synapses (synaptic efficacy), changes in synapse number or size, or changes in morphological structures that contain synapses (e.g., dendritic spines and presynaptic boutons) (De Roo et al. 2008; Sala and Segal 2014). Sleep has long been suspected to play an important role in synaptic plasticity, and glial cells may mediate this important putative function. Astrocytes and microglia profoundly influence synaptogenesis and synaptic pruning during early development. There is also accumulating evidence that these glial cells modulate both Hebbian and non-Hebbian forms of plasticity in vitro and in vivo (Volterra 2013; Stogsdill and Eroglu 2017). Glia cells influence plasticity through several processes, including metabolic support; glutamate uptake; exocytosis of growth factors, adhesion molecules, and gliotransmitters; and phagocytosis (Volterra 2013; Stogsdill and Eroglu 2017).

It is currently unknown if glial cells mediate sleep-dependent plasticity. However, this is suggested by several findings. Sleep deprivation increases glial-mediated phagocytosis of cortical synapses, which is consistent with synaptic pruning during sleep (Bellesi et al. 2017). Sleep is also accompanied by higher brain concentrations of the glial-derived cytokine TNF-α, which promotes non-Hebbian synaptic potentiation (Krueger 2008). In addition astrocytes (Imamura et al. 1993) and microglia (Sipe et al. 2016) are required for a classic form of visual cortical plasticity (ocular

dominance plasticity) that is highly sleep-dependent and involves both synaptic weakening and strengthening (Frank 2015). Collectively, these findings suggest that glia may mediate diverse forms of plastic remodeling in the sleeping brain.

4 Conclusions

The precise role of glia in sleep regulation and function is only now beginning to be explored. Consequently, there are many important unanswered questions. The relative contribution of different glia subtypes to sleep is poorly understood, as is the relative importance of various glial mechanisms (e.g., gliotransmission, ion buffering, glutamate clearance, metabolic support, and non-vesicle-mediated exocytosis (Magistretti 2011; Nedergaard and Verkhratsky 2012; Verkhratsky et al. 2012)). It is also not clear where in the brain glia exert their effects on sleep. Astrocytes, for example, are dispersed widely in subcortical and cortical brain areas (Zhang and Haydon 2005) including regions known to trigger sleep and wakefulness (Halassa et al. 2009b). Therefore, they may regulate sleep and brain activity by acting globally throughout the cerebrum, via modulation of canonical sleep and arousal centers, or both (Benington and Heller 1995; Strecker et al. 2000; Szymusiak et al. 2007; Krueger et al. 2008).

The precise signaling pathways linking neuronal activity and glial-mediated sleep processes are also unknown. Astrocytes and microglia in vivo express a number of membrane-bound receptors for neurotransmitters and peptides, which could mediate neuronal-glial transduction pathways critical for sleep regulation and function. Theoretically, these receptors as well as other membrane-bound peptides could provide new pharmacological targets for arousal and sleep disorders. For example, the astrocyte-specific water transporting protein aquaporin 4 influences processes that could impact sleep and wake (neuronal excitation and neuroinflammation). Several laboratories are developing compounds that target this protein (Verkman et al. 2017). A different strategy is to repurpose existing compounds (which may have failed as neuronal-targeted compounds) as novel glial pharmacopeia (Dale et al. 2016). The development of novel delivery systems that specifically deliver drugs to glial cells provides yet another strategy. This may be achieved through the development of nanoparticles or polymers coated with surface peptides or lipids that result in the selective uptake by glia (Madhusudanan et al. 2017). Despite these promising steps, the development of glial-specific compounds remains very challenging (Möller and Boddeke 2016; Madhusudanan et al. 2017).

Investigating glia in vivo with the same precision as used in neurons has historically been very difficult. This situation is rapidly improving with the development of new tools designed for use in glia. A number of glial-specific promoters have been identified, and when expressed in conjunction with *Cre-Lox*, or viral vector strategies, they can be used to selectively delete or express molecules of interest in glial cells in vivo. The use of calcium indicator fluorescent proteins (i.e., GCaMPs) has revolutionized the study of astrocytes, as these cells are electrically silent and instead signal with changes in intracellular calcium (Bazargani and Attwell 2016, 2017; Kjaerby et al. 2017; Losi et al. 2017). Optogenetics has also been used to

trigger gliotransmission (although the mechanisms at play are unclear) (Bazargani and Attwell 2016, 2017; Kjaerby et al. 2017; Losi et al. 2017). The future application of these techniques in freely behaving animals will be key to a deeper understanding of how glia and neurons contribute to sleep regulation and function.

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References

Achariyar TM, Li B, Peng W et al (2016) Glymphatic distribution of CSF-derived apoE into brain is isoform specific and suppressed during sleep deprivation. Mol Neurodegener 11:74. https://doi.org/10.1186/s13024-016-0138-8

Amzica F (2002) In vivo electrophysiological evidences for cortical neuron-glia interactions during slow (<1 Hz) and paroxysmal sleep oscillations. J Physiol Paris 96:209–219

Amzica F, Massimini M (2002) Glial and neuronal interactions during slow wave and paroxysmal activities in the neocortex. Cereb Cortex 12:1101–1113

Amzica F, Neckelmann D (1999) Membrane capacitance of cortical neurons and glia during sleep oscillations and spike-wave seizures. J Neurophysiol 82:2731–2746

Bazargani N, Attwell D (2016) Astrocyte calcium signaling: the third wave. Nat Neurosci 19:182–189. https://doi.org/10.1038/nn.4201

Bazargani N, Attwell D (2017) Amines, astrocytes, and arousal. Neuron 94:228–231. https://doi.org/10.1016/j.neuron.2017.03.035

Bellesi M, Pfister-Genskow M, Maret S, Keles S, Tononi G, Cirelli C (2013) Effects of sleep and wake on oligodendrocytes and their precursors. J Neurosci 33:14288–14300

Bellesi M, de Vivo L, Tononi G, Cirelli C (2015) Effects of sleep and wake on astrocytes: clues from molecular and ultrastructural studies. BMC Biol 13:66. https://doi.org/10.1186/s12915-015-0176-7

Bellesi M, de Vivo L, Chini M, Gilli F, Tononi G, Cirelli C (2017) Sleep loss promotes astrocytic phagocytosis and microglial activation in mouse cerebral cortex. J Neurosci 37:5263

Benington J, Heller HC (1995) Restoration of brain energy metabolism as the function of sleep. Prog Neurobiol 45:347–360

Borbély AA, Achermann P (2000) Sleep homeostasis and models of sleep regulation. In: Kryger M, Roth T, Dement WC (eds) Principles and practice of sleep medicine, 3rd edn. Saunders, Philadelphia, pp 377–390

Clasadonte J, Scemes E, Wang Z, Boison D, Haydon PG (2017) Connexin 43-mediated astroglial metabolic networks contribute to the regulation of the sleep-wake cycle. Neuron 95:1365–1380. e1365. https://doi.org/10.1016/j.neuron.2017.08.022

Clegern WC, Moore ME, Schmidt MA, Wisor J (2012) Simultaneous electroencephalography, real-time measurement of lactate concentration and optogenetic manipulation of neuronal activity in the rodent cerebral cortex. J Vis Exp 70:e4328. https://doi.org/10.3791/4328

Crunelli V, Hughes SW (2010) The slow (<1 Hz) rhythm of non-REM sleep: a dialogue between three cardinal oscillators. Nat Neurosci 13:9–17. https://doi.org/10.1038/nn.2445

Crunelli V, Blethyn KL, Cope DW et al (2002) Novel neuronal and astrocytic mechanisms in thalamocortical loop dynamics. Philos Trans R Soc Lond Ser B Biol Sci 357:1675–1693. https://doi.org/10.1098/rstb.2002.1155

Crunelli V, Errington AC, Hughes SW, Tóth TI (2011) The thalamic low-threshold Ca2+ potential: a key determinant of the local and global dynamics of the slow (<1 Hz) sleep oscillation in thalamocortical networks. Philos Trans R Soc A Math Phys Eng Sci 369:3820–3839

Dale E, Staal RGW, Eder C, Möller T (2016) KCa3.1 – a microglial target ready for drug repurposing? Glia 64:1733–1741. https://doi.org/10.1002/glia.22992

Dash MB, Tononi G, Cirelli C (2012) Extracellular levels of lactate, but not oxygen, reflect sleep homeostasis in the rat cerebral cortex. Sleep 35:909–919. https://doi.org/10.5665/sleep.1950

De Roo M, Klauser P, Garcia PM, Poglia L, Muller D (2008) Spine dynamics and synapse remodeling during LTP and memory processes. Prog Brain Res 169:199–207

Dijk DJ, Lockley SW (2002) Integration of human sleep-wake regulation and circadian rhythmicity. J Appl Physiol 92:852–862

Dworak M, McCarley RW, Kim T, Kalinchuk AV, Basheer R (2010) Sleep and brain energy levels: ATP changes during sleep. J Neurosci 30:9007–9016

Faraguna U, Vyazovskiy VV, Nelson AB, Tononi G, Cirelli C (2008) A causal role for brain-derived neurotrophic factor in the homeostatic regulation of sleep. J Neurosci 28:4088–4095

Fellin T, Halassa MM, Terunuma M et al (2009) Endogenous nonneuronal modulators of synaptic transmission control cortical slow oscillations in vivo. Proc Natl Acad Sci U S A 106:15037–15042. https://doi.org/10.1073/pnas.0906419106

Fiacco TA, Agulhon C, McCarthy KD (2009) Sorting out astrocyte physiology from pharmacology. Annu Rev Pharmacol Toxicol 49:151–174. https://doi.org/10.1146/annurev.pharmtox.011008.145602

Frank MG (2006) The mystery of sleep function: current perspectives and future directions. Rev Neurosci 17:375–392

Frank MG (2010) The functions of sleep. In: Winkelman JW, Plante DT (eds) Foundations of psychiatric sleep medicine. Cambridge University Press, Cambridge, pp 59–78

Frank M (2015) Sleep and synaptic plasticity in the developing and adult brain. Curr Top Behav Neurosci 25:123–149. https://doi.org/10.1007/7854_2014_305

Franken P, Gip P, Hagiwara G, Ruby NF, Heller HC (2003) Changes in brain glycogen after sleep deprivation vary with genotype. Am J Physiol Regul Integr Comp Physiol 285:R413–R419

Fuller P, Sherman D, Pedersen NP, Saper CB, Lu J (2011) Reassessment of the structural basis of the ascending arousal system. J Comp Neurol 519:933–956. https://doi.org/10.1002/cne.22559

García-Marín V, García-López P, Freire M (2007) Cajal's contributions to glia research. Trends Neurosci 30:479–487

Gerstner JR, Vanderheyden WM, LaVaute T et al (2012) Time of day regulates subcellular trafficking, tripartite synaptic localization, and polyadenylation of the astrocytic Fabp7 mRNA. J Neurosci 32:1383

Gerstner JR, Perron IJ, Riedy S et al (2017) Normal sleep requires the astrocyte brain-type fatty acid binding protein FABP7. Sci Adv 3(4):e1602663

Gip P, Hagiwara G, Ruby NF, Heller HC (2002) Sleep deprivation decreases glycogen in the cerebellum but not in the cortex of young rats. Am J Physiol Regul Integr Comp Physiol 283: R54–R59

Gip P, Hagiwara G, Sapolsky RM, Cao VH, Heller HC, Ruby NF (2004) Glucocorticoids influence brain glycogen levels during sleep deprivation. Am J Physiol Regul Integr Comp Physiol 286: R1057–R1062

Greene RW, Bjorness TE, Suzuki A (2017) The adenosine-mediated, neuronal-glial, homeostatic sleep response. Curr Opin Neurobiol 44:236–242. https://doi.org/10.1016/j.conb.2017.05.015

Gyoneva S, Orr AG, Traynelis SF (2009) Differential regulation of microglial motility by ATP/ADP and adenosine. Parkinsonism Relat Disord 15(Suppl 3):S195–S199

Halassa MM, Haydon PG (2010) Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. Annu Rev Physiol 72:335–355. https://doi.org/10.1146/annurev-physiol-021909-135843

Halassa MM, Fellin T, Haydon PG (2009a) Tripartite synapses: roles for astrocytic purines in the control of synaptic physiology and behavior. Neuropharmacology 57:343–346. https://doi.org/10.1016/j.neuropharm.2009.06.031

Halassa MM, Florian C, Fellin T et al (2009b) Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. Neuron 61:213–219. https://doi.org/10.1016/j.neuron.2008.11.024

Hamilton NB, Atwell D (2010) Do astrocytes really exocytose neurotransmitters? Nat Rev Neurosci 11:227–238
Hayaishi O (2002) Functional genomics of sleep and circadian rhythm: invited review: molecular genetic studies on sleep-wake regulation, with special emphasis on the prostaglandin D2 system. J Appl Physiol 92:863–868
Haynes SE, Hollopeter G, Yang G, Kurpius D, Dailey ME, Gan W-B, Julius D (2006) The P2Y12 receptor regulates microglial activation by extracellular nucleotides. Nat Neurosci 9:1512–1519
Huang Z-L, Qu W-M, Eguchi N et al (2005) Adenosine A2A, but not A1, receptors mediate the arousal effect of caffeine. Nat Neurosci 8:858–859
Huang Z-L, Urade Y, Hayaishi O (2007) Prostaglandins and adenosine in the regulation of sleep and wakefulness. Curr Opin Pharmacol 7:33–38
Hyden H, Lange PW (1965) Rhythmic enzyme changes in neurons and glia during sleep. Science 149:654–656
Hyder F, Fulbright RK, Shulman RG, Rothman DL (2013) Glutamatergic function in the resting awake human brain is supported by uniformly high oxidative energy. J Cereb Blood Flow Metab 33(3):339–347
Imamura K, Mataga N, Watanabe Y (1993) Gliotoxin-induced suppression of ocular dominance plasticity in kitten visual cortex. Neurosci Res 16:117–124
Inagaki N, Wada H (1994) Histamine and prostanoid receptors on glial cells. Glia 11:102–109
Inoue S, Honda K, Komoda Y (1995) Sleep as neuronal detoxification and restitution. Behav Brain Res 69:91–96
Ishimori K (1909) True cause of sleep: a hypnogenic substance as evidenced in the brain of sleep-deprived animals. Tokyo Igakkai Zasshi 23:429–457
Kjaerby C, Rasmussen R, Andersen M, Nedergaard M (2017) Does global astrocytic calcium signaling participate in awake brain state transitions and neuronal circuit function? Neurochem Res 42:1810–1822. https://doi.org/10.1007/s11064-017-2195-y
Kong J, Shepel PN, Holden CP, Mackiewicz M, Pack AI, Geiger JD (2002) Brain glycogen decreases with increased periods of wakefulness: implications for homeostatic drive to sleep. J Neurosci 22:5581–5587
Kreft M, Bak LK, Waagepetersen HS, Schousboe A (2012) Aspects of astrocyte energy metabolism, amino acid neurotransmitter homoeostasis and metabolic compartmentation. ASN Neuro 4:e00086
Krueger JM (2008) The role of cytokines in sleep regulation. Curr Pharm Des 14:3408–3416
Krueger JM, Rector DM, Roy S, Van Dongen HP, Belenky G, Panksepp J (2008) Sleep as a fundamental property of neuronal assemblies. Nat Rev Neurosci 9:910–919. https://doi.org/10.1038/nrn2521
Krueger JM, Taishi P, De A et al (2010) ATP and the purine type 2 X7 receptor affect sleep. J Appl Physiol 109:1318–1327
Kushikata T, Fang J, Krueger JM (1999) Brain-derived neurotrophic factor enhances spontaneous sleep in rats and rabbits. Am J Physiol Regul Integr Comp Physiol 276:R1334–R1338
Losi G, Mariotti L, Sessolo M, Carmignoto G (2017) New tools to study astrocyte Ca2+ signal dynamics in brain networks in vivo. Front Cell Neurosci 11:134
Madhusudanan P, Reade S, Shankarappa SA (2017) Neuroglia as targets for drug delivery systems: a review. Nanomedicine 13:667–679. https://doi.org/10.1016/j.nano.2016.08.013
Magistretti PJ (2011) Neuronal-glia metabolic coupling and plasticity. Exp Physiol 96:407–410
Matsui T, Svensson CI, Hirata Y, Mizobata K, Hua X-Y, Yaksh TL (2010) Release of prostaglandin E2 and nitric oxide from spinal microglia is dependent on activation of p38 mitogen-activated protein kinase. Anesth Analg 111:554–560
Möller T, Boddeke HWGM (2016) Glial cells as drug targets: what does it take? Glia 64:1742–1754. https://doi.org/10.1002/glia.22993
Morozov A, Kellendonk C, Simpson E, Tronche F (2003) Using conditional mutagenesis to study the brain. Biol Psychiatry 54:1125–1133

Naylor E, Aillon DV, Barrett BS et al (2012) Lactate as a biomarker for sleep. Sleep 35:1209–1222. https://doi.org/10.5665/sleep.2072

Nedergaard M, Verkhratsky A (2012) Artifact versus reality – how astrocytes contribute to synaptic events. Glia 60:1013–1023. https://doi.org/10.1002/glia.22288

Parpura V, Zorec R (2010) Gliotransmission: exocytotic release from astrocytes. Brain Res Rev 63:83–92

Pascual O, Casper KB, Kubera C et al (2005) Astrocytic purinergic signaling coordinates synaptic networks. Science 310:113–116

Pelluru D, Konadhode RR, Bhat NR, Shiromani PJ (2016) Optogenetic stimulation of astrocytes in the posterior hypothalamus increases sleep at night in C57BL/6J mice. Eur J Neurosci 43:1298–1306. https://doi.org/10.1111/ejn.13074

Petit J-M, Tobler I, Allaman I, Borbely AA, Magistretti PJ (2002) Sleep deprivation modulates brain mRNAs encoding genes of glycogen metabolism. Eur J Neurosci 16:1163–1167

Petit JM, Tobler I, Kopp C, Morgenthaler F, Borbely AA, Magistretti PJ (2010) Metabolic response of the cerebral cortex following gentle sleep deprivation and modafinil administration. Sleep 33:901–908

Petit JM, Gyger J, Burlet-Godinot S, Fiumelli H, Martin J-M, Magistretti PJ (2013) Genes involved in the astrocyte-neuron lactate shuttle (ANLS) are specifically regulated in cortical astrocytes following sleep deprivation in mice. Sleep 36:1445–1458

Poskanzer KE, Yuste R (2016) Astrocytes regulate cortical state switching in vivo. Proc Natl Acad Sci 113:E2675–E2684

Ramm P, Smith CT (1990) Rates of cerebral protein synthesis are linked to slow-wave sleep in the rat. Physiol Behav 48:749–753

Reimund E (1994) The free radical theory of sleep. Med Hypotheses 43:231–233

Sala C, Segal M (2014) Dendritic spines: the locus of structural and functional plasticity. Physiol Rev 94:141–188

Scales SJ, Bock JB, Scheller RH (2000) The specifics of membrane fusion. Nature 407:144–146. https://doi.org/10.1038/35025176

Schmitt LI, Sims RE, Dale N, Haydon PG (2012) Wakefulness affects synaptic and network activity by increasing extracellular astrocyte-derived adenosine. J Neurosci 32:4417–4425

Schulze G (2004) Sleep protects excitatory cortical circuits against oxidative damage. Med Hypotheses 63:203–207

Seibt J, Dumoulin M, Aton SJ, Naidoo J, Watson A, Coleman T, Frank MG (2012) Protein synthesis during sleep consolidates cortical plasticity in vivo. Curr Biol 22:676–682

Shannon BJ, Dosenbach RA, Su Y et al (2012) Morning-evening variation in human brain metabolism and memory circuits. J Neurophysiol 109:1444–1456

Sipe GO, Lowery RL, Tremblay MÈ, Kelly EA, Lamantia CE, Majewska AK (2016) Microglial P2Y12 is necessary for synaptic plasticity in mouse visual cortex. Nat Commun 7:10905. https://doi.org/10.1038/ncomms10905. https://www.nature.com/articles/ncomms10905#supplementary-information

Stogsdill JA, Eroglu C (2017) The interplay between neurons and glia in synapse development and plasticity. Curr Opin Neurobiol 42:1–8. https://doi.org/10.1016/j.conb.2016.09.016

Strecker RE, Morairty S, Thakkar MM et al (2000) Adenosinergic modulation of basal forebrain and preoptic/anterior hypothalamic neuronal activity in the control of behavioral state. Behav Brain Res 115:183–204

Szabó Z, Héja L, Szalay G et al (2017) Extensive astrocyte synchronization advances neuronal coupling in slow wave activity in vivo. Sci Rep 7:6018. https://doi.org/10.1038/s41598-017-06073-7

Szymusiak R, Gvilia I, McGinty D (2007) Hypothalamic control of sleep. Sleep Med 8:291–301

Takano T, Tian G-F, Peng W, Lou N, Libionka W, Han X, Nedergaard M (2006) Astrocyte-mediated control of cerebral blood flow. Nat Neurosci 9:260–267

Tobler I, Borbély AA, Schwyzer M, Fontana A (1984) Interleukin-1 derived from astrocytes enhances slow wave activity in sleep EEG of the rat. Eur J Pharmacol 104:191–192

Urade Y, Hayaishi O (2011) Prostaglandin D2 and sleep/wake regulation. Sleep Med Rev 15:411–418
Verkhratsky A, Rodriguez J, Parpura V (2012) Calcium signalling in astroglia. Mol Cell Endocrinol 353:45–56
Verkman AS, Smith AJ, Phuan P-W, Tradtrantip L, Anderson MO (2017) The aquaporin-4 water channel as a potential drug target in neurological disorders. Expert Opin Ther Targets 21:1161–1170. https://doi.org/10.1080/14728222.2017.1398236
Volterra A (2013) Astrocytes: modulation of synaptic function and network activity. In: Kettenmann H, Ransom B (eds) Neuroglia. Oxford University Press, New York, pp 481–493
Wisor JP, Clegern WC (2011) Quantification of short-term slow wave sleep homeostasis and its disruption by minocycline in the laboratory mouse. Neurosci Lett 490:165–169. https://doi.org/10.1016/j.neulet.2010.11.034
Wisor JP, Clegern WC, Schmidt MA (2011a) Toll-like receptor 4 is a regulator of monocyte and electroencephalographic responses to sleep loss. Sleep 34:1335–1345. https://doi.org/10.5665/SLEEP.1274
Wisor JP, Schmidt MA, Clegern WC (2011b) Evidence for neuroinflammatory and microglial changes in the cerebral response to sleep loss. Sleep 34:261–272
Wisor JP, Rempe MJ, Schmidt MA, Moore ME, Clegern WC (2012) Sleep slow-wave activity regulates cerebral glycolytic metabolism. Cereb Cortex 23:1978–1987
Xie L, Kang H, Xu Q et al (2013) Sleep drives metabolite clearance from the adult brain. Science 342:373–377. https://doi.org/10.1126/science.1241224
Yamamoto K, Miwa T, Ueno R, Hayaishi O (1988) Muramyl dipeptide-elicited production of PGD2 from astrocytes in culture. Biochem Biophys Res Commun 156:882–888
Yulug B, Hanoglu L, Kilic E (2017) Does sleep disturbance affect the amyloid clearance mechanisms in Alzheimer's disease? Psychiatry Clin Neurosc 71(10):673–677. https://doi.org/10.1111/pcn.12539
Zhang Q, Haydon PG (2005) Roles for gliotransmission in the nervous system. J Neural Transm 112:121–125
Zimmerman JE, Mackiewicz M, Galante RJ et al (2004) Glycogen in the brain of *Drosophila melanogaster*: diurnal rhythm and the effect of rest deprivation. J Neurochem 88:32–40

Sleep- and Wake-Like States in Small Networks In Vivo and In Vitro

Laura E. McKillop and Vladyslav V. Vyazovskiy

Contents

Abstract

Wakefulness and sleep are highly complex and heterogeneous processes, involving multiple neurotransmitter systems and a sophisticated interplay between global and local networks of neurons and non-neuronal cells. Macroscopic approaches applied at the level of the whole organism, view sleep as a global behaviour and allow for investigation into aspects such as the effects of insufficient or disrupted sleep on cognitive function, metabolism, thermoregulation and sensory processing. While significant progress has been achieved using such large-scale approaches, the inherent complexity of sleep-wake regulation has necessitated the development of methods which tackle specific aspects of sleep in isolation. One way this may be achieved is by investigating specific cellular or molecular phenomena in the whole organism in situ, either during spontaneous or induced sleep-wake states. This approach has greatly advanced our knowledge

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about the electrophysiology and pharmacology of ion channels, specific receptors, intracellular pathways and the small networks implicated in the control and regulation of the sleep-wake cycle. Importantly though, there are a variety of external and internal factors that influence global behavioural states which are difficult to control for using these approaches. For this reason, over the last few decades, ex vivo experimental models have become increasingly popular and have greatly advanced our understanding of many fundamental aspects of sleep, including the neuroanatomy and neurochemistry of sleep states, sleep regulation, the origin and dynamics of specific sleep oscillations, network homeostasis as well as the functional roles of sleep. This chapter will focus on the use of small neuronal networks as experimental models and will highlight the most significant and novel insights these approaches have provided.

Keywords
Global · In vitro · In vivo · Local · Sleep · Small networks

1 Introduction: From Global Sleep to Small Networks

Waking and sleep are distinguished by characteristic changes in behaviour, sensory processing and brain activity. The majority, if not all organisms investigated to date exhibit sleep-like behaviour, which spans a diverse range of vertebrate and invertebrate species from the well-studied rodent models to the microscopic nematode worms (Allada and Siegel 2008; Campbell and Tobler 1984; Raizen et al. 2008; Tobler 2005). While the minimum requirements and sufficient conditions for sleep remain unclear, a prevailing view is that sleep occurs in any organism with a neuronal/glial network (Krueger et al. 2016). This view suggests that the size of the network and/or its specific cellular composition may play a significant role in determining the amount, architecture and spatio-temporal dynamics of sleep, as well as many other characteristics. The size of the nervous system in general, and the brain in particular, differs several orders of magnitude among mammalian species, and even more so if invertebrates are included (Buzsaki et al. 2013). According to recent estimates, human brains are around five times larger than would be expected for a mammal of its body size, consisting of approximately 86 billion neurons, of which 16 billion are located in the cerebral cortex and 69 billion in the cerebellum (Azevedo et al. 2009). On the other hand, the entire nervous system of *C. elegans* consists of only 302 neurons, yet these simple organisms are still able to enter putative sleep-like states (Mackiewicz et al. 2008). Human studies have shown that large-scale brain networks exhibit complex dynamics during waking and sleep, including the emergence of stage-specific spatial patterns of functional organisation during sleep (Ramot et al. 2013; Watanabe et al. 2014). The many complex and highly dynamic interactions between neuronal networks, as well as their biochemical and genetic underpinnings, represent significant obstacles for investigating the cellular and network properties of sleep, its possible functions and how pharmacological interventions may be used to manipulate sleep. As

human studies are limited by numerous technical and ethical implications, in vitro and in vivo models have instead been developed to perturb localised networks and therefore further our basic understanding of sleep. Importantly, these approaches also enable external factors such as the environmental conditions to be controlled, which are known to have profound influences on many aspects of sleep and associated network dynamics.

The functional role of sleep in larger-scale processes, such as behaviour or metabolism, can be investigated using in vivo approaches, through stimulation, ablation or recording specific neuronal populations in situ, during physiological waking, sleep or a sleep-like state such as anaesthesia. Although these approaches typically only target small parts of the network, their function can be investigated in the context of the rest of the brain. These models are however constrained by technical limitations, such as the number of neurons or brain regions that can be recorded simultaneously. Furthermore, the causal relationship between specific patterns of activity in a small brain region and global changes in behaviour are usually difficult to ascertain using these methodologies. It is therefore important to utilise small network model systems, both in vitro and in vivo, to provide further insights into the neural circuitry and the biochemical molecules governing sleep regulation.

Simple ex vivo/in vitro/in silico models have reliably reproduced several of the essential characteristics of sleep, allowing relevant phenomena to be studied at the level of small circuits, rather than across the entire brain. Importantly, these methodologies have important shortcomings, namely, that the afferent and efferent connectivity of the network is largely disrupted, which may result in the occurrence of nonphysiological activity patterns, such as epileptic discharges or a persistent hyperpolarisation, not present under physiological conditions in vivo (Lemieux et al. 2014). Furthermore, while such a reductionist approach is necessary for understanding sleep as a whole, it is becoming increasingly clear that local level activity likely dictates larger-scale brain organisation. Therefore, it is crucial to utilise an integrated approach which takes into account the complex nonlinear interactions between smaller and simpler processes at many spatial and temporal scales. In this chapter we highlight some of the most important advancements these model systems have provided to the sleep field and discuss their pharmacological applications.

2 Local Network Activity and Sleep Regulation

Brain activity during waking and sleep is traditionally studied by recording the electroencephalogram (EEG, electrodes typically on cortical surface) or local field potentials (LFP, electrodes in deeper brain structures) (Buzsaki et al. 2012). It is now well established that rhythmic activity in the neocortex arises from specific interactions between excitatory and inhibitory neurons, intrinsic membrane properties and synaptic activity (Buzsáki 2006). The interaction between a positive feedback loop (e.g. recurrent excitation) and negative feedback control (e.g. inhibition) contributes to the generation of oscillatory activities in the neocortex

and subcortical areas (Buzsaki et al. 2012; McGinley et al. 2015). There are several types of brain oscillations during waking and sleep, which are arranged in a hierarchical system to maintain the local-global integration of network activity (Buzsáki 2006; Molle and Born 2011; Staresina et al. 2015). Notably, these network oscillations are conserved across organisms, regardless of brain size (Buzsaki et al. 2013), and so the results obtained from various laboratory animals are likely to generalise across species.

The brain is generally more metabolically and electrically active during waking (Braun et al. 1997; Kennedy et al. 1982; McKillop et al. 2018; Vyazovskiy et al. 2009). During sleep, slow waves recorded with cortical EEG or LFP electrodes reflect the synchronous occurrence of periods of reduced spiking and synaptic activity within thalamocortical networks (Crunelli et al. 2015; Destexhe et al. 1999; Neske 2016; Timofeev 2013; Vyazovskiy and Harris 2013). These so-called OFF periods become increasingly frequent the longer the duration of preceding wakefulness and are most prominent during the initial deep NREM sleep occurring immediately after periods of wakefulness (McKillop et al. 2018). These changes at the network level modulate the amplitude and incidence of EEG or LFP slow waves in a sleep-dependent manner, observed both in laboratory animals and humans (Lazar et al. 2015; Riedner et al. 2007; Vyazovskiy et al. 2009). This in turn underpins the well-established dynamics of EEG slow-wave activity (SWA, 0.5–4 Hz), which increases as a function of preceding wake duration and decreases during subsequent sleep (Borbély 1982; Dijk et al. 1987; Franken et al. 1991; Guillaumin et al. 2018; Vyazovskiy 2015; Vyazovskiy et al. 2002, 2007). Sleep homeostasis is a ubiquitous phenomenon found in all animal species carefully studied so far, suggesting that it is a fundamental function (Tobler 2005).

The notion that sleep is not a global process, but rather concerns local circuitries or specific parts of the brain goes back almost a century. Maria Manasseina noted that "the scientists recognizing sleep for stopping or diastole of cerebral activity are mistaken, for during sleep the brain as a whole does not sleep at all, it does not stay idle entirely, but only those parts of it which constitute an anatomical basis, anatomic substrate of consciousness are under the process of sleeping" (Kovalzon 2009). Similarly, Giuseppe Moruzzi suggested that "sleep concerns primarily not the whole cerebrum, nor even the entire neocortex, but only those neurons or synapses . . . which during wakefulness are responsible for, or related to, the brain functions concerned with conscious behavior" (Moruzzi 1972). In vitro studies have provided the most consistent evidence for sleep being a fundamentally local process, showing that cortical networks are capable of generating localised activity independent of the rest of the brain. In 1949 Kristiansen and Courtois observed the occurrence of sleep- and wake-like states in isolated portions of the cortex, similar to that observed in the intact cortex. An important impetus for this research direction was provided by the theory of local, activity-dependent regulation of sleep, which postulated that rather than being a global process that is bottom-up regulated, sleep instead originates locally in neuronal circuits and it is rather the synchronisation of many circuits together which leads to global sleep at the macroscopic level (Krueger and Obal 1993; Krueger et al. 2008; Roy et al. 2008; Van Dongen et al. 2011).

According to the early version of this theory, "sleep serves to stimulate the use of structures insufficiently activated during wakefulness", or in other words, the function of sleep is to reinforce specific synapses relative to others (Krueger and Obal 1993). This theory was subsequently developed to take a broader view in which global sleep and wake states are the result of bottom-up processes whereby large numbers of localised yet highly interconnected semi-autonomous cellular networks of neurons and glia oscillate in a semi-autonomous manner (Krueger et al. 2013).

Considerable evidence for the local regulation of sleep was provided by studies in which multiple electrodes were used to simultaneously record activity from multiple brain regions (Finelli et al. 2001; Siclari et al. 2014). These studies revealed that EEG or LFP slow-wave activity (SWA, 0.5–4 Hz) occurs in a regional use-dependent fashion, with brain regions more active during wakefulness showing higher SWA during subsequent sleep (Kattler et al. 1994; Riedner et al. 2007; Vyazovskiy et al. 2011a). For example, unilateral vibration of the hand of human subjects or whiskers of rodents led to an increase in SWA during subsequent sleep, specifically in those cortical areas stimulated by the vibration (Kattler et al. 1994; Vyazovskiy et al. 2000). Localised sleep-like activity in the EEG has also been shown to occur in behaviourally awake animals, with the increased incidence of localised slow waves leading to a reduced performance in a food pellet reaching task (Vyazovskiy et al. 2011b). This led to the theory that optimal performance involves an interaction between multiple networks and that the occurrence of sleep-like activity in one or more of these networks can have a detrimental effect on performance (Krueger et al. 2013; Vyazovskiy 2013). Therefore, given the extensive evidence for the local regulation of sleep in both cortical and subcortical networks (see below), multiple aspects of sleep can be understood by investigating sleep network dynamics in simple models.

The idea that sleep is locally controlled is now supported by numerous studies that utilised in vivo small network systems and in vitro studies to investigate the cellular and network properties of sleep initiation and regulation. In vivo chronic recordings have been instrumental in investigating the activity of localised distinct cortical regions, without disrupting the connectivity to other brain networks. For example, Pigarev et al. (1997) was one of the first studies to suggest that sleep may indeed initiate at the level of local small cortical networks. This study showed that in monkeys a topographically defined subset of neurons in the visual cortex failed to fire in response to stimulation while performing a visual task. Individual cortical columns have also been shown to have localised state-dependent activity, oscillating between sleep- and wake-like states, as defined by the amplitude of event related potentials (ERPs), with higher amplitude ERPs identified during sleep (Krueger et al. 2013; Rector et al. 2005, 2009). Cortical columns have therefore been suggested to be the most basic unit capable of oscillating between sleep and wake states (Rector et al. 2005). Asynchronous transitions between waking and sleep have also been shown at the level of individual cortical neurons (Vyazovskiy et al. 2014), while local slow waves have also been observed in awake behaviourally active animals (Einstein et al. 2017; Fisher et al. 2016; Polack et al. 2013; Vyazovskiy and Harris 2013; Vyazovskiy et al. 2011b).

The local occurrence of sleep-like states has a profound implication for sensory processing and cognitive function in general. Various aspects of behaviour, brain state and network activity account for the trial-to-trial variability in response to stimuli (Ahissar et al. 1992; Arieli et al. 1996; Crochet et al. 2005; Fontanini and Katz 2008; Haider et al. 2007; Krueger et al. 2008; Rector et al. 2005; Sanchez-Vives and McCormick 2000; Scheeringa et al. 2011; Vyazovskiy et al. 2013). Important determinants of neuronal responsiveness are preceding activity (Abbott and Nelson 2000) and the brain-state-specific levels of neuromodulators (McCormick et al. 1991), which impose both global and local synapse-specific control (Gil et al. 1997; Laplante et al. 2005; Lawrence 2008; Marrocco et al. 1987). It is well established that brain states have a great influence on information processing and local neuronal interactions (Zagha and McCormick 2014). Slow oscillations have also been shown to affect the response of networks to synaptic inputs (Reig et al. 2015), although this may depend on the cortical area being studied and the type of stimulus (Sela et al. 2016). As during OFF periods all or most local neurons are simultaneously deactivated or inhibited (Chauvette et al. 2011; Sanchez-Vives et al. 2010; Timofeev et al. 2001) and do not produce spikes (Logothetis et al. 2010; Vyazovskiy et al. 2009), it is perhaps not surprising that the occurrence of OFF periods would result in a disruption of short-range neuronal coordination which may therefore contribute to behavioural lapses or a loss of consciousness (Castelnovo et al. 2018; Nir et al. 2017; Pigorini et al. 2015; Vyazovskiy et al. 2011b).

It is important to remember that although local and global states likely represent a continuum, the mechanistic origin of "local sleep" or "local wake" and global states of vigilance may be fundamentally distinct. While more research is needed in this area, there is little doubt that considering multiple levels of organisation may provide important advancements in our understanding of sleep and its complexity (Olbrich et al. 2011; Vyazovskiy 2015; Vyazovskiy and Delogu 2014). A recent study provided evidence that in contrast to humans, where ageing is associated with substantial changes in global sleep characteristics, including its architecture and the EEG (Landolt and Borbely 2001; Mander et al. 2017), cortical neural activity and local signatures of sleep homeostasis remain intact in laboratory rodents across the lifespan (McKillop et al. 2018). Such studies highlight the importance of investigating the network activity underpinning well-established global phenomena, such as the reduction in SWA or increase in spindle-frequency spectral power after administration of commonly used hypnotics and related substances (Borbely et al. 1985; Kopp et al. 2004a; Lancel 1999; Seibt et al. 2008).

3 Cortical Activity During Sleep: Insights from In Vivo Approaches

A broad range of techniques are available for investigating network activity in vivo at several spatio-temporal scales (Olcese and Faraguna 2015). These techniques often involve the implantation of electrodes into the brain to record neuronal activity

from either head fixed (Blumberg et al. 2015; Saleem et al. 2010; Seibt et al. 2017; Yüzgeç et al. 2018) or freely moving animals (Fisher and Vyazovskiy 2014; McKillop et al. 2018; Meisel et al. 2017; Vyazovskiy et al. 2013; Watson et al. 2016). Early studies identified that during NREM sleep, oscillatory activity in the slow-wave activity frequency range (SWA, 0.4–5 Hz) predominates the EEG/LFP signal. At the neuronal level, this activity is underpinned by a bistable slow (<1 Hz) oscillatory alternation in the membrane potential of cortical neurons, from a depolarised *up* state caused by active neuronal firing and synaptic activity to a hyperpolarised *down* state where both spiking and synaptic activity are absent (Neske 2016; Steriade 1978; Steriade et al. 1993). These hyperpolarisation periods of synaptic silence and the decrease in neuronal firing (named OFF periods when referring to the synchronous silence across neuronal populations recorded extracellularly) are important defining features of NREM sleep and are largely the result of a disfacilitation process, with leak currents (predominantly potassium currents) dominating the underlying activity in the absence of other synaptic currents (Timofeev et al. 2001). Rodent studies have performed continuous chronic recordings of extracellular neuronal activity have been performed in rodents, allowing for the association between ongoing vigilance state and network activity to be investigated in vivo. These studies have shown that network *down* states in the neocortex are consistently associated with surface negativity in the EEG signal or positive potentials if recordings are performed from deeper cortical layers (using LFP recordings), while slow waves correlate with near-synchronous transitions between *up* and *down* states across cortical neurons (Steriade et al. 1993; Vyazovskiy et al. 2009). Importantly, evidence suggests that the higher the spatial synchrony and the longer the network down state, the larger the amplitude and steeper the slope of the resultant EEG/LFP slow waves (Vyazovskiy et al. 2009). As SWA is used as a physiological correlate of sleep homeostasis (Borbély 1982), this indicates that both neuronal activity and global network EEG can reflect the homeostatic regulation of sleep.

Importantly, these techniques have technical limitations which only allow a small number of cortical or subcortical networks to be recorded simultaneously. In contrast, calcium imaging has provided a relatively unbiased way of simultaneously imaging a large area of the cortex; although such recordings typically only record from superficial cortical layers, where neuronal population activity may differ from deeper layers (Beltramo et al. 2013; Sakata and Harris 2012). Calcium imaging has also been useful for recording cortical activity from zebrafish (Leung et al. 2013) and drosophila (Bushey et al. 2015); however recordings of small networks during physiological sleep remain scarce. In vivo calcium imaging has revealed a global reduction in neuronal network activity during sleep compared to waking in mice, both in superficial and deep cortical layers (Niethard et al. 2016). Curiously during REM sleep neuronal activity was found to be further reduced (Niethard et al. 2016), contradicting the increase identified by electrophysiological recordings (McKillop et al. 2018; Vyazovskiy et al. 2009).

4 Cortical Activity During Sleep: Insights from In Vitro Approaches

In vitro and ex vivo studies have utilised neuronal cultures and brain slices to study network phenomena, such as the slow oscillation. In vitro brain slice experiments have provided important insights into the processes underlying sleep oscillations, such as the role of rhythmic dendritic Ca2+ entry in thalamocortical neurons (Errington et al. 2012), low-threshold discharges (Connelly et al. 2015), and network bistability (Crunelli et al. 2005). Both in vivo and in vitro studies have consistently shown that deep layers of the neocortex contribute substantially to the generation of network slow oscillations (Beltramo et al. 2013; Chauvette et al. 2010; Fiath et al. 2016; Sakata and Harris 2012; Sanchez-Vives and McCormick 2000). It has also been established that slow waves and their cellular counterpart – the slow oscillation – have a preferential origin within frontal cortical areas and higher-order and intralaminar thalamic regions (Sheroziya and Timofeev 2014). Therefore, different dynamics may be identified depending on the cortical area used to prepare the brain slices or neuronal cultures. It is possible that some cortical areas may be capable of intrinsically generating slow oscillations, while others merely follow a driver or modulatory inputs from distant areas. Notably, excitatory pyramidal neurons and inhibitory interneurons in the neocortex exhibit different activity levels during waking and likely have distinct contributions to the network slow oscillation (Beltramo et al. 2013; Funk et al. 2017; Lemieux et al. 2015; Neske 2016; Timofeev 2013; Zucca et al. 2017). Many types of inhibitory neurons come together to form the local inhibitory circuitry and have unique and distinct contributions to cortical network activity and the regulation of global states (Gerashchenko et al. 2008; Isaacson and Scanziani 2011; Jackson et al. 2016; Kvitsiani et al. 2013; Lemieux et al. 2015; Morairty et al. 2013; Neske and Connors 2016; Staiger et al. 2009).

The main shortcoming of cortical slice preparations is that they may lack important inputs from other cortical areas, and different cortical layers may be affected to different extents (Stepanyants et al. 2009). It has been shown that the beginning of an *up* state is associated with increased excitatory currents (Haider et al. 2006) and is characterised by highly structured activity within a population of neurons (Luczak et al. 2007). Notably, it has been shown that early EPSPs evoked by long-range inputs arising from supragranular levels are remarkably voltage-dependent (Hirsch and Gilbert 1991). Specifically, at the resting membrane potential, EPSPs are too small and brief to lead to action potentials but grow several hundred folds with membrane depolarisation (Hirsch and Gilbert 1991). Such state dependency implies that specific long-range connections between distant cortical areas may only be fully functional in an alert waking state, when supragranular neurons across the neocortex are tonically depolarised (Petersen et al. 2003; Poulet and Petersen 2008). Severing these inputs in slices could therefore make it impossible to achieve the full manifestation of an alert wake state.

Finally, in silico small network models have also provided important insights into various aspects of sleep research, including the generation of network slow oscillations within the thalamocortical network and the roles of specific ionic

conductances, such as persistent sodium and Ca2+-dependent K+ currents in synaptic plasticity (Bazhenov et al. 2002; Esser et al. 2007; Hill and Tononi 2005). More recently, modelling approaches have been used to investigate the relationship between synaptic pruning and the decline of slow-wave activity, well documented to occur during adolescence (Hoel et al. 2016). Consistent with experimental data, this model was capable of producing a reorganisation of intralaminar connections to establish preferential connectivity between neurons with similar receptive fields and preferred orientations, which in turn resulted in a reduction in slow-wave activity and changes to slow-wave characteristics (Hoel et al. 2016).

5 Sleep as a Default State of Cortical Networks

It has recently been suggested that sleep has features of a "default state" (Sanchez-Vives and Mattia 2014). This is supported by experimental evidence which showed that neuronal cultures or isolated cortical slabs deprived of synaptic inputs can spontaneously generate slow oscillations, usually characteristic of a sleep-like state (Corner 2013; Hinard et al. 2012; Lemieux et al. 2014; Saberi-Moghadam et al. 2018). Therefore, in vitro mammalian cortical cell cultures have been useful for providing a detailed understanding of the network dynamics of states reminiscent of sleep (Wagenaar et al. 2005). It has been shown that after approximately 4 days of cell culture, neocortical cells from embryonic day 18 rats begin to fire and soon after globally synchronise their firing to form a recurring burst-pause firing pattern around 0.5–2 s in duration (Wagenaar et al. 2005). In addition, after 2 weeks in culture, dissociated cortical primary cultures from 3-day-old mice show action potential burstiness and a synchronisation of slow electrical potentials between recording electrodes (Jewett et al. 2015). High-density cultures of dissociated mammalian neuronal/glial cell cultures show properties of sleep-like burst-pause firing patterns in vitro, reminiscent of that seen during NREM sleep in vivo (Chiappalone et al. 2006; Corner 2008; Hinard et al. 2012; Jewett et al. 2015; Krueger et al. 2016). It should be noted that these cultures have longer inter-burst intervals compared to in vivo recordings, and this can persist for the lifetime of the culture. It was proposed that burst-firing activity in culture may be due to the deafferentation of thalamocortical neurons which in turn strengthens network connectivity (Wagenaar et al. 2005). This view is consistent with the emergence of sleep-like activity in deafferented cortical slabs (Lemieux et al. 2014; Timofeev et al. 2000).

Synchronised bursting is a network phenomenon, reflecting synaptic interactions between a large number of disperse cells (Hinard et al. 2012; Saberi-Moghadam et al. 2018; Wagenaar et al. 2005). The extent of synchronisation is dependent on the fraction of active, tonically firing cells, with evidence that a reduction in the number of steadily firing cells leads to bursting activity (Latham et al. 2000). Application of a mixture of excitatory neurotransmitters (chemical stimulation) was able to change the default sleep-like bustiness activity into a transient tonic firing wake-like state, which then spontaneously returned to a sleep-like state (Hinard et al. 2012; Saberi-Moghadam et al. 2018). Cholinergic neuromodulation has been shown to play a

significant role in spontaneous rhythmic activity in neuronal cultures (Hammond et al. 2013). Interestingly, application of excitatory neurotransmitters to cultures also caused them to exhibit a gene expression profile (transcriptome) similar to that of an awake mouse or that seen after sleep deprivation (Hinard et al. 2012). However, this study only applied the excitatory neurotransmitters to cultures once sleep-like activity was observed and not before.

Electrical pulse stimulation of mature cultures has also been shown to result in wake-like firing patterns, suggesting that electrical stimulation is a sufficient substitute for natural afferent inputs to neurons, enabling the transition into wake-like cortical activity (Jewett et al. 2015; Wagenaar et al. 2005). Notably, a subsequent rebound was found in both the synchronisation between electrodes and slow-wave power values poststimulation, suggesting that some manifestations of sleep homeostasis can also be observed in vitro (Jewett et al. 2015; Saberi-Moghadam et al. 2018; Wagenaar et al. 2005). Consistently, in vitro optogenetic stimulation of mature neuronal cultures induced the release of adenosine triphosphate (ATP) and the expression of interleukin-1 beta (IL1) and tumour necrosis factor (TNF) (Jewett et al. 2015), all of which are known to be involved in sleep regulation (Clinton et al. 2011; Krueger et al. 2008; Van Dongen et al. 2011). Furthermore, application of TNF to the culture was able to enhance burstiness, synchronisation and the magnitude of slow-wave power, suggesting that TNF application can induce a sleep-like state reminiscent of deeper sleep stages (Jewett et al. 2015).

6 Thalamocortical Networks and Sleep Spindles

The thalamocortical network is essential for generating the full manifestation of cortical activity patterns observed during sleep (Crunelli et al. 2015; David et al. 2013; Sheroziya and Timofeev 2014). According to the traditional model, the dorsal thalamus receives inputs from ascending sensory pathways, including brainstem cholinergic, noradrenergic or serotoninergic modulatory systems (Franks 2008; Jones 2009; Steriade and Amzica 1998; Steriade et al. 1990). Thalamocortical neurons send excitatory glutamatergic projections to the reticular (RE) thalamic nucleus and the cerebral cortex, where they terminate in layers III, IV and VI of the neocortex. The RE thalamic nucleus receives collaterals of thalamocortical neurons, as well as corticothalamic glutamatergic inputs originating from layer VI of the cortex (Steriade et al. 1986). All neurons within the RE thalamic nucleus are GABAergic, although functional diversity between these neurons has been reported (Halassa et al. 2014). In vitro brain slice experiments have been useful for investigating intrathalamic network activity patterns and synaptic plasticity (Pigeat et al. 2015). Specifically, it has been demonstrated that stimulation of brain slices in the slow-wave activity frequency range during sleep leads to the development of long-term depression (LTD) in the inhibitory synaptic connections between RE and TC neurons (Pigeat et al. 2015). On the other hand, slice preparations have also been useful for investigating the functional relationship between the thalamus and the neocortex during slow oscillations (Crunelli and Hughes 2009; Neske 2016).

Specifically, slices containing thalamocortical and cortical networks showed reduced postsynaptic potentials during cortical *up* states, suggesting a reduction in the thalamocortical input and a functional disconnection during network activity (Watson et al. 2008). However, conflicting results have also shown that paired-pulse facilitation was significantly larger during network *up* states and persisted for longer as compared to silent slices (Reig and Sanchez-Vives 2007).

Although the cellular and network mechanisms of the slow oscillation have been extensively investigated using simple model preparations, other thalamocortical oscillations are more difficult to study. A notable example of a sleep-related phenomenon which is difficult to reproduce in vitro is the sleep spindle. EEG spindles are oscillatory events (Olbrich and Achermann 2005) that occur predominantly during NREM sleep (Astori et al. 2013; Vyazovskiy et al. 2004) and have been described in several mammalian species (Buzsaki et al. 2013). Spindles are generated within the reticular thalamic nuclei, where neurons typically exhibit a bursting discharge pattern at frequencies 7–14 Hz (Bartho et al. 2014; Halassa et al. 2014; Marks and Roffwarg 1993; Steriade et al. 1986). The rhythmic hyperpolarisation of thalamocortical neurons leads to rebound spike bursts, which are transferred to the neocortex as spindles (Steriade and Amzica 1998). As it is difficult to replicate, and therefore study sleep spindles in vitro, this has slowed the advancement of understanding their functional role in normal brain function, as well as how spindle deficits may underlie diseases such as schizophrenia (Manoach et al. 2016).

7 Recordings Local Network Activity from Subcortical Areas In Vivo and In Vitro

Simple model approaches usually target cortical networks and so other brain regions remain under investigated. According to the prevailing view, global sleep and wake are generated and maintained via a set of subcortical sleep- and wake-promoting areas (Eban-Rothschild et al. 2018; Saper et al. 2010). Numerous subcortical areas have been shown to play important roles in global sleep-wake control (Lee and Dan 2012) including the locus coeruleus (Aston-Jones and Bloom 1981), the tuberomammillary nucleus of the hypothalamus (Liu et al. 2010; Scammell et al. 2000), dorsal raphe nuclei (Gervasoni et al. 2000; Jones 2004), the orexinergic area of the hypothalamus (Kosse et al. 2015; Mileykovskiy et al. 2005), preoptic area of the hypothalamus (Suntsova et al. 2007; Szymusiak et al. 1998) and other areas. These subcortical neuromodulatory systems exert local influences on other wake- and sleep-promoting neurons but also send distant projections to the neocortex, thalamus and hippocampus, thus contributing fundamentally to the regulation of sleep oscillations, local cortical states and global behaviours. The extensive projections of these nuclei make it especially difficult to investigate their functional role in sleep-wake control, both in vitro and in vivo. Nevertheless, several studies have been successful in investigating the response of specific neurons to common neuromodulators and other substances.

Most studies addressing the role of the ventrolateral preoptic area (VLPO) in sleep regulation have utilised Fos immunoreactivity experiments (Sherin et al. 1996) or electrophysiological recordings in slice preparations or neuronal cultures (Tabarean 2013). VLPO neurons have been shown to produce powerful low-threshold Ca^{2+} spikes (LTS) and are inhibited by the major wakefulness-related neurotransmitters such as noradrenaline. Use of isolated VLPO tissue in acute slices allows the potential interactions between subtypes of VLPO neurons to be studied. VLPO neurons can be identified by their coexpression of GABA and galanin, their low-threshold spike firing and their sensitivity to noradrenaline (Chamberlin et al. 2003; Gallopin et al. 2000; Li et al. 2009; Liu et al. 2010). In vitro electrophysiological classification of VLPO neurons has been performed in mice, according to their response to bath application of noradrenaline (NA) (Moore et al. 2012). These experiments revealed that the VLPO contains a mixed population of both noradrenaline-hyperpolarised (NA−) and depolarised (NA+) neurons, in an approximate 2:1 ratio, of which NA− VLPO neurons are thought to be the putative sleep-promoting subset.

Local network activity has also been successfully recorded from several other subcortical structures in vivo. For example, it has been shown that the discharging of neurons in the pedunculopontine tegmentum, although highly diverse, generally correlates with wakefulness and REM sleep (Datta and Siwek 2002). VTA GABAergic neurons were especially active during waking and movement, reduced their spiking activity during NREM sleep and increased it again during periods of REM sleep (Lee et al. 2001). Calcium imaging studies have shown that glutamatergic neurons in the dorsal pons are also active during REM sleep (Cox et al. 2016). Finally, local network interactions between MCH and orexin neurons in the lateral hypothalamus have been investigated using localised recordings and optogenetic stimulation (Apergis-Schoute et al. 2015).

8 Anaesthesia as a Sleep-Like State Model to Investigate Network Activity

Anaesthesia is a widely used tool to study the circuit mechanisms of sleep oscillations, subcortical control of sleep-wake states and the role of sleep in plasticity (Beltramo et al. 2013; Ferron et al. 2009; González-Rueda et al. 2018; Kroeger and Amzica 2007; Neske 2016; Zhang et al. 2015). Much of the existing knowledge about the cellular and network mechanisms of slow oscillations was originally obtained using anesthesia preparations. As anaesthesia shares several important similarities with sleep, including the patterns of cortical activity and the involvement of specific subcortical circuits, both electrophysiological and imaging approaches often use anaesthesia as an experimental model for studying physiological sleep (Civillico and Contreras 2012; Franks 2008; Hwang et al. 2013; Kuki et al. 2013; Neske 2016; Zhang et al. 2015). In addition, comparisons between sleep and anesthesia have furthered our understanding of the mechanisms underlying the effects of anaesthetics (Franks 2008). Two-photon calcium imaging of neuronal activity in layer 2/3 of the mouse barrel cortex revealed that there is an

increased network synchronisation under isoflurane, ketamine and urethane anaesthesia (Lissek et al. 2016). Cross-correlation analysis identified stable temporal relationships between neurons under ketamine anaesthesia, where individual neurons preferentially fired during population *up* states (Luczak and Bartho 2012). Putative inhibitory interneurons were found to be more active at the beginning of *up* states, while the spiking activity of putative pyramidal cells was evenly distributed across the entire *up* state (Luczak and Bartho 2012). Interestingly, the slow oscillation has similar dynamics across several cortical areas (primary visual, somatosensory, motor and medial prefrontal cortex), with the exception of the prefrontal cortex which shows faster *down*-to-*up* state transitions and higher firing rates during *up* states (Ruiz-Mejias et al. 2011). Another study found that the propagation of slow-wave activity in anesthetised animals shows a clear preferred direction and often originates from the same location in the neocortex (Fucke et al. 2011). Under anesthesia the membrane potential during active states shows a low correlation between distant neurons, despite transitions between periods of activity and silence being synchronised (Volgushev et al. 2011). Importantly, properties of slow waves are also used to clinically assess the depth of anaesthesia, through the use of automated systems which provide continuous estimations of anaesthesia depth using spectral analysis of the EEG (Berezowskyj et al. 1976; Olofsen et al. 2008; Voss and Sleigh 2007).

As different anaesthetics as well as anaesthesia depth may have differential effects on parameters of slow waves, important differences between anaesthesia and physiological sleep may be expected. However, surprisingly, only a few studies have quantitatively addressed this. A recent study showed that cats under ketamine-xylazine anaesthesia had slower slow waves that were more rhythmic and more synchronous across the cortex (Chauvette et al. 2011). In contrast, the same study found that *down* states were longer with a larger amplitude difference in membrane potential around transitions between active and silent states, as compared to sleep. It is well known that neuronal excitability is strongly correlated with ongoing network fluctuations between *up* and *down* states (Steriade 2001). This was highlighted by a study which showed that in anesthetised animals the shape of evoked responses varied significantly from one trial to the next, with the variation highest under medium levels of anaesthesia where cortical activity exhibits rhythmic population bursting activity (Kisley and Gerstein 1999).

9 Pharmacological Manipulation of Sleep and Associated Network Activities

Sleep disorders are highly prevalent in the population, commonly classified into insomnia, hypersomnia and parasomnias. There are a number of ways to improve sleep ranging from behavioural techniques using cognitive behavioural therapy (CBT) for insomnia (Espie et al. 2007; Freeman et al. 2015; Morin et al. 2006) or physiological techniques either targeting the circadian system through chronotherapeutics (Wirz-Justice 2009) or sleep through pharmacological interventions (Olfson et al. 2015). Although the use of drugs to improve sleep is

associated with a number of disadvantages such as dependency and tolerance, hypnotic drugs remain a prevalent treatment for improving sleep (Kripke 2000; Olfson et al. 2015; Schutte-Rodin et al. 2008; Watson et al. 2010). Historically these drugs have been developed using largely empirical approaches, where the mechanisms of actions are often not well understood (Watson et al. 2010). This has led to the development of drugs which often have serious side effects associated with their use, further highlighting the necessity to learn more about the interactions drugs may have with sleep-wake system and the mechanisms underlying their efficacy. Over the past few decades, there have been great advancements in the field of sleep medicine, and it is now more widely recognised that rational drug discovery approaches are necessary for the development of drugs which not only have less adverse effects but also have more desirable ones (Watson et al. 2010). For example, drugs may instead be developed based on known principles of the receptor-binding properties of a molecule as well as the network activity underlying sleep and wakefulness.

Simple model systems have provided important new insights into the effects of common drugs, such as psychostimulants and hypnotics, crucial for the development of new pharmacological treatments. Many of the current drugs either act on the sleep-promoting GABAergic system (including benzodiazepines and barbiturates) and wake-promoting systems such as histaminergic, serotonergic and orexinergic systems or modulate arousal through systems such as melatonin or adenosine (Amar 2018). It is now well established that these systems act through a complex interaction between various neurons and neurotransmitters to control sleep and wakefulness (Amar 2018; Watson et al. 2010).

Most of the hypnotic drugs currently available target GABA type A ($GABA_A$) receptors, which are well studied, and their physiology and pharmacology are reviewed elsewhere (Mohler 2011; Winsky-Sommerer 2009). Sedative/hypnotic drugs that target $GABA_A$ receptors can be subdivided into two main kinds: traditional benzodiazepines and non-benzodiazepines or Z-drugs, both of which are discussed below. Benzodiazepines are among the most common drugs used for the treatment of insomnia; however these have important adverse effects associated with their use such as tolerance and addiction. Benzodiazepines act to potentiate inhibitory GABAergic neurotransmission by increasing the affinity of GABA for $GABA_A$ receptors. In vitro studies have revealed that the binding of benzodiazepines increases GABA-induced chloride influx resulting in a general inhibitory effect on neuronal activity (Rudolph and Mohler 2006). This has downstream effects on specific brain oscillations, leading to a reduction in EEG power in the slow-wave activity range (0.5–4 Hz) and an enhancement of activity in the spindle frequency range (9–14 Hz) during NREM sleep, which has been noted in both humans and rodent studies (Aeschbach et al. 1994; Borbely et al. 1985; Kopp et al. 2003, 2004a; Lancel 1999; Lancel et al. 1996, 1997; Tobler et al. 2001). However, benzodiazepines do not affect the homeostatic regulation of sleep, as evidence suggests they do not greatly affect the time course of SWA, despite reducing absolute power (Aeschbach et al. 1994). Non-benzodiazepine "Z" drugs, such as zolpidem, are also widely used as sedative drugs (Dijk et al. 2010; Lancel 1999; Sanger et al. 1996), but these can have adverse effects on memory and cognitive

function. Calcium imaging using a miniaturised fluorescent microscope revealed a state-independent suppression of neuronal activity after systemic administration of zolpidem in freely behaving mice (Berdyyeva et al. 2014). However, benzodiazepines do not affect the homeostatic regulation of sleep, as evidence suggests they do not greatly affect the time course of SWA, despite reducing absolute power (Aeschbach et al. 1994).

It is well established that the molecular structure of $GABA_A$ receptors is highly heterogeneous, with a large repertoire of receptor subtypes identified (Mohler 2011; Winsky-Sommerer 2009). Investigations into these distinct subtypes has revealed great diversity in their function and pharmacological properties which provides a great opportunity to develop drugs that may have more specific effects. In vitro studies using Xenopus oocytes or cell culture systems in which $GABA_A$ receptors are expressed have been crucial in the profiling of the specificity of sedative drugs (Dämgen and Lüddens 1999; Winsky-Sommerer 2009). In vivo studies have also aided the identification of the $GABA_A$ receptor subtypes involved in the efficacy of common benzodiazepine and Z-drugs. As α1 and α3 $GABA_A$ subtypes are known to be present in the corticothalamic network (Jean-Marc and Hanns 2004), which is involved in the generation of delta and spindle oscillations (McCormick and Bal 1997; Saper et al. 2001; Steriade et al. 1987), these subtypes were originally thought to be responsible for the efficacy of benzodiazepines. However, in vivo studies in which mice were genetically modified so that they were insensitive to α1 or α3 $GABA_A$ receptors revealed that these mice still showed the typical reduction in delta power postinjection of diazepam (Kopp et al. 2004a; Tobler et al. 2001). It is now more widely thought that α2 $GABA_A$ receptors may instead be responsible for the action of benzodiazepines as they are present or project from the basal forebrain or preoptic regions of the ascending system (Jean-Marc and Hanns 2004) and they are involved in the generation of slow oscillations during NREM sleep through the hyperpolarisation of ascending projections to the corticothalamic system (McCormick and Bal 1997; Steriade et al. 1987). Importantly, mice genetically modified to be insensitive to diazepam showed an attenuation of the reduction in delta power, an increase in theta activity and an absence of the increase in high frequencies (above 16–18 Hz) after diazepam injection (Tobler et al. 2001). In contrast, the Z-drug, zolpidem, has been shown to preferentially bind to the α1 receptor subtype in vitro (Dämgen and Lüddens 1999). This was confirmed in vivo using α1(H101R) mice (Crestani et al. 2000), which did not show the reduction in SWA classically observed after zolpidem administration (Kopp et al. 2004b). Therefore, it is likely that the sleep EEG effects of zolpidem are mediated through the α1 subunit, while the sleep-promoting effects have instead been shown to involved α2 and α3 subtypes (Winsky-Sommerer 2009).

Together the combination of in vitro and in vivo studies have highlighted the complexity in interpreting the mechanisms of action of sedative drugs. With a single drug targeting numerous subtypes it is not surprising that the currently used drugs have diverse and unwanted effects associated with their use. Further studies to investigate the molecular and physiological mechanisms of drugs and their targets, as well as the sleep-wake system in general, should allow for the development of drugs with more specific actions.

10 Conclusions

In order to address the complex and dynamic processes involved in sleep, it has been essential to develop small network model systems to break sleep networks into smaller components and address each of them individually. Evidence suggests that in vitro cultured mature neuronal-glial networks can display (induced and controlled) sleep- and wake-like activity similar to that seen in vivo, suggesting that sleep is an activity-dependent emergent local network property. Combining the use of these systems with whole animal in vivo experiments allows us to gain a fuller understanding of sleep-wake networks and address fundamental, yet still unanswered, questions such as "what is the function of sleep?" Though over the past few decades, much progress has been made to address these questions, there remains much to learn in this ever-expanding field of sleep research.

References

Abbott LF, Nelson SB (2000) Synaptic plasticity: taming the beast. Nat Neurosci 3(Suppl):1178–1183

Aeschbach D, Dijk DJ, Trachsel L, Brunner DP, Borbely AA (1994) Dynamics of slow-wave activity and spindle frequency activity in the human sleep EEG: effect of midazolam and zopiclone. Neuropsychopharmacology 11:237–244

Ahissar E, Vaadia E, Ahissar M, Bergman H, Arieli A, Abeles M (1992) Dependence of cortical plasticity on correlated activity of single neurons and on behavioral context. Science 257:1412–1415

Allada R, Siegel JM (2008) Unearthing the phylogenetic roots of sleep. Curr Biol 18:R670–R679

Amar A (2018) Drugs affecting sleep and wakefulness: a review. Int J Basic Clin Pharmacol 7:1057–1064

Apergis-Schoute J, Iordanidou P, Faure C, Jego S, Schone C, Aitta-Aho T, Adamantidis A, Burdakov D (2015) Optogenetic evidence for inhibitory signaling from orexin to MCH neurons via local microcircuits. J Neurosci 35:5435–5441

Arieli A, Sterkin A, Grinvald A, Aertsen A (1996) Dynamics of ongoing activity: explanation of the large variability in evoked cortical responses. Science 273:1868–1871

Aston-Jones G, Bloom FE (1981) Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. J Neurosci 1:876–886

Astori S, Wimmer RD, Luthi A (2013) Manipulating sleep spindles – expanding views on sleep, memory, and disease. Trends Neurosci 36:738–748

Azevedo FA, Carvalho LR, Grinberg LT, Farfel JM, Ferretti RE, Leite RE, Jacob Filho W, Lent R, Herculano-Houzel S (2009) Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. J Comp Neurol 513:532–541

Bartho P, Slezia A, Matyas F, Faradzs-Zade L, Ulbert I, Harris KD, Acsady L (2014) Ongoing network state controls the length of sleep spindles via inhibitory activity. Neuron 82:1367–1379

Bazhenov M, Timofeev I, Steriade M, Sejnowski TJ (2002) Model of thalamocortical slow-wave sleep oscillations and transitions to activated States. J Neurosci 22:8691–8704

Beltramo R, D'Urso G, Dal Maschio M, Farisello P, Bovetti S, Clovis Y, Lassi G, Tucci V, De Pietri Tonelli D, Fellin T (2013) Layer-specific excitatory circuits differentially control recurrent network dynamics in the neocortex. Nat Neurosci 16:227–234

Berdyyeva T, Otte S, Aluisio L, Ziv Y, Burns LD, Dugovic C, Yun S, Ghosh KK, Schnitzer MJ, Lovenberg T et al (2014) Zolpidem reduces hippocampal neuronal activity in freely behaving mice: a large scale calcium imaging study with miniaturized fluorescence microscope. PLoS One 9:e112068

Berezowskyj JL, McEwen JA, Anderson GB, Jenkins LC (1976) A study of anaesthesia depth by power spectral analysis of the electroencephalogram (EEG). Can Anaesth Soc J 23:1–8

Blumberg MS, Sokoloff G, Tiriac A, Del Rio-Bermudez C (2015) A valuable and promising method for recording brain activity in behaving newborn rodents. Dev Psychobiol 57:506–517

Borbély AA (1982) A two process model of sleep regulation. Hum Neurobiol 1:195–204

Borbely AA, Mattmann P, Loepfe M, Strauch I, Lehmann D (1985) Effect of benzodiazepine hypnotics on all-night sleep EEG spectra. Hum Neurobiol 4:189–194

Braun AR, Balkin TJ, Wesenten NJ, Carson RE, Varga M, Baldwin P, Selbie S, Belenky G, Herscovitch P (1997) Regional cerebral blood flow throughout the sleep-wake cycle. An H2(15)O PET study. Brain 120(Pt 7):1173–1197

Bushey D, Tononi G, Cirelli C (2015) Sleep- and wake-dependent changes in neuronal activity and reactivity demonstrated in fly neurons using in vivo calcium imaging. Proc Natl Acad Sci U S A 112:4785–4790

Buzsáki G (2006) Rhythms of the brain. Oxford University Press, Oxford and New York

Buzsaki G, Anastassiou CA, Koch C (2012) The origin of extracellular fields and currents – EEG, ECoG, LFP and spikes. Nat Rev Neurosci 13:407–420

Buzsaki G, Logothetis N, Singer W (2013) Scaling brain size, keeping timing: evolutionary preservation of brain rhythms. Neuron 80:751–764

Campbell SS, Tobler I (1984) Animal sleep: a review of sleep duration across phylogeny. Neurosci Biobehav Rev 8:269–300

Castelnovo A, Lopez R, Proserpio P, Nobili L, Dauvilliers Y (2018) NREM sleep parasomnias as disorders of sleep-state dissociation. Nat Rev Neurol 14:470–481

Chamberlin NL, Arrigoni E, Chou TC, Scammell TE, Greene RW, Saper CB (2003) Effects of adenosine on gabaergic synaptic inputs to identified ventrolateral preoptic neurons. Neuroscience 119:913–918

Chauvette S, Volgushev M, Timofeev I (2010) Origin of active states in local neocortical networks during slow sleep oscillation. Cereb Cortex 20:2660–2674

Chauvette S, Crochet S, Volgushev M, Timofeev I (2011) Properties of slow oscillation during slow-wave sleep and anesthesia in cats. J Neurosci 31:14998–15008

Chiappalone M, Bove M, Vato A, Tedesco M, Martinoia S (2006) Dissociated cortical networks show spontaneously correlated activity patterns during in vitro development. Brain Res 1093:41–53

Civillico EF, Contreras D (2012) Spatiotemporal properties of sensory responses in vivo are strongly dependent on network context. Front Syst Neurosci 6:25

Clinton JM, Davis CJ, Zielinski MR, Jewett KA, Krueger JM (2011) Biochemical regulation of sleep and sleep biomarkers. J Clin Sleep Med 7:S38–S42

Connelly WM, Crunelli V, Errington AC (2015) The global spike: conserved dendritic properties enable unique Ca2+ spike generation in low-threshold spiking neurons. J Neurosci 35:15505–15522

Corner MA (2008) Spontaneous neuronal burst discharges as dependent and independent variables in the maturation of cerebral cortex tissue cultured in vitro: a review of activity-dependent studies in live 'model' systems for the development of intrinsically generated bioelectric slow-wave sleep patterns. Brain Res Rev 59:221–244

Corner MA (2013) From neural plate to cortical arousal-a neuronal network theory of sleep derived from in vitro "model" systems for primordial patterns of spontaneous bioelectric activity in the vertebrate central nervous system. Brain Sci 3:800–820

Cox J, Pinto L, Dan Y (2016) Calcium imaging of sleep-wake related neuronal activity in the dorsal pons. Nat Commun 7:10763

Crestani F, Martin JR, Möhler H, Rudolph U (2000) Mechanism of action of the hypnotic zolpidem in vivo. Br J Pharmacol 131:1251–1254

Crochet S, Chauvette S, Boucetta S, Timofeev I (2005) Modulation of synaptic transmission in neocortex by network activities. Eur J Neurosci 21:1030–1044

Crunelli V, Hughes SW (2009) The slow (<1 Hz) rhythm of non-REM sleep: a dialogue between three cardinal oscillators. Nat Neurosci 13:9–17

Crunelli V, Toth TI, Cope DW, Blethyn K, Hughes SW (2005) The 'window' T-type calcium current in brain dynamics of different behavioural states. J Physiol 562:121–129

Crunelli V, David F, Lorincz ML, Hughes SW (2015) The thalamocortical network as a single slow wave-generating unit. Curr Opin Neurobiol 31:72–80

Dämgen K, Lüddens H (1999) Zaleplon displays a selectivity to recombinant GABAA receptors different from zolipdem, zopiclone and benzodiazepines. Neurosci Res Commun 25:139–148

Datta S, Siwek DF (2002) Single cell activity patterns of pedunculopontine tegmentum neurons across the sleep-wake cycle in the freely moving rats. J Neurosci Res 70:611–621

David F, Schmiedt JT, Taylor HL, Orban G, Di Giovanni G, Uebele VN, Renger JJ, Lambert RC, Leresche N, Crunelli V (2013) Essential thalamic contribution to slow waves of natural sleep. J Neurosci 33:19599–19610

Destexhe A, Contreras D, Steriade M (1999) Spatiotemporal analysis of local field potentials and unit discharges in cat cerebral cortex during natural wake and sleep states. J Neurosci 19:4595–4608

Dijk DJ, Beersma DG, Daan S (1987) EEG power density during nap sleep: reflection of an hourglass measuring the duration of prior wakefulness. J Biol Rhythm 2:207–219

Dijk DJ, James LM, Peters S, Walsh JK, Deacon S (2010) Sex differences and the effect of gaboxadol and zolpidem on EEG power spectra in NREM and REM sleep. J Psychopharmacol 24:1613–1618

Eban-Rothschild A, Appelbaum L, de Lecea L (2018) Neuronal mechanisms for sleep/wake regulation and modulatory drive. Neuropsychopharmacology 43:937–952

Einstein MC, Polack PO, Tran DT, Golshani P (2017) Visually evoked 3-5 Hz membrane potential oscillations reduce the responsiveness of visual cortex neurons in awake behaving mice. J Neurosci 37:5084–5098

Errington AC, Hughes SW, Crunelli V (2012) Rhythmic dendritic Ca2+ oscillations in thalamocortical neurons during slow non-REM sleep-related activity in vitro. J Physiol 590:3691–3700

Espie CA, MacMahon KMA, Kelly H-L, Broomfield NM, Douglas NJ, Engleman HM, McKinstry B, Morin CM, Walker A, Wilson P (2007) Randomized clinical effectiveness trial of nurse-administered small-group cognitive behavior therapy for persistent insomnia in general practice. Sleep 30:574–584

Esser SK, Hill SL, Tononi G (2007) Sleep homeostasis and cortical synchronization: I. Modeling the effects of synaptic strength on sleep slow waves. Sleep 30:1617–1630

Ferron JF, Kroeger D, Chever O, Amzica F (2009) Cortical inhibition during burst suppression induced with isoflurane anesthesia. J Neurosci 29:9850–9860

Fiath R, Kerekes BP, Wittner L, Toth K, Beregszaszi P, Horvath D, Ulbert I (2016) Laminar analysis of the slow wave activity in the somatosensory cortex of anesthetized rats. Eur J Neurosci 44:1935–1951

Finelli LA, Borbely AA, Achermann P (2001) Functional topography of the human nonREM sleep electroencephalogram. Eur J Neurosci 13:2282–2290

Fisher SP, Vyazovskiy VV (2014) Local sleep taking care of high-maintenance cortical circuits under sleep restriction. Sleep 37:1727–1730

Fisher SP, Cui N, McKillop LE, Gemignani J, Bannerman DM, Oliver PL, Peirson SN, Vyazovskiy VV (2016) Stereotypic wheel running decreases cortical activity in mice. Nat Commun 7:13138

Fontanini A, Katz DB (2008) Behavioral states, network states, and sensory response variability. J Neurophysiol 100:1160–1168

Franken P, Dijk DJ, Tobler I, Borbely AA (1991) Sleep deprivation in rats: effects on EEG power spectra, vigilance states, and cortical temperature. Am J Phys 261:R198–R208

Franks NP (2008) General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nat Rev Neurosci 9:370–386

Freeman D, Sheaves B, Goodwin GM, Yu L-M, Harrison PJ, Emsley R, Bostock S, Foster RG, Wadekar V, Hinds C et al (2015) Effects of cognitive behavioural therapy for insomnia on the mental health of university students: study protocol for a randomized controlled trial. Trials 16:236

Fucke T, Suchanek D, Nawrot MP, Seamari Y, Heck DH, Aertsen A, Boucsein C (2011) Stereotypical spatiotemporal activity patterns during slow-wave activity in the neocortex. J Neurophysiol 106:3035–3044

Funk CM, Peelman K, Bellesi M, Marshall W, Cirelli C, Tononi G (2017) Role of somatostatin-positive cortical interneurons in the generation of sleep slow waves. J Neurosci 37:9132–9148

Gallopin T, Fort P, Eggermann E, Cauli B, Luppi PH, Rossier J, Audinat E, Muhlethaler M, Serafin M (2000) Identification of sleep-promoting neurons in vitro. Nature 404:992–995

Gerashchenko D, Wisor JP, Burns D, Reh RK, Shiromani PJ, Sakurai T, de la Iglesia HO, Kilduff TS (2008) Identification of a population of sleep-active cerebral cortex neurons. Proc Natl Acad Sci U S A 105:10227–10232

Gervasoni D, Peyron C, Rampon C, Barbagli B, Chouvet G, Urbain N, Fort P, Luppi PH (2000) Role and origin of the GABAergic innervation of dorsal raphe serotonergic neurons. J Neurosci 20:4217–4225

Gil Z, Connors BW, Amitai Y (1997) Differential regulation of neocortical synapses by neuromodulators and activity. Neuron 19:679–686

González-Rueda A, Pedrosa V, Feord RC, Clopath C, Paulsen O (2018) Activity-dependent downscaling of subthreshold synaptic inputs during slow-wave-sleep-like activity in vivo. Neuron 97:1244–1252

Guillaumin MCC, McKillop LE, Cui N, Fisher SP, Foster RG, de Vos M, Peirson SN, Achermann P, Vyazovskiy VV (2018) Cortical region-specific sleep homeostasis in mice: effects of time of day and waking experience. Sleep 41

Haider B, Duque A, Hasenstaub AR, McCormick DA (2006) Neocortical network activity in vivo is generated through a dynamic balance of excitation and inhibition. J Neurosci 26:4535–4545

Haider B, Duque A, Hasenstaub AR, Yu Y, McCormick DA (2007) Enhancement of visual responsiveness by spontaneous local network activity in vivo. J Neurophysiol 97:4186–4202

Halassa MM, Chen Z, Wimmer RD, Brunetti PM, Zhao S, Zikopoulos B, Wang F, Brown EN, Wilson MA (2014) State-dependent architecture of thalamic reticular subnetworks. Cell 158:808–821

Hammond MW, Xydas D, Downes JH, Bucci G, Becerra V, Warwick K, Constanti A, Nasuto SJ, Whalley BJ (2013) Endogenous cholinergic tone modulates spontaneous network level neuronal activity in primary cortical cultures grown on multi-electrode arrays. BMC Neurosci 14:38

Hill S, Tononi G (2005) Modeling sleep and wakefulness in the thalamocortical system. J Neurophysiol 93:1671–1698

Hinard V, Mikhail C, Pradervand S, Curie T, Houtkooper RH, Auwerx J, Franken P, Tafti M (2012) Key electrophysiological, molecular, and metabolic signatures of sleep and wakefulness revealed in primary cortical cultures. J Neurosci 32:12506–12517

Hirsch JA, Gilbert CD (1991) Synaptic physiology of horizontal connections in the cat's visual cortex. J Neurosci 11:1800–1809

Hoel EP, Albantakis L, Cirelli C, Tononi G (2016) Synaptic refinement during development and its effect on slow-wave activity: a computational study. J Neurophysiol 115:2199–2213

Hwang E, McNally JM, Choi JH (2013) Reduction in cortical gamma synchrony during depolarized state of slow wave activity in mice. Front Syst Neurosci 7:107

Isaacson JS, Scanziani M (2011) How inhibition shapes cortical activity. Neuron 72:231–243

Jackson J, Ayzenshtat I, Karnani MM, Yuste R (2016) VIP+ interneurons control neocortical activity across brain states. J Neurophysiol 115:3008–3017

Jean-Marc F, Hanns M (2004) GABAA-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. J Comp Neurol 359:154–194

Jewett KA, Taishi P, Sengupta P, Roy S, Davis CJ, Krueger JM (2015) Tumor necrosis factor enhances the sleep-like state and electrical stimulation induces a wake-like state in co-cultures of neurons and glia. Eur J Neurosci 42:2078–2090

Jones BE (2004) Paradoxical REM sleep promoting and permitting neuronal networks. Arch Ital Biol 142:379–396

Jones EG (2009) Synchrony in the interconnected circuitry of the thalamus and cerebral cortex. Ann N Y Acad Sci 1157:10–23

Kattler H, Dijk DJ, Borbely AA (1994) Effect of unilateral somatosensory stimulation prior to sleep on the sleep EEG in humans. J Sleep Res 3:159–164

Kennedy C, Gillin JC, Mendelson W, Suda S, Miyaoka M, Ito M, Nakamura RK, Storch FI, Pettigrew K, Mishkin M et al (1982) Local cerebral glucose utilization in non-rapid eye movement sleep. Nature 297:325–327

Kisley MA, Gerstein GL (1999) Trial-to-trial variability and state-dependent modulation of auditory-evoked responses in cortex. J Neurosci 19:10451–10460

Kopp C, Rudolph U, Keist R, Tobler I (2003) Diazepam-induced changes on sleep and the EEG spectrum in mice: role of the α3-GABAA receptor subtype. Eur J Neurosci 17:2226–2230

Kopp C, Rudolph U, Low K, Tobler I (2004a) Modulation of rhythmic brain activity by diazepam: GABA(A) receptor subtype and state specificity. Proc Natl Acad Sci U S A 101:3674–3679

Kopp C, Rudolph U, Tobler I (2004b) Sleep EEG changes after zolpidem in mice. Neuroreport 15:2299–2302

Kosse C, Gonzalez A, Burdakov D (2015) Predictive models of glucose control: roles for glucose-sensing neurones. Acta Physiol (Oxf) 213:7–18

Kovalzon VM (2009) Some notes on the biography of Maria Manasseina. J Hist Neurosci 18:312–319

Kripke DF (2000) Chronic hypnotic use: deadly risks, doubtful benefit. REVIEW ARTICLE. Sleep Med Rev 4:5–20

Kroeger D, Amzica F (2007) Hypersensitivity of the anesthesia-induced comatose brain. J Neurosci 27:10597–10607

Krueger JM, Obal F (1993) A neuronal group theory of sleep function. J Sleep Res 2:63–69

Krueger JM, Rector DM, Roy S, Van Dongen HP, Belenky G, Panksepp J (2008) Sleep as a fundamental property of neuronal assemblies. Nat Rev Neurosci 9:910–919

Krueger JM, Huang YH, Rector DM, Buysse DJ (2013) Sleep: a synchrony of cell activity-driven small network states. Eur J Neurosci 38:2199–2209

Krueger JM, Frank MG, Wisor JP, Roy S (2016) Sleep function: toward elucidating an enigma. Sleep Med Rev 28:42–50

Kuki T, Ohshiro T, Ito S, Ji ZG, Fukazawa Y, Matsuzaka Y, Yawo H, Mushiake H (2013) Frequency-dependent entrainment of neocortical slow oscillation to repeated optogenetic stimulation in the anesthetized rat. Neurosci Res 75:35–45

Kvitsiani D, Ranade S, Hangya B, Taniguchi H, Huang JZ, Kepecs A (2013) Distinct behavioural and network correlates of two interneuron types in prefrontal cortex. Nature 498:363–366

Lancel M (1999) Role of GABAA receptors in the regulation of sleep: initial sleep responses to peripherally administered modulators and agonists. Sleep 22:33–42

Lancel M, Crönlein TAM, Faulhaber J (1996) Role of $GABA_A$ receptors in sleep regulation. Neuropsychopharmacology 15:63–74

Lancel M, Faulhaber J, Schiffelholz T, Mathias S, Deisz RA (1997) Muscimol and midazolam do not potentiate each other's effects on sleep EEG in the rat. J Neurophysiol 77:1624–1629

Landolt HP, Borbely AA (2001) Age-dependent changes in sleep EEG topography. Clin Neurophysiol 112:369–377

Laplante F, Morin Y, Quirion R, Vaucher E (2005) Acetylcholine release is elicited in the visual cortex, but not in the prefrontal cortex, by patterned visual stimulation: a dual in vivo microdialysis study with functional correlates in the rat brain. Neuroscience 132:501–510

Latham PE, Richmond BJ, Nirenberg S, Nelson PG (2000) Intrinsic dynamics in neuronal networks. II. Experiment. J Neurophysiol 83:828–835

Lawrence JJ (2008) Cholinergic control of GABA release: emerging parallels between neocortex and hippocampus. Trends Neurosci 31:317–327
Lazar AS, Lazar ZI, Dijk DJ (2015) Circadian regulation of slow waves in human sleep: topographical aspects. NeuroImage 116:123–134
Lee SH, Dan Y (2012) Neuromodulation of brain states. Neuron 76:209–222
Lee RS, Steffensen SC, Henriksen SJ (2001) Discharge profiles of ventral tegmental area GABA neurons during movement, anesthesia, and the sleep-wake cycle. J Neurosci 21:1757–1766
Lemieux M, Chen JY, Lonjers P, Bazhenov M, Timofeev I (2014) The impact of cortical deafferentation on the neocortical slow oscillation. J Neurosci 34:5689–5703
Lemieux M, Chauvette S, Timofeev I (2015) Neocortical inhibitory activities and long-range afferents contribute to the synchronous onset of silent states of the neocortical slow oscillation. J Neurophysiol 113:768–779
Leung LC, Wang GX, Mourrain P (2013) Imaging zebrafish neural circuitry from whole brain to synapse. Front Neural Circ 7:76
Li KY, Guan YZ, Krnjevic K, Ye JH (2009) Propofol facilitates glutamatergic transmission to neurons of the ventrolateral preoptic nucleus. Anesthesiology 111:1271–1278
Lissek T, Obenhaus HA, Ditzel DA, Nagai T, Miyawaki A, Sprengel R, Hasan MT (2016) General anesthetic conditions induce network synchrony and disrupt sensory processing in the cortex. Front Cell Neurosci 10:64
Liu YW, Li J, Ye JH (2010) Histamine regulates activities of neurons in the ventrolateral preoptic nucleus. J Physiol 588:4103–4116
Logothetis NK, Augath M, Murayama Y, Rauch A, Sultan F, Goense J, Oeltermann A, Merkle H (2010) The effects of electrical microstimulation on cortical signal propagation. Nat Neurosci 13:1283–1291
Luczak A, Bartho P (2012) Consistent sequential activity across diverse forms of UP states under ketamine anesthesia. Eur J Neurosci 36:2830–2838
Luczak A, Bartho P, Marguet SL, Buzsaki G, Harris KD (2007) Sequential structure of neocortical spontaneous activity in vivo. Proc Natl Acad Sci U S A 104:347–352
Mackiewicz M, Naidoo N, Zimmerman JE, Pack AI (2008) Molecular mechanisms of sleep and wakefulness. Ann N Y Acad Sci 1129:335–349
Mander BA, Winer JR, Walker MP (2017) Sleep and human aging. Neuron 94:19–36
Manoach DS, Pan JQ, Purcell SM, Stickgold R (2016) Reduced sleep spindles in schizophrenia: a treatable endophenotype that links risk genes to impaired cognition? Biol Psychiatry 80:599–608
Marks GA, Roffwarg HP (1993) Spontaneous activity in the thalamic reticular nucleus during the sleep/wake cycle of the freely-moving rat. Brain Res 623:241–248
Marrocco RT, Lane RF, McClurkin JW, Blaha CD, Alkire MF (1987) Release of cortical catecholamines by visual stimulation requires activity in thalamocortical afferents of monkey and cat. J Neurosci 7:2756–2767
McCormick DA, Bal T (1997) Sleep and arousal: thalamocortical mechanisms. Annu Rev Neurosci 20:185–215
McCormick DA, Pape HC, Williamson A (1991) Actions of norepinephrine in the cerebral cortex and thalamus: implications for function of the central noradrenergic system. Prog Brain Res 88:293–305
McGinley MJ, David SV, McCormick DA (2015) Cortical membrane potential signature of optimal states for sensory signal detection. Neuron 87:179–192
McKillop LE, Fisher SP, Cui N, Peirson SN, Foster RG, Wafford KA, Vyazovskiy VV (2018) Effects of ageing on cortical neural dynamics and local sleep homeostasis in mice. J Neurosci 38:3911–3928
Meisel C, Klaus A, Vyazovskiy VV, Plenz D (2017) The interplay between long- and short-range temporal correlations shapes cortex dynamics across vigilance states. J Neurosci 37:10114–10124

Mileykovskiy BY, Kiyashchenko LI, Siegel JM (2005) Behavioral correlates of activity in identified hypocretin/orexin neurons. Neuron 46:787–798
Mohler H (2011) The rise of a new GABA pharmacology. Neuropharmacology 60:1042–1049
Molle M, Born J (2011) Slow oscillations orchestrating fast oscillations and memory consolidation. Prog Brain Res 193:93–110
Moore JT, Chen J, Han B, Meng QC, Veasey SC, Beck SG, Kelz MB (2012) Direct activation of sleep-promoting VLPO neurons by volatile anesthetics contributes to anesthetic hypnosis. Curr Biol 22:2008–2016
Morairty SR, Dittrich L, Pasumarthi RK, Valladao D, Heiss JE, Gerashchenko D, Kilduff TS (2013) A role for cortical nNOS/NK1 neurons in coupling homeostatic sleep drive to EEG slow wave activity. Proc Natl Acad Sci U S A 110:20272–20277
Morin CM, Bootzin RR, Buysse DJ, Edinger JD, Espie CA, Lichstein KL (2006) Psychological and behavioral treatment of insomnia: update of the recent evidence (1998–2004). Sleep 29:1398–1414
Moruzzi G (1972) Sleep-waking cycle. Ergebnisse Der Physiologie Biologischen Chemie Und Experimentellen Pharmakologie 64:1–165
Neske GT (2016) The slow oscillation in cortical and thalamic networks: mechanisms and functions. Front Neural Circ 9:88
Neske GT, Connors BW (2016) Distinct roles of SOM and VIP interneurons during cortical up states. Front Neural Circ 10:52
Niethard N, Hasegawa M, Itokazu T, Oyanedel CN, Born J, Sato TR (2016) Sleep-stage-specific regulation of cortical excitation and inhibition. Curr Biol 26:2739–2749
Nir Y, Andrillon T, Marmelshtein A, Suthana N, Cirelli C, Tononi G, Fried I (2017) Selective neuronal lapses precede human cognitive lapses following sleep deprivation. Nat Med 23:1474–1480
Olbrich E, Achermann P (2005) Analysis of oscillatory patterns in the human sleep EEG using a novel detection algorithm. J Sleep Res 14:337–346
Olbrich E, Achermann P, Wennekers T (2011) The sleeping brain as a complex system. Philos Trans A Math Phys Eng Sci 369:3697–3707
Olcese U, Faraguna U (2015) Slow cortical rhythms: from single-neuron electrophysiology to whole-brain imaging in vivo. Arch Ital Biol 153:87–98
Olfson M, King M, Schoenbaum M (2015) Benzodiazepine use in the United States. JAMA Psychiat 72:136–142
Olofsen E, Sleigh JW, Dahan A (2008) Permutation entropy of the electroencephalogram: a measure of anaesthetic drug effect. Br J Anaesth 101:810–821
Petersen CC, Hahn TT, Mehta M, Grinvald A, Sakmann B (2003) Interaction of sensory responses with spontaneous depolarization in layer 2/3 barrel cortex. Proc Natl Acad Sci U S A 100:13638–13643
Pigarev IN, Nothdurft HC, Kastner S (1997) Evidence for asynchronous development of sleep in cortical areas. Neuroreport 8:2557–2560
Pigeat R, Chausson P, Dreyfus FM, Leresche N, Lambert RC (2015) Sleep slow wave-related homo and heterosynaptic LTD of intrathalamic GABAAergic synapses: involvement of T-type Ca2+ channels and metabotropic glutamate receptors. J Neurosci 35:64–73
Pigorini A, Sarasso S, Proserpio P, Szymanski C, Arnulfo G, Casarotto S, Fecchio M, Rosanova M, Mariotti M, Lo Russo G et al (2015) Bistability breaks-off deterministic responses to intracortical stimulation during non-REM sleep. NeuroImage 112:105–113
Polack PO, Friedman J, Golshani P (2013) Cellular mechanisms of brain state-dependent gain modulation in visual cortex. Nat Neurosci 16:1331–1339
Poulet JF, Petersen CC (2008) Internal brain state regulates membrane potential synchrony in barrel cortex of behaving mice. Nature 454:881–885
Raizen DM, Zimmerman JE, Maycock MH, Ta UD, You YJ, Sundaram MV, Pack AI (2008) Lethargus is a Caenorhabditis elegans sleep-like state. Nature 451:569–572
Ramot M, Fisch L, Davidesco I, Harel M, Kipervasser S, Andelman F, Neufeld MY, Kramer U, Fried I, Malach R (2013) Emergence of sensory patterns during sleep highlights differential dynamics of REM and non-REM sleep stages. J Neurosci 33:14715–14728

Rector DM, Topchiy IA, Carter KM, Rojas MJ (2005) Local functional state differences between rat cortical columns. Brain Res 1047:45–55
Rector DM, Schei JL, Van Dongen HPA, Belenky G, Krueger JM (2009) Physiological markers of local sleep. Eur J Neurosci 29:1771–1778
Reig R, Sanchez-Vives MV (2007) Synaptic transmission and plasticity in an active cortical network. PLoS One 2:e670
Reig R, Zerlaut Y, Vergara R, Destexhe A, Sanchez-Vives MV (2015) Gain modulation of synaptic inputs by network state in auditory cortex in vivo. J Neurosci 35:2689–2702
Riedner BA, Vyazovskiy VV, Huber R, Massimini M, Esser S, Murphy M, Tononi G (2007) Sleep homeostasis and cortical synchronization: III. A high-density EEG study of sleep slow waves in humans. Sleep 30:1643–1657
Roy S, Krueger JM, Rector DM, Wan Y (2008) A network model for activity-dependent sleep regulation. J Theor Biol 253:462–468
Rudolph U, Mohler H (2006) GABA-based therapeutic approaches: GABAA receptor subtype functions. Curr Opin Pharmacol 6:18–23
Ruiz-Mejias M, Ciria-Suarez L, Mattia M, Sanchez-Vives MV (2011) Slow and fast rhythms generated in the cerebral cortex of the anesthetized mouse. J Neurophysiol 106:2910–2921
Saberi-Moghadam S, Simi A, Setareh H, Mikhail C, Tafti M (2018) In vitro cortical network firing is homeostatically regulated: a model for sleep regulation. Sci Rep 8:6297
Sakata S, Harris KD (2012) Laminar-dependent effects of cortical state on auditory cortical spontaneous activity. Front Neural Circ 6:109
Saleem AB, Chadderton P, Apergis-Schoute J, Harris KD, Schultz SR (2010) Methods for predicting cortical UP and DOWN states from the phase of deep layer local field potentials. J Comput Neurosci 29:49–62
Sanchez-Vives MV, Mattia M (2014) Slow wave activity as the default mode of the cerebral cortex. Arch Ital Biol 152:147–155
Sanchez-Vives MV, McCormick DA (2000) Cellular and network mechanisms of rhythmic recurrent activity in neocortex. Nat Neurosci 3:1027–1034
Sanchez-Vives MV, Mattia M, Compte A, Perez-Zabalza M, Winograd M, Descalzo VF, Reig R (2010) Inhibitory modulation of cortical up states. J Neurophysiol 104:1314–1324
Sanger DJ, Morel E, Perrault G (1996) Comparison of the pharmacological profiles of the hypnotic drugs, zaleplon and zolpidem. Eur J Pharmacol 313:35–42
Saper CB, Chou TC, Scammell TE (2001) The sleep switch: hypothalamic control of sleep and wakefulness. Trends Neurosci 24:726–731
Saper CB, Fuller PM, Pedersen NP, Lu J, Scammell TE (2010) Sleep state switching. Neuron 68:1023–1042
Scammell TE, Estabrooke IV, McCarthy MT, Chemelli RM, Yanagisawa M, Miller MS, Saper CB (2000) Hypothalamic arousal regions are activated during modafinil-induced wakefulness. J Neurosci 20:8620–8628
Scheeringa R, Mazaheri A, Bojak I, Norris DG, Kleinschmidt A (2011) Modulation of visually evoked cortical FMRI responses by phase of ongoing occipital alpha oscillations. J Neurosci 31:3813–3820
Schutte-Rodin S, Broch L, Buysse D, Dorsey C, Sateia M (2008) Clinical guideline for the evaluation and management of chronic insomnia in adults. J Clin Sleep Med 4:487–504
Seibt J, Aton SJ, Jha SK, Coleman T, Dumoulin MC, Frank MG (2008) The non-benzodiazepine hypnotic zolpidem impairs sleep-dependent cortical plasticity. Sleep 31:1381–1391
Seibt J, Richard CJ, Sigl-Glöckner J, Takahashi N, Kaplan DI, Doron G, de Limoges D, Bocklisch C, Larkum ME (2017) Cortical dendritic activity correlates with spindle-rich oscillations during sleep in rodents. Nat Commun 8:684
Sela Y, Vyazovskiy VV, Cirelli C, Tononi G, Nir Y (2016) Responses in rat core auditory cortex are preserved during sleep spindle oscillations. Sleep 39:1069–1082
Sherin JE, Shiromani PJ, McCarley RW, Saper CB (1996) Activation of ventrolateral preoptic neurons during sleep. Science 271:216–219

Sheroziya M, Timofeev I (2014) Global intracellular slow-wave dynamics of the thalamocortical system. J Neurosci 34:8875–8893

Siclari F, Bernardi G, Riedner BA, LaRocque JJ, Benca RM, Tononi G (2014) Two distinct synchronization processes in the transition to sleep: a high-density electroencephalographic study. Sleep 37:1621

Staiger JF, Zuschratter W, Luhmann HJ, Schubert D (2009) Local circuits targeting parvalbumin-containing interneurons in layer IV of rat barrel cortex. Brain Struct Funct 214:1–13

Staresina BP, Bergmann TO, Bonnefond M, van der Meij R, Jensen O, Deuker L, Elger CE, Axmacher N, Fell J (2015) Hierarchical nesting of slow oscillations, spindles and ripples in the human hippocampus during sleep. Nat Neurosci 18:1679–1686

Stepanyants A, Martinez LM, Ferecsko AS, Kisvarday ZF (2009) The fractions of short- and long-range connections in the visual cortex. Proc Natl Acad Sci U S A 106:3555–3560

Steriade M (1978) Cortical long-axoned cells and putative interneurons during the sleep-waking cycle. Behav Brain Sci 1:465–485

Steriade M (2001) Impact of network activities on neuronal properties in corticothalamic systems. J Neurophysiol 86:1–39

Steriade M, Amzica F (1998) Coalescence of sleep rhythms and their chronology in corticothalamic networks. Sleep Res Online 1:1–10

Steriade M, Domich L, Oakson G (1986) Reticularis thalami neurons revisited: activity changes during shifts in states of vigilance. J Neurosci 6:68–81

Steriade M, Domich L, Oakson G, Deschenes M (1987) The deafferented reticular thalamic nucleus generates spindle rhythmicity. J Neurophysiol 57:260–273

Steriade M, Datta S, Pare D, Oakson G, Curro Dossi RC (1990) Neuronal activities in brain-stem cholinergic nuclei related to tonic activation processes in thalamocortical systems. J Neurosci 10:2541–2559

Steriade M, Nunez A, Amzica F (1993) A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. J Neurosci 13:3252–3265

Suntsova N, Guzman-Marin R, Kumar S, Alam MN, Szymusiak R, McGinty D (2007) The median preoptic nucleus reciprocally modulates activity of arousal-related and sleep-related neurons in the perifornical lateral hypothalamus. J Neurosci 27:1616–1630

Szymusiak R, Alam N, Steininger TL, McGinty D (1998) Sleep-waking discharge patterns of ventrolateral preoptic/anterior hypothalamic neurons in rats. Brain Res 803:178–188

Tabarean IV (2013) Functional pharmacology of H1 histamine receptors expressed in mouse preoptic/anterior hypothalamic neurons. Br J Pharmacol 170:415–425

Timofeev I (2013) Local origin of slow EEG waves during sleep. Zh Vyssh Nerv Deiat Im I P Pavlova 63:105–112

Timofeev I, Grenier F, Bazhenov M, Sejnowski TJ, Steriade M (2000) Origin of slow cortical oscillations in deafferented cortical slabs. Cereb Cortex 10:1185–1199

Timofeev I, Grenier F, Steriade M (2001) Disfacilitation and active inhibition in the neocortex during the natural sleep-wake cycle: an intracellular study. Proc Natl Acad Sci U S A 98:1924–1929

Tobler I (2005) Phylogeny of sleep regulation. In: Kryger MH, Roth T, Dement WC (eds) Principles and practice of sleep medicine. W. B. Saunders, Philadelphia

Tobler I, Kopp C, Deboer T, Rudolph U (2001) Diazepam-induced changes in sleep: role of the α1 GABAA receptor subtype. Proc Natl Acad Sci 98:6464–6469

Van Dongen HP, Belenky G, Krueger JM (2011) A local, bottom-up perspective on sleep deprivation and neurobehavioral performance. Curr Top Med Chem 11:2414–2422

Volgushev M, Chauvette S, Timofeev I (2011) Long-range correlation of the membrane potential in neocortical neurons during slow oscillation. Prog Brain Res 193:181–199

Voss L, Sleigh J (2007) Monitoring consciousness: the current status of EEG-based depth of anaesthesia monitors. Best Pract Res Clin Anaesthesiol 21:313–325. Awareness during Anaesthesia

Vyazovskiy VV (2013) Cortical neuronal mechanisms of sleep homeostasis. Zh Vyssh Nerv Deiat Im I P Pavlova 63:13–23
Vyazovskiy VV (2015) Sleep, recovery, and metaregulation: explaining the benefits of sleep. Nat Sci Sleep 7:171–184
Vyazovskiy VV, Delogu A (2014) NREM and REM sleep: complementary roles in recovery after wakefulness. Neuroscientist 20:203–219
Vyazovskiy VV, Harris KD (2013) Sleep and the single neuron: the role of global slow oscillations in individual cell rest. Nat Rev Neurosci 14:443–451
Vyazovskiy V, Borbely AA, Tobler I (2000) Unilateral vibrissae stimulation during waking induces interhemispheric EEG asymmetry during subsequent sleep in the rat. J Sleep Res 9:367–371
Vyazovskiy VV, Deboer T, Rudy B, Lau D, Borbely AA, Tobler I (2002) Sleep EEG in mice that are deficient in the potassium channel subunit K.v.3.2. Brain Res 947:204–211
Vyazovskiy VV, Achermann P, Borbely AA, Tobler I (2004) The dynamics of spindles and EEG slow-wave activity in NREM sleep in mice. Arch Ital Biol 142:511–523
Vyazovskiy VV, Achermann P, Tobler I (2007) Sleep homeostasis in the rat in the light and dark period. Brain Res Bull 74:37–44
Vyazovskiy VV, Olcese U, Lazimy YM, Faraguna U, Esser SK, Williams JC, Cirelli C, Tononi G (2009) Cortical firing and sleep homeostasis. Neuron 63:865–878
Vyazovskiy VV, Cirelli C, Tononi G (2011a) Electrophysiological correlates of sleep homeostasis in freely behaving rats. Prog Brain Res 193:17–38
Vyazovskiy VV, Olcese U, Hanlon EC, Nir Y, Cirelli C, Tononi G (2011b) Local sleep in awake rats. Nature 472:443–447
Vyazovskiy VV, Olcese U, Cirelli C, Tononi G (2013) Prolonged wakefulness alters neuronal responsiveness to local electrical stimulation of the neocortex in awake rats. J Sleep Res 22:264–271
Vyazovskiy VV, Cui N, Rodriguez AV, Funk C, Cirelli C, Tononi G (2014) The dynamics of cortical neuronal activity in the first minutes after spontaneous awakening in rats and mice. Sleep 37:1337–1347
Wagenaar DA, Madhavan R, Pine J, Potter SM (2005) Controlling bursting in cortical cultures with closed-loop multi-electrode stimulation. J Neurosci 25:680–688
Watanabe T, Kan S, Koike T, Misaki M, Konishi S, Miyauchi S, Miyahsita Y, Masuda N (2014) Network-dependent modulation of brain activity during sleep. NeuroImage 98:1–10
Watson BO, MacLean JN, Yuste R (2008) UP states protect ongoing cortical activity from thalamic inputs. PLoS One 3:e3971
Watson CJ, Baghdoyan HA, Lydic R (2010) Neuropharmacology of sleep and wakefulness. Sleep Med Clin 5:513–528
Watson BO, Levenstein D, Greene JP, Gelinas JN, Buzsaki G (2016) Network homeostasis and state dynamics of neocortical sleep. Neuron 90:839–852
Winsky-Sommerer R (2009) Role of GABAA receptors in the physiology and pharmacology of sleep. Eur J Neurosci 29:1779–1794
Wirz-Justice A (2009) From the basic neuroscience of circadian clock function to light therapy for depression: on the emergence of chronotherapeutics. J Affect Disord 116:159–160
Yüzgeç Ö, Prsa M, Zimmermann R, Huber D (2018) Pupil size coupling to cortical states protects the stability of deep sleep via parasympathetic modulation. Curr Biol 28:392–400.e393
Zagha E, McCormick DA (2014) Neural control of brain state. Curr Opin Neurobiol 29:178–186
Zhang Z, Ferretti V, Guntan I, Moro A, Steinberg EA, Ye Z, Zecharia AY, Yu X, Vyssotski AL, Brickley SG et al (2015) Neuronal ensembles sufficient for recovery sleep and the sedative actions of alpha2 adrenergic agonists. Nat Neurosci 18:553–561
Zucca S, D'Urso G, Pasquale V, Vecchia D, Pica G, Bovetti S, Moretti C, Varani S, Molano-Mazón M, Chiappalone M et al (2017) An inhibitory gate for state transition in cortex. elife 6:e26177

Optogenetic Dissection of Sleep-Wake States In Vitro and In Vivo

Antoine Adamantidis and Anita Lüthi

Contents

Abstract

Optogenetic tools have revolutionized insights into the fundamentals of brain function. This is particularly true for our current understanding of sleep-wake regulation and sleep rhythms. This is illustrated here through a comprehensive and step-by-step review over the major brain areas involved in transitions between sleep and wake states and in sleep rhythmogenesis.

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1 Introductory Remarks

1.1 Identify Brain Areas That Are Key for Transitions Between Vigilance States

Sleep or sleep-like activity is present in practically all animals studied to date and the vital necessity of sleep has been demonstrated in organisms ranging in complexity from fruit flies to humans (Siegel 2008). Over the past half-century several potential functions of sleep have been identified including, but not limited to, restoration and homeostasis (Siegel 2008), adaptive energy conservation (Schmidt 2014), development and brain clearance (Xie et al. 2013), and memory consolidation (Rasch and Born 2013). However, despite the overt prevalence and importance of sleep throughout the animal kingdom, its specific function(s) remain(s) to be clarified (Krueger et al. 2016; Siegel 2009; Tononi and Cirelli 2014). The basic neurobiological processes involved in sleep are still the subject of intense studies that resulted in the identification of the genetic basis of neural circuits and their correlated activity across sleep-wake states (Fort et al. 2009; Pace-Schott and Hobson 2002). A fundamental role of sleep for the "brain," the "mind," and the "body" has been supported by its conservation throughout evolution. Indeed, fluctuating levels of consciousness and circulating factors (hormones, metabolites, etc.) as well as changes in ionic composition of the interstitial fluid (Ding et al. 2016) occur during sleep.

Since the seminal experiment in the 1930s that identified the brainstem as a critical area for sleep-wake control (Moruzzi and Magoun 1949) and suggested sleep as a default, or passive, behavioral state, pieces of experimental evidence from the last decades have progressively defined a revised framework wherein sleep is considered an active brain state that results from the coordinated activity of multiple neuronal circuits distributed across the brain. In this view, the neural underpinnings of the sleep-wake state involve interactions between sleep-promoting areas such as the anterior hypothalamus, and arousal systems (most are wake-promoting) located in the posterior hypothalamus, the basal forebrain and the brainstem (Brown et al. 2012; Lee and Dan 2012; Saper et al. 2010). The arousal systems include the histaminergic cell group in the tuberomammillary nucleus (TMN) of the hypothalamus and the hypocretinergic (Hcrt) neurons in the lateral hypothalamus, the cholinergic neurons, located in the pedunculopontine tegmentum (PPT) and the basal forebrain (BF), the noradrenergic neurons of locus coeruleus (LC), the dopaminergic and serotonergic raphe neurons of the brainstem (Aston-Jones and Bloom 1981; Dahan et al. 2007; Hassani et al. 2009; Takahashi et al. 2008). Further details on the neurobiology of sleep-wake states are reviewed in other chapters of this book.

1.2 Principal Goals of Using Optogenetic Techniques for Sleep-Wake Studies

In an effort to draw more causal evidence from experimental testing, genetic engineering, pharmacology, and electrical stimulation strategies have been used to identify molecular/cellular mechanisms of sleep-wake systems in the brain. However, achieving high temporal and spatial resolutions simultaneously has been challenging due to the inherent limitations of the existing techniques. Importantly, genetic engineering approaches target single gene mutations (i.e., high "spatial" resolution in the gene domain) from early embryonic ages in the whole, or in part of the organism (conditional gene targeting). Although conditional approaches or viral targeting limit the genetic modification to the desired brain areas and may additionally reduce the temporal window of action, their timescale remains longer than most of the physiological changes and processes occurring during sleep-wake states. Finally, transient pharmacological approaches, although selective to precisely identified receptors, often rely on non-physiological doses of (ant)agonists molecules that stay in the synaptic cleft where they induce long and persistent (in-)activation of the targeted receptors, as well as, to a lesser extent, other nonselective targets. Thus, the advent of optogenetic technologies to both image and control the activity of neural circuits has overcome most of those limitations through high temporal (millisecond timescale) and spatial (genetic targeting) resolution offering temporally precise activation/silencing of cells/circuits.

Why Is Optogenetics an Appropriate Tool for Experimental Sleep Research?
Both hypothalamus and brainstem, nodal points of sleep-wake control, consist of intricate networks of excitatory and inhibitory neuronal populations, each of which has a specific chemical nature and has separate roles in vigilance state control. All of the evidence supporting a functional role for these circuits in sleep and wakefulness stems from in vitro and in vivo techniques (electrical stimulation, pharmacological approaches, unit recordings, knockout/knockin models, etc.) that have low spatial and temporal resolution and that suffer from possible compensatory mechanisms. Optogenetic technology ("*opto*" for optical control/recording and "*genetics*" for genetically targeted cells/cell activities) is a system neuroscience tool that allows remote control of specific neural circuits with physiologically relevant spatial and temporal resolution. When expressed in a genetically targeted neuronal population, the light-sensitive proteins Channelrhodopsin-2 (ChR2, E123T mutation in ChR2 – ChETA, etc.) and the halo- or archeo-rhodopsin (eNpHR and ArchT, respectively) allow bimodal modulation of electrical signals (activation or inhibition) with millisecond timescale precision. ChR2/ChETA is a monovalent cation channel that allows cations to enter the cell following exposure to ~473 nm blue light and activate cells, whereas the eNpHR and ArchT are a chloride and a proton pump, respectively, that activates upon illumination with ~580 nm yellow light and silences cells. Their fast temporal kinetics makes it possible to drive reliable trains of high

frequency action potentials in vitro and in vivo using ChR2 or suppress single action potentials within high frequency spike trains using NpHR or ArchT in vitro/vivo. Hence, optogenetics allow for frequency-dependent interrogation of the neural system properties in vitro and in vivo without inadvertent stimulation of neighboring neurons.

Ten years after the birth of optogenetics (Adamantidis et al. 2015; Boyden 2015; Deisseroth 2015; Hegemann and Nagel 2013; Miesenbock 2009), major implementations have overcome some biological limitations, however, some experimental challenges remain that will be discussed further in this chapter. Here, we summarize the rise of optogenetics-based experimental studies that are progressively identifying key circuits in the brain controlling sleep-wake state architecture, oscillations, and function(s). We describe key studies that used neuron-inspired patterns of optical stimulation to optogenetically investigate the neural substrate of sleep-wake states, from ChR2-assisted functional circuit mapping to state switching, sleep rhythms, and sleep functions. This chapter is divided into distinct sections, including: (1) optogenetic dissection of sleep-wake state initiation, maintenance, and termination; (2) optogenetic unraveling of circuit mechanisms of sleep homeostasis; (3) optogenetic identification of neural substrates of sleep rhythms and their biological function.

2 Optogenetics and Sleep-Wake Transitions

The recurrent cycle of sleep-wake episodes results from a coordinated activation/inhibition of distributed, yet partially defined, neural networks in the brain that are under a strong internal and external modulation (e.g., hormone, metabolism, homeostatic, circadian, environment, etc.). In mammals, after a consolidated period of wakefulness, the activity of wake-promoting systems slowly decreases and the organism progressively enters into NREM sleep (or slow-wave sleep). A transition back to wakefulness signals the termination of NREM sleep. Depending on species, NREM sleep is followed by REM sleep (or paradoxical sleep) that is usually terminated by a transition to wakefulness (see Fig. 1 for representative cortical EEG/EMG traces typical for the different vigilance states). It is hypothesized that the neural circuits involved are highly specialized in controlling the initiation, maintenance, and termination of distinct states or oscillations in the sleeping brain. Therefore, the following sections will focus on specific transitions and hallmark brain oscillations that are typical for NREM or REM sleep states. For each transition, we summarized recent findings and emphasized optogenetic dissection of neural circuits communication in vitro (example shown in Fig. 2a–d) and in vivo (Fig. 2e–k).

2.1 Transitions from Sleep to Wakefulness

Classically, the onset of wakefulness follows the activation of the reticular ascending system from the brainstem, which projects to forebrain cortical and subcortical structures (Brown et al. 2012; Fort et al. 2009). We will review here some of the

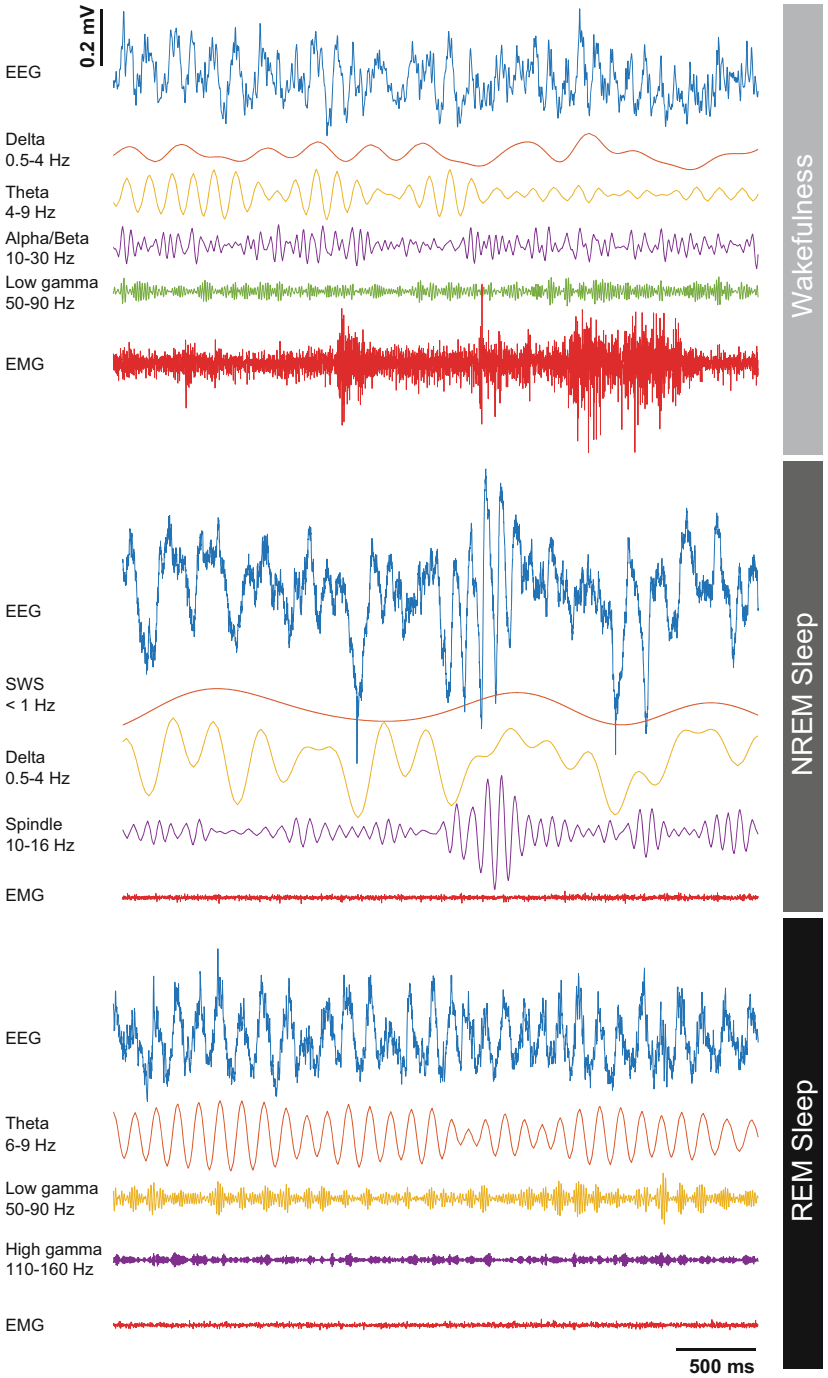

Fig. 1 Brain oscillations during sleep-wake states. A complete sleep-wake cycle in rodents is composed of repeated transitions from wakefulness to NREM sleep and then to REM sleep.

main findings related to the identification of wake-promoting circuitry in the mammalian brain following a somewhat chronological order of experimental optogenetic findings, rather than a sequence based on their individual importance or hierarchical positions, should there be any.

Hcrt/Ox ($LH_{Hcrt/Ox}$) In the first in vivo optogenetic study (Adamantidis et al. 2007), the role of lateral hypothalamic hypocretins/orexins ($LH_{Hcrt/Ox}$)-expressing neurons in arousal and sleep-to-wake transitions was investigated. After genetic targeting of ChR2 expression to $LH_{Hcrt/Ox}$ neurons using a lentivirus, optical stimulation of Hcrt neurons at frequencies between 5 and 30 Hz during NREM and REM sleep induced a reduction of the latency of transition to wakefulness of ChR2-mCherry animals compared to controls (Adamantidis et al. 2007). This response was blocked by a single dose of an Hcrt receptor 1 antagonist, suggesting an implication of Hcrt peptides on one of their receptors. In contrast, 1-Hz light pulses did not result in a significant change. This study established, for the first time, a causal link between frequency-dependent electrical activity of $LH_{Hcrt/Ox}$ neurons and sleep-to-wake transitions.

This arousal effect was strongly dependent on sleep pressure and activation of norepinephrine neurons from the Locus coeruleus (LC_{NE}). Indeed, sleep pressure gated the arousal action of LH_{HCRT} neuron on sleep (Carter et al. 2009). Interestingly, and in addition to already described $LH_{Hcrt/Ox}$-TMN_{Hist} intra-hypothalamic circuits (Brown et al. 2012; Eriksson et al. 2001), norepinephrine cells from the LC, rather than histaminergic cells, mediate part of this arousal effect (Carter et al. 2010) at least for spontaneous sleep-wake transitions. Importantly, optogenetic stimulation of LC_{NE} neurons induced a rapid arousal transition (<2 s) from both NREM and REM sleep, while their silencing extended sleep duration. Interestingly, prolonged stimulation of LC_{NE} neurons was able to maintain wakefulness for a much longer period than Hcrt; however, accumulating sleep pressure eventually induced sleep despite the strong LC_{NE} tone induced by semi-chronic optogenetics (trains of 200 5-ms pulses delivered at 20 Hz every minute for 1 h). These findings demonstrated the reliability of optogenetics to functionally dissect circuits and functions of sleep-to-wake transitions and arousal systems in general and opened new avenues to systematically manipulate specific features of sleep in the mammalian brain.

One question that arose from these behavioral studies was the synaptic role of Hcrt peptides and glutamate, which is also produced by $LH_{Hcrt/Ox}$ cells. To address this, Schöne et al. used optogenetics to study the release of Hcrt peptide and

Fig. 1 (continued) Termination of the REM sleep episode is always followed by a transition to wakefulness. This figure shows representative cortical EEG (top) and neck muscle EMG recording (bottom) across sleep-wake states in freely moving mice. In between the EEG and the EMG recordings, the same EEG trace, yet filtered in different frequency bands characteristic for the three different vigilance states (indicated to the left of each trace), is presented

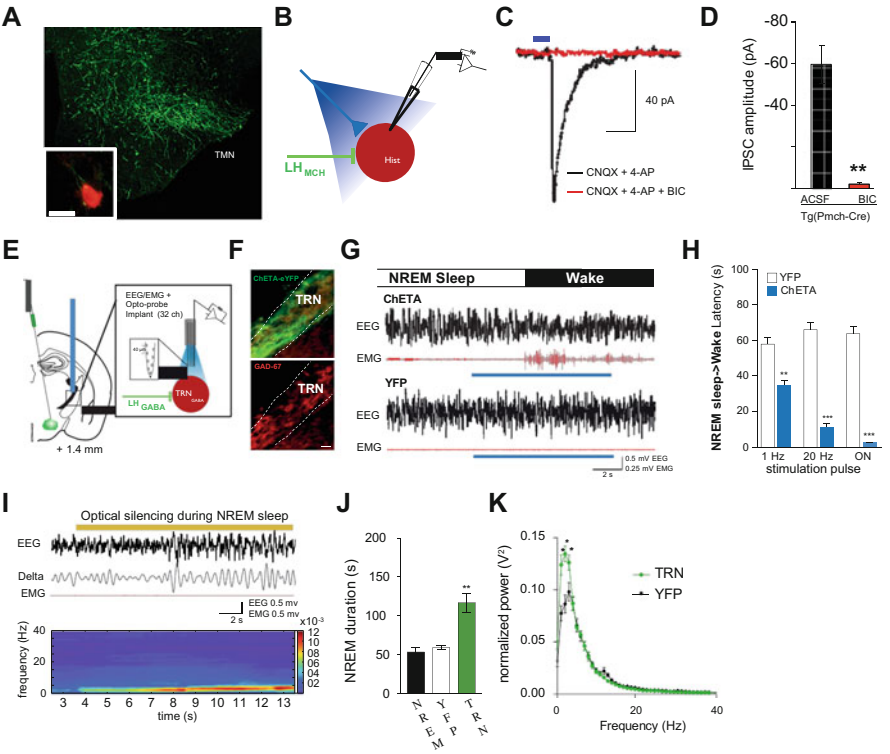

Fig. 2 Example recordings from in vitro or in vivo characterizations of projections involved in sleep-wake control. (**a**) Photomicrograph showing ChETA-EYFP-expressing MCH terminals in TMN area. Scale bar: 200 µm. *Inset*, ChETA-EYFP-expressing MCH terminals (green) contacting histamine cell (red). Scale bar: 10 µm. (**b**, **c**) IPSCs (black) were recorded in TMN histamine and non-histamine neurons from Tg (*Pmch-Cre*) animals transduced with ChETA-EYFP viruses. (**c**) Optically evoked responses (black traces) were blocked by bicuculline (BIC, red traces). (**d**) Mean amplitude of evoked IPSCs from Tg (*Pmch-Cre*) before and after bath application of BIC. (**e**) Schematic of genetic targeting and surgical implantation of opto-tetrode (32 ch) in the TRN. EEG/EMG electrodes are not shown. (**f**) Photomicrographs of coronal section showing ChETA-EYFP-expressing LH_{GABA} terminals (top) intermingled with GAD-67+ cells in the posterior TRN (bottom). Scale bar: 20 µm. (**g**) Representative EEG/EMG recordings before, during, and after single optical stimulation (blue bars) in ChETA-YFP (top) and YFP (bottom) animals during spontaneous NREM sleep induced rapid transitions to wakefulness in freely moving mouse. (**h**) Mean latencies of NREM sleep-to-wake transitions upon optogenetic stimulation in ChETA ($N = 7$) and control animals ($N = 5$). ** $P < 0.001$, *** $P < 0.0001$. (**h**) Mean latencies of NREM sleep-to-wake transitions upon optogenetic stimulation in ChETA and control animals. ** $P < 0.001$, *** $P < 0.0001$. (**i**) Representative EEG (top) delta (0.5–4 Hz) oscillation (middle), EMG (red), and corresponding heat map EEG power spectrum (bottom) in response to local optical silencing (yellow bar, 593 nm) of LH_{GABA}-TRN circuit during NREM sleep. (**j**) Mean duration ± S.E.M of NREM sleep episodes during baseline (left, black bar), optogenetic silencing in control (white bar), and ArchT-EYFP (black bar) mice. ** $P < 0.01$, one-way multiple ANOVA followed by Bonferoni post-hoc test. (**k**) Mean ± S.E.M average of normalized spectral power distribution of cortical EEG signal in control animals (black trace) and ArchT-EYFP (green trace) animals upon optical silencing. * $P < 0.05$ using unpaired two-tailed Student's t-test between control and ArchT animals. Modified from Jego et al. (2013) and Herrera et al. (2015)

glutamate from Hcrt neurons by recording monosynaptically connected histamine cells in an in vitro preparation (Schöne et al. 2014). They identified that $LH_{Hcrt/Ox}$ neuron optical stimulation evoked Hcrt receptor 2-dependent slow postsynaptic currents. Blocking glutamate-driven spiking did not affect $LH_{Hcrt/Ox}$-driven spiking and vice versa, suggesting isolation of outputs. Therefore, co-transmission rules in $LH_{Hcrt/Ox}$-TMN_{Hist} circuit, and sleep-wake circuits in general, may translate distinct features of cellular activity into parallel, nonredundant control signals through downstream effectors.

Histamine (TMN_{Hist}) Beside the glutamatergic $LH_{Hcrt/Ox}$ neurons, the posterior hypothalamus contains histamine-secreting neurons that represent another cluster of wake-active neurons. TMN_{Hist} cells possess all the machinery necessary to synthetize GABA and this mode of transmission had not been tested until recently. Optogenetic activation of histamine cell in vitro disinhibits TMN cells themselves, while indirectly suppressing the activity of sleep-promoting cells from the anterior hypothalamus through local GABA interneurons (Williams et al. 2014). Furthermore, in vivo optogenetic activation of histaminergic neuron terminals in the striatum and cortex evoked tonic (extra-synaptic) $GABA_A$ receptor chloride currents onto medium spiny neurons and pyramidal neurons, respectively. Importantly, these currents were abolished following genetic deletion of the vesicular GABA transporter (VGAT) from histamine cell terminals, suggesting an inhibitory mode of action (Yu et al. 2015). Altogether, these studies confirmed the inhibitory nature of the histamine cells, while the role of specific histamine receptor in mediating these responses awaits further investigation.

Basal Forebrain Acetylcholine (BF_{Ach}) Several studies have probed the role of cholinergic neurons from the basal forebrain in sleep-wake state modulation. Consistently, it was found that optogenetic activation of BF_{Ach} neurons during NREM sleep is sufficient to elicit cortical activation and facilitate wakefulness (Han et al. 2014; Irmak and de Lecea 2014). Prolonged duration of poststimulation arousal was noticed (Irmak and de Lecea 2014). These effects were accompanied by an overall decrease in the power across the 1–14 Hz frequency band. Consistent with these findings, transient bilateral inactivation of BF_{Ach} neurons genetically targeted with Arch-T prolonged NREM sleep and decreased the probability of awakening in mice. Chronic silencing had minor effects on overall sleep-wake durations (Shi et al. 2015). This arousal effect is thought to be mediated by cholinergic modulation of local cells including cortex-projecting inhibitory GABAergic cells (Brown and McKenna 2015; Kim et al. 2015), rather than cholinergic modulation of cortical circuits (Zant et al. 2016).

A careful analysis of these results reveals some interesting findings. *First*, in contrast to $LH_{Hcrt/Ox}$ and LC_{NE}, the arousal effects of cholinergic modulation were dependent on the ongoing sleep states, as photostimulation of cholinergic neurons during REM sleep did not increase wakefulness. This last result suggests a highly specialized, rather than a redundant, organization of arousal circuits in the

mammalian brain. *Second*, the wake-promoting effects of cholinergic activation were not significant for stimulations preceded by long periods of NREM (Irmak and de Lecea 2014). Although this could be due to the use of unilateral stimulation instead of bilateral, or single 10 Hz pattern, it is consistent with other systems, such as the $LH_{Hcrt/Ox}$ and to a lesser extent the LC_{NE}, whose wake-promoting effect strongly depends on/is gated by sleep pressure (Carter et al. 2009). *Third*, this absence of arousal transitions upon stimulation occurring at the end of NREM episodes might actually favor a switch to REM sleep and its typical "cortical arousal." *Finally*, and in contrast to the melanin-concentrating hormone (MCH) neurons in the LH that promote and extend REM sleep episodes when stimulated during REM sleep (*see below*), the ratio of NREM-Wake and NREM-REM upon BF_{Ach} optical stimulation was similar to natural transitions, suggesting a modulatory, rather than a switch-promoting, role of these cells. A recent study systematically recorded from ChR2-tagged cholinergic, glutamatergic, and parvalbumin-positive (PV+) GABAergic neurons in the BF and reported that they were more active during wakefulness and REM sleep (wake/REM active) than during NREM sleep. Optogenetic activation of each cell type rapidly induced wakefulness. In contrast, activation of somatostatin-positive (SOM+) GABA neurons promoted NREM sleep, although only some of them were NREM sleep-active (Xu et al. 2015).

LH_{GABA} Although most of the wake-promoting systems were thought to rely on excitatory neurotransmission, inhibitory transmission in the hypothalamus was recently identified as a strong arousal circuit. Juxtacellular studies originally showed that the activity of GABAergic cells in the LH correlates mainly with wakefulness or REM sleep states, suggesting specialized LH-based inhibitory circuits that control both states (Hassani et al. 2009, 2010; Koyama et al. 2003). Indeed, a subset of LH_{GABA} cells makes monosynaptic contact onto GABAergic thalamus reticular nucleus (TRN) neurons of the thalamus (LH_{GABA}-TRN_{GABA}) where they exert a strong inhibitory tone both in vitro and in vivo (Herrera et al. 2015) (Fig. 2e–h). Optogenetic activation of this circuit recapitulated state-dependent changes of TRN neuron activity in behaving mice and induced rapid arousal (<2 s) from NREM, but not REM sleep (i.e., similar to BF_{Ach}, but at a much faster rate). Importantly, selective activation of the LH_{GABA}-TRN_{GABA} circuit during deep anesthesia induced a sustained cortical arousal, and often a recovery of muscle tone that is interpreted as a recovery of consciousness in animals. In contrast, optogenetic silencing of LH_{GABA}-TRN_{GABA} increased the duration of NREM sleep and the amplitude of delta (1–4 Hz) oscillations (Fig. 2i–k). This later results suggest that modulation of TRN cell activity may represent a permissive state to sleep homeostasis (Process S, see Borbély et al. 1989). Collectively, this is the first study to demonstrate that TRN cells integrate subcortical arousal inputs selectively during NREM sleep and that they may participate in sleep intensity. A recent study further identified a potent inhibition of anterior hypothalamus VLPO cells by LH_{GABA} neurons, which provides a possible additional pathway for promoting rapid wakefulness (Venner et al. 2016). Chemogenetic stimulation of this pathway

strongly promoted wakefulness, whereas its inhibition augmented NREM sleep without altering REM sleep. Optogenetic manipulation of this circuit will be required to compare the dynamics of wake promotion to those of the LH_{GABA}-TRN_{GABA} projection.

VTA-Dopaminergic Circuit Midbrain dopamine neurons are classically involved in reward processing, suggesting their participation in arousal, attention, and, possibly, wakefulness. This modulation was confirmed recently using fiber photometry of VTA dopaminergic cell signals (Eban-Rothschild et al. 2016). Furthermore, the same authors showed that VTA dopaminergic neurons are necessary for arousal and that their inhibition suppresses wakefulness and promotes sleep-related nesting behavior using opto- and pharmacogenetics approaches. Following these correlative observations, chemogenetic and optogenetic activation and inactivation of VTA dopaminergic neurons are both necessary and sufficient for arousal and the effects might be target specific.

Interestingly, the activity of these cells is also modulated across sleep-wake cycle, with higher burst mode discharges during REM sleep than during NREM sleep (Dahan et al. 2007), while reactivation of previously activated VTA dopaminergic cell assemblies has been observed during slow wave oscillations in NREM sleep (Valdés et al. 2015).

Sympathetic Tone One interesting study investigated the role of adrenergic C1 neurons that are located in the lower brainstem and that exert a sympatho-excitatory effect in cardiovascular organs, yet that also project to the wake-promoting LC (Burke et al. 2014). So far, these cells were known to act together with other cell groups to mediate increases in breathing, blood pressure and to cause arousal in response to hypoxia. Burke et al. (2014) now demonstrate that selective optical activation of the C1 neurons in transgenic rats expressing Cre in tyrosine hydroxylase-positive neurons increases breathing and blood pressure and produces arousal from NREM sleep, suggesting a possible contribution to sleep disruption associated with cardiovascular effects of apneas.

2.2 Transitions from Wakefulness to Sleep

Along with the many arousal centers identified in the brain, experimental evidence for the existence of a sleep center has been converging towards the anterior hypothalamus and the lateral-preoptic area in particular.

AN_{GABA} and AN_{GABA} Amassing correlative evidence identified the anterior hypothalamus (AN), both the VLPO and the LPO, as sleep-promoting centers. This was originally suggested by Constantin von Economo who described that damage to this area in the brains of patients with persistent insomnia following the influenza pandemic in the early years of the twentieth century (Von Economo 1930).

This clinical finding was confirmed in lesional studies in cat, rat, and mouse (Lu et al. 2000; McGinty and Sterman 1968; Nauta 1946; Sallanon et al. 1989; Saper et al. 2010), whereas warming of this region promoted sleep, probably through activation of local GABAergic neurons whose activity is modulated across sleep-wake states, and particularly high during NREM sleep. These neurons strongly project to the arousal circuitry of the brain where they are thought to inhibit wake-promoting neurons, which eventually induces sleep (Brown et al. 2012). However, the VLPO area does not contain a neurochemically and functionally homogeneous cell population (Modirrousta et al. 2004; Szymusiak et al. 1998), and its lesion dramatically, reduces, but not fully suppresses sleep time (Lu et al. 2000). Of importance, it is also involved in thermoregulation (Boulant 2000) and, possibly, parental behaviors (Dulac et al. 2014).

In a recent chemo-genetic study, Zhang et al. showed that anesthesia- or sleep recovery-tagged neurons from the lateral preoptic area were able to induce NREM sleep, with the accompanying drop in body temperature. This result is important since it is the first study to demonstrate that (1) sedation (induced by dexmedetomidine, an α2-adrenergic agonist) and NREM sleep shares functional hypothalamic pathways, but importantly, (2) activation of a subset of cells in the anterior hypothalamus is causally involved in sleep regulation (Zhang et al. 2015). However, the precise identification of possible VLPO subpopulations and functional connectivity is awaiting further investigation.

Pontine Control of NREM Sleep Recent optogenetic study identified a potential additional "sleep center" that includes the GABAergic cells from the Parafacial zone (PZ), a small nucleus located in the rostral medullary brainstem, adjacent to the cell bodies of the ascending arousal system (Anaclet et al. 2014). Anaclet et al. showed that PZ neurons express c-Fos after sleep but not after wakefulness, suggesting that they are sleep-active, and that lesions of the PZ cells result in large and sustained increases (50%) in daily wakefulness at the expense of NREM sleep. Furthermore, half of these PZ cells express a marker for inhibitory neurons (VGAT) and genetic removal of vesicular GABA/glycine transporter in PZ cells resulted in increased wakefulness concomitant with a decrease of both NREM and REM sleep (Anaclet et al. 2012). Causal evidence for a role of these PZ_{GABA} cells as NREM sleep-promoting neurons was further shown using an optogenetic approach, possibly through PZ_{GABA}->Parabrachial (PB)glu->BF (magnocellular neurons)-Cortex" circuitry (Anaclet et al. 2014), although this functional circuit relies on in vitro studies so far.

Beside cholinergic inputs, pontine glycinergic inputs have been shown to inhibit arousal-promoting midline thalamic circuits, through polysynaptic GABA and glycinergic pathways (Giber et al. 2015). Interestingly, their optogenetic activation evokes transient interruption of awake cortical activity in freely moving animals,

while prolonged stimulation induced behavioral arrest. Altogether, these findings suggest that long-range projecting inhibitory cells from the brainstem also participate in the forebrain and thalamic control of sleep and support a functional alternative dissociated from the "classical" anterior hypothalamus model.

Cholinergic Inputs to Thalamus Beside the PZ-PB complex, and together with the progressive decrease of monoaminergic tone during transition from wakefulness to NREM sleep, cholinergic inputs to the thalamus, and the TRN in particular, have recently been shown to be involved in sleep transitions (Sun et al. 2013; Ye et al. 2010). Indeed, Ni et al. showed that optical stimulation of cholinergic inputs to the TRN exerts a net activation of local GABAergic neurons through α7-containing nicotinic acetylcholine receptors, although contrasting studies rather suggest the involvement of muscarinic receptors (Beierlein 2014). Chronic optical stimulation patterns ultimately promote sleep by decreasing sleep onset latency and extension of NREM, but not REM, sleep duration, possibly by increasing sleep spindle occurrence (Ni et al. 2016). Although these findings contrast to previous reports on cholinergic control of arousal, they strongly suggest that arousal, and likewise sleep-circuits, are organized in a specialized, rather than a redundant, scheme.

Following the convergence of these circuits onto thalamo-cortical (TC) networks, several studies have tested whether direct activation of TC and cortico-thalamic (CT) elements (TC-CT-TRN) induced sleep-like oscillations and sleep states. Indeed, direct phasic optogenetic activation of TRN cells induced spindle-like oscillations (Halassa and Acsády 2016; Halassa et al. 2011; Kim et al. 2012), while its tonic activation rapidly induces slow wave activity (similar to sleep slow waves) in a spatially restricted cortical area. These slow wave events are associated with behaviorally relevant signs of sleep (however, arousal threshold not tested) (Lewis et al. 2015).

We will finish this section by referring the reader to two studies that somewhat confirmed previous findings. Tsunematsu et al. showed that optical silencing of $LH_{Hcrt/Ox}$ reduced NREM sleep onset whereas optical stimulation of ChR2-expressing astrocytes (using glial fibrillary acidic protein (GFAP) promoter) in the LH promoted both NREM and REM sleep during the 6-h stimulation sessions (Tsunematsu et al. 2011, 2013). However, both studies are somewhat limited by the absence of proper control experiments and the precise molecular and cellular consequence of optogenetic activation of astrocytes (e.g., release mechanism), respectively.

2.3 Transitions from NREM to REM Sleep

LH_{MCH} Lesion studies link the AN with sleep, while the LH encompasses neurons with arousal properties. However, in 2003, Verret et al. provided the first evidence for a role of MCH neurons in REM sleep control. As evidenced by functional neuroanatomy, MCH neurons are active during a REM sleep rebound and icv

injection of MCH peptides induced REM, and to a lesser extent, NREM sleep (Verret et al. 2003). This hypothesis was further confirmed by juxtacellular recordings showing that MCH neurons maximally discharged during REM sleep (Hassani et al. 2009). Acute optogenetic activation of MCH neurons (ChETA, SSFO) at the onset of REM sleep extended the duration of REM, but not non-REM sleep episodes. In contrast, their acute silencing (eNpHR3.0, ArchT) reduced the frequency and amplitude of hippocampal theta rhythm, without affecting REM sleep duration (Jego et al. 2013). In vitro activation of MCH neuron terminals induced $GABA_A$-mediated inhibitory postsynaptic currents (IPSCs) in wake-promoting histaminergic neurons of the tubero-mammillary nucleus (TMN) (Fig. 2a–d), while in vivo activation of MCH neuron terminals in TMN or medial septum also extended the duration of REM sleep episodes. Interestingly, acute stimulation of MCH neurons during NREM sleep facilitated the transitions towards REM sleep (Jego et al. 2013; Tsunematsu et al. 2014). Consistent with these studies, chronic optogenetic activation over hours of MCH cells increase sleep (Konadhode et al. 2013).

Collectively, these results suggest that activation of MCH neurons promotes and stabilizes REM sleep, possibly through inhibition of arousal circuits in the mammalian brain. Although the effect on REM sleep was consistent across different experimental approaches, they also revealed a facilitatory role for MCH neurons on NREM sleep onset that strongly depends on the technology, stimulation parameter, and species considered.

Consistent with Jouvet's work on the role of brainstem nuclei in REM sleep, and according to recent recording of cholinergic neurons firing across sleep-wake states (Boucetta et al. 2014), acute optogenetic stimulation of cholinergic neurons in the LDT/PPT nuclei during NREM sleep promotes REM sleep with some delay (~1 min) (Van Dort et al. 2015), similarly to MCH neurons (Jego et al. 2013). However, in this set of experiment, the duration of REM sleep was not affected by the optogenetic stimulation.

The medulla contains neurons that send rostral projections to the pons and midbrain (dorsal part) and caudal projections to the spinal cord (ventral part) and are maximally active during REM sleep (Fort et al. 2009; Weber et al. 2015). For instance, cell-type-specific microendoscopic Ca^{2+} imaging confirms previous functional anatomy results showing that glutamatergic neurons in the laterodorsal tegmental nucleus are maximally active during REM sleep, while the majority of GABAergic neurons is maximally active during wakefulness (Cox et al. 2016). Their optogenetic activation showed that GABA inhibitory circuit originating from the ventral medulla reliably initiated REM sleep episodes and prolonged their durations in mice, whereas inactivating these neurons had the opposite effect (Weber et al. 2015). Although the ventral part of this nuclei is thought to control motor atonia, rather than REM sleep itself, a possible pathway involves an inhibition of REM-suppressing GABAergic neurons in the ventrolateral periaqueductal grey.

A recent study took advantage of the ontogeny of cells in the brainstem to manipulate both NREM and REM sleep in this area using chemo-genetic tools

(Hayashi et al. 2015). Although these cells share a common developmental origin with neurons promoting wakefulness, interestingly, the authors identified excitatory glutamatergic neurons that inhibit REM sleep and promote NREM sleep, whereas some other GABA-releasing neurons act downstream to inhibit REM sleep. Chemogenetic reduction or prolongation of REM sleep was found to also affect slow-wave activity during subsequent NREM sleep, implicating REM sleep in the regulation of NREM sleep. Yet, the precise underlying mechanisms still remain unclear and the time course of the effects described in this study is not compatible with that of homeostatic sleep regulation, suggesting possible side-effect pertaining to pharmacogenetics.

3 Optogenetics and Sleep Homeostasis

After prolonged periods of wakefulness, sleep pressure – which reflects a process called "*sleep homeostasis*" (or process S) (Borbély et al. 1989; Franken et al. 2001; Rechtschaffen et al. 1989; Saper et al. 2010) – increases and favors the onset of sleep. The increased sleep pressure is accompanied by enhanced amplitude of low frequency oscillations (delta frequency band: 0.5–4 Hz) in the cortical EEG in both animals and human. Although its precise underlying mechanism remains unclear, alterations in receptor and messenger signaling pathways, neural network synchronization, and/or phase coupling (Fattinger et al. 2014; Mang and Franken 2015; Urry and Landolt 2015; Dauvilliers et al. 2015; Greene and Frank 2010) from cortical or subcortical structures support this evolutionarily conserved phenomenon.

Sleep homeostasis has been shown to block $LH_{Hcrt/Ox}$-mediated arousal during sleep, suggesting a gating mechanism (Carter et al. 2009). More recently, we showed that optogenetic silencing of LH_{GABA}-TRN_{GABA} circuitry alone was sufficient to enhance the amplitude of NREM sleep delta oscillations (Herrera et al. 2015), suggesting a possible mechanisms for local and global modulation of sleep homeostasis from subcortical structures. Finally, spindles are typically detected during normal NREM sleep and consist of characteristic "waxing-and-waning" field potentials grouped into 9–15 Hz oscillations that last for ~1 s and occur several time per minutes in the thalamus and the cortex (Barthó et al. 2014; Lüthi 2014).

4 Optogenetics and Brain Rhythms

Optogenetic approaches have also advanced our understanding of the mechanistic and functional substrates of neural rhythms associated with sleep and wake states. The separate consideration of how optogenetics help advance this field is justified for several reasons.

Brain rhythms represent the hallmarks of distinct states of sleep. Such electrical patterns necessitate activation of particular ion channel classes, modes of synaptic transmission, and discharge patterns over repetitive cycles of activity. For these reasons, constant light stimulation will need to be replaced by appropriately temporally patterned stimuli to initiate or maintain the cellular events underlying network oscillations.

Moreover, whether or not brain rhythms can be generated depends on the particular state of the neural circuitry, which includes its neuromodulatory conditions, cellular excitabilities, and the synaptic strengths. The goal of this chapter is to highlight cases in which optogenetically manipulated rhythms were successfully obtained and analyzed in terms of similarity to their natural counterparts. These cases help to illustrate the revolutionary power of optogenetics and to foresee its capacity to generate further landmark insights on the role of these rhythms for sleep.

4.1 Optogenetic Manipulation of Brain Rhythms in Sleep and Wake States

Sleep states represent an assembly of super-imposed rhythms that are key to the scoring of these in both humans and animals. Although many of the cellular details of these rhythms are known, a better definition and understanding of their initiation, spread, and termination mechanisms, as well as their function in sleep-specific alterations in arousal threshold, consciousness, and offline processing are warranted.

Sleep Spindles Optogenetic induction of brain rhythms has been particularly successful in cases in which some of the key pacemaker elements were known from prior electrophysiological work. One well-documented case is sleep spindles, known to occur predominantly during human sleep stage N2 and to arise from reverberating neuronal activity within the reciprocal circuits of TC cells and TRN. Optogenetic control of spindles is of great interest as sleep spindles are key elements in the operations of the sleeping brain that control arousal threshold and memory consolidation (Astori et al. 2013).

The basic mechanism of sleep spindle generation was re-probed through optogenetically activating TRN cells that were rendered light-sensitive through using Cre-driver lines for genes involved in the life cycle of the neurotransmitter GABA. Halassa et al. (2011) were the first to use animals expressing ChR2 under the control of the vesicular GABA transporter (VGAT). In these animals, they stimulated the TRN in vivo unilaterally using an optic fiber implanted over its sensory sectors. EEG spindles could be observed through giving single light pulses to the TRN, which induced 4–5 waves in the frequency band of 10–15 Hz. These clearly outlasted the duration of the light stimulus, indicating that a network rhythm had been generated. The same light stimulus also caused a pause in TC cell activity that was followed by rebound burst discharge, a pattern required for sleep spindle generation. Importantly, the success of spindle induction was dependent on brain state, being most easily induced during NREM sleep and hardly at all in wake and REM sleep. A similar approach was taken by Barthó et al. (2014), who used PV-Cre or VGAT-Cre driver lines to express ChR2 and implanted the optic fiber over sensory TRN. Single light pulses elicited spindles that were detected as rhythmic multiunit activity in TC cells in urethane anesthesia. In this study, optogenetic triggering of spindles was also used

to demonstrate that the duration of sleep spindles is the result of a complex interaction between the burst propensity of TRN cells and background modulatory effects.

In these two studies, spindle patterns were produced that resembled closely those observed naturally. Moreover, both studies demonstrated a state-dependence of spindle occurrence that indicated a preference for synchronized brain states typical for non-REM sleep.

Kim et al. (2012) used Thy1-ChR2 mice to excite TRN neurons through bilateral delivery of repetitive 8 Hz light pulses. EEG patterns followed these repeated light pulses faithfully as long as they were continued, but stopped immediately thereafter, indicating that no self-sustained rhythm was produced that outlasted the light exposure. "Spindle-like" rhythms could thus be produced through entraining a network into a rhythm using enforced neuronal recruitment independently of the current circuit state. Supporting this interpretation is the fact that spindles were observed throughout all vigilance states, with no major difference between waking and non-REM sleep.

Slow Waves The cellular mechanisms of slow waves, which are characteristic for stage N3 of human NREM sleep, are the focus of three studies. These investigated the recruitability of the anesthetized corticothalamic system when exposed to light stimulation at frequencies corresponding to the slow waves (1–2 Hz). Stroh et al. (2013) optogenetically stimulated layer 5 neurons in a mouse line with ChR2 expression confined to layer 5 in ketamine-xylazine anesthesia. Anesthetized cortical circuits show an elevated excitability in 1-s intervals that facilitates an avalanche-like network recruitment to both synaptically and intrinsically generated excitation. A similar conclusion was taken by Kuki et al. (2013) who stimulated at various depths in motor cortex of the W-TChR2V4 rat line that expresses ChR2 throughout excitatory and inhibitory neurons predominantly in deeper layers. David et al. (2013) found that repeated light stimuli (0.75–1.5 Hz) to ChR2-expressing somatosensory thalamus entrained cortical activity to this frequency through generating an up-down-state sequence that closely followed the evoked response to the thalamic input. This sequence was clearest in amplitude at 1.5 Hz, whereas it became markedly smaller at frequencies >2 Hz. The entrainment was dependent on the occurrence of low-threshold calcium spikes in the thalamus, known to underlie burst discharge in these cells. Low-frequency synchronized rhythmic activity in the thalamus can thus shift the frequency of slow waves in the cortex within the range of 1–2 Hz, and exert a desynchronizing effect if occurring more rapidly.

Beltramo et al. (2013) used the rbp4-cre mouse line to drive ChR2 expression selectively in layer 5. Although ~14% of pyramidal neurons expressed ChR2, low-intensity light with a 0.5 s on-off periodicity was sufficient to reliably generate depolarized states in layer 5 neurons through synaptic inputs driven by ChR2 that propagated to upper cortical layers. Conversely, comparable optogenetic manipulation of layer 2/3 neurons did not lead to persistent recruitment of other cortical layers. Moreover, optogenetic inhibition of a subgroup of layer 5 neurons was

sufficient to interrupt spontaneous up states. 1 Hz rhythmic light exposure also boosted slow waves in non-REM sleep of freely moving mice. This study confirms earlier observations in carnivores in favor of layer 5 neurons as hubs for the generation of slow waves (Sanchez-Vives and McCormick 2000).

Gamma Rhythms Two in vitro studies demonstrate an induction of rhythms in the gamma frequency range (~40–75 Hz) through using temporally varying optogenetic stimuli, such as ramps in somatosensory cortex of mice expressing ChR2 in layer 2/3 cortical neurons (Adesnik and Scanziani 2010) or theta-modulated sinusoidal stimuli in the CA1 area of the hippocampus (Butler et al. 2016). The generation of activity in this frequency range in reduced preparations, notably including layer 2/3 of single cortical barrels (Adesnik and Scanziani 2010), supports the idea that local circuits are able to generate gamma rhythms in response to specific sensory drives or states of memory processing.

Hippocampal Rhythms Optogenetic approaches recently were successfully used for probing mechanisms of hippocampal ripple generation. The hippocampus generates fast field oscillations at 150–300 Hz that appear as short-lasting events prominently in the CA1 area during states of NREM sleep, but also during quiet wakefulness (Colgin 2016). Basic mechanisms of generation and propagation within hippocampus remain open (Buzsáki 2015). Ripples are central to sleep-dependent memory consolidation and implied in the replay of fragments of wake-related events. To date, however, only two studies provide evidence that chronic (>10 days) attenuation of hippocampal ripples during the hour following learning slows down the acquisition of hippocampus-dependent spatial memory tasks (Ego-Stengel and Wilson 2010; Girardeau et al. 2009). Optogenetic control over ripples promises to clarify the exact role of these rhythms in sleep.

To date, several studies successfully have demonstrated optogenetic control over ripples. Using a hippocampal slice preparation in which spontaneous ripple generation occurs, Schlingloff et al. (2014) found that, in slices from PV::ChR2 expressing mice, light pulses evoked ripples closely resembling spontaneous events. Interestingly, these evoked ripples could also be elicited even when excitatory synaptic transmission was pharmacologically abolished, suggesting that PV neuron-dependent inhibitory circuitry can generate activity within ripple frequencies. Using the light-gated chloride pump eNpHR 3.0, silencing PV-neurons largely abolished the ripples. This study thus demonstrates that combining activating and silencing optogenetic approaches provides valuable insight into mechanisms of rhythm generation.

Two studies have identified powerful mechanisms of how ripple occurrence is regulated through external inputs. Vandecasteele et al. (2014) show that optogenetic stimulation of cholinergic afferents into the hippocampus rapidly and almost completely suppressed ripples in both urethane-anesthetized and freely moving animals, while enhancing theta oscillations. Cholinergic hippocampal modulation thus promotes a theta-dominated hippocampal state that is typical for active

behaviors, such as exploration and learning, while suppressing sleep-related rhythms, such as the ripples.

A modulatory action of median raphe on hippocampal ripples was identified through driving ChR2 or eNpHR in median raphe neurons. Ripple occurrence was suppressed when median raphe projections were stimulated, whereas ripple frequency increased when median raphe activity was silenced (Wang et al. 2015). Further optogenetic analysis revealed that serotoninergic but also non-serotoninergic neurons within the median raphe each accounted for part of this effect. Wang et al. also found that suppressing ripples for 4 h after contextual fear conditioning attenuates recall the next day (Wang et al. 2015), thus relating ripple occurrence to learning.

Another important case that documents the usefulness of optogenetic tools is the hippocampal theta rhythm that is found during both active exploration and REM sleep. In addition to cholinergic inputs (see Schlingloff et al. 2014), septal glutamatergic (Fuhrmann et al. 2015) or GABAergic inputs (Bender et al. 2015) in awake animals contribute to this rhythm, yet the role of the distinct cellular groups in exploration or REM sleep-related brain states has not been defined yet. Fuhrmann et al. (2015) used awake mice expressing ChR2 under the promoter of the vesicular glutamate transporter 2 (VGlut2) and found that rhythmically entrained hippocampal rhythms occurred when illuminating glutamatergic medial septal neurons at stimulation frequencies of 6–12 Hz. Intriguingly, the frequency of these rhythms was proportional to the running speed of the mice, indicating that glutamatergic neurons within the septum correlate locomotion with hippocampal firing rates. Hippocampal rhythms, measured through local field potential recordings in head-fixed animals, were tightly phase-locked to the light pulses, but continued occasionally once light stimulation was terminated. Combining these light stimulation patterns with in vivo whole-cell recording, a cellular mechanism involving dis-inhibition of pyramidal cells through stratum oriens interneurons was described. The theta rhythm associated with REM sleep is the subject of a separate chapter (see above) and necessitates hypothalamic MCH neurons (Jego et al. 2013) as well as septal GABAergic neurons (Boyce et al. 2016).

In conclusion, many of the major vigilance state-related related rhythms have gained in understanding through optogenetic investigation, with the following major results. (1) Key pacemaker elements such as TRN neurons for spindles, hippocampal PV neurons for ripples, or layer 5 cortical neurons for slow waves were identified and confirmed prior studies. (2) Novel cellular mechanisms of rhythm generation were described and linked to behavioral aspects of vigilance states. (3) Novel afferent modulatory pathways controlling these rhythms were described. Next important steps in the field, to which optogenetics will undoubtedly contribute, are the exact temporal coordination between rhythms during vigilance states and their role in sensory perception and memory consolidation. Furthermore, the exact role of modulatory pathways, such as those converging on thalamus and hippocampus, will need to be elucidated in terms of the control they occur on the global and local occurrence of rhythms in the brain.

4.2 Optogenetic Probing of Brain Rhythms in Memory Processes

Optogenetic suppression of rhythms has proved extremely useful in assessing their role in memory consolidation. Wang et al. (2015) took a first step in this direction through specifically suppressing ripples through median raphe input following a hippocampal-dependent contextual fear conditioning procedure. Such manipulation deteriorated recall in the animals in which light suppressed the ripples, but not in control animals. This finding extends pioneering studies on the role of ripples in hippocampal memory formation, in which electrical stimulation of ventral hippocampus disrupted ripple occurrence and attenuated performance in a spatial maze (Ego-Stengel and Wilson 2010; Girardeau et al. 2009). Most recently, instead of suppressing neural rhythms, a study exploited the idea of reproducing slow wave activity following a sensorimotor detection task (Miyamoto et al. 2016). Slow waves were generated in mice expressing ChR2 specifically in layer 5 in a manner that reproduced the coherence between somatosensory and motor circuits during the task. This artificially induced synchronization enhanced memory and was even sufficient to overcome the memory deficits caused by sleep deprivation (Miyamoto et al. 2016). In this elegant study, optogenetics could thus help to identify, for the first time, the critical synaptic pathways and interactions between cortical areas that lead to perceptual memory.

The advantage of optogenetic inhibition was also used recently to specifically suppress the hippocampal theta rhythm during REM sleep while leaving sleep architecture, including the timing of REM sleep, largely untouched (Boyce et al. 2016). This was achieved through optogenetic suppression of medial septal GABAergic neurons in a VGAT Cre driver line previously injected with AAV containing a floxed ChR2 gene. Continuous illumination of the medial septum temporally locked to REM sleep episodes suppressed theta rhythm generation specifically and thus allowed to probe behavior in animals that had spent REM sleep time without theta rhythm. This led to an impairment of contextual memory consolidation as assessed by a novel object recognition task and fear conditioning, while other memories remain intact.

In addition to manipulating rhythms to elucidate their role, optogenetic tools also help to disrupt sleep architecture in specific ways, thereby overcoming the limitations of conventional sleep deprivation techniques. These latter ones are typically associated with many confounding factors, such as stress or sensory overstimulation (Longordo et al. 2009). The idea to disrupt sleep specifically was tested by Rolls et al. (2011) who used optogenetic activation of hypocretin neurons to wake up mice from non-REM sleep regularly, yet without interfering with other vigilance states or with total sleep duration. Sleep thus essentially became more fragmented, yet its architectural and spectral properties were otherwise preserved. Under these conditions, mice showed deficits in a hippocampus-dependent object recognition task. To date, this is the cleanest approach relating fragmented sleep to altered cognitive performance.

5 Perspectives

Optogenetic tools provide bidirectional control of genetically defined neuronal populations with unprecedented temporal resolution and neurochemical specificity. Therefore, it is not exaggerating to state that optogenetic methods have leveraged research on sleep and wake states to a truly systems-oriented field with causal and dynamic insights from molecular to behavioral levels. As methods continue to be refined, long-awaited landmark progress in both mechanistic and functional aspects of sleep and wakefulness is within reach. First and foremost, the neural circuitry underlying the control of vigilance state will be further defined and this will range from understanding the deepest sleep stage up to the highest levels of arousal and attention. To give just one example, optogenetic control of a major sleep-controlling brain area, the ventrolateral preoptic (VLPO) circuitry has just started (Venner et al. 2016). It is likely that the cellular identities of VLPO neurons, the role of peptidergic synaptic transmission, and the projection pathways involved in sleep will unravel a hitherto unrecognized complexity of NREM sleep control that will allow to understand further the regulation of NREM sleep states, the transitions to REM sleep, the homeostatic control of NREM, as well as the circuitry control of NREM sleep depth. In addition to VLPO, NREM sleep-promoting brainstem areas were recently described, indicating that a hierarchy of structures is responsible for NREM-sleep specific attributes of brain, muscular, and autonomic system. Clearly, a major task will be to elaborate on these hierarchies to determine the "leaders" and "followers," the communication between these, the distinct tasks they take, and the signals they integrate. Optogenetic silencing strategies are ideal for this goal, as already exemplified by the studies of Carter et al., who started elaborating the hierarchical links between wake-promoting systems (Carter et al. 2013). In yet another step, a full understanding of a vigilance state will need to provide a comprehensive definition of physiological parameters, moving towards a unifying model of sleep that delineates the crosstalk with the sensory periphery, and with cardiac, metabolic, and temperature control systems. Optogenetic approaches, combined with novel genetic methods, hold the potential to deconstruct vigilance states as bodily states to which major organs contribute.

Light-triggered brain rhythms extend the mechanistic understanding of vigilance states, to which classic electrophysiology has provided many of the cellular and circuit basics. The topology of rhythms on local and global scales, the temporal integration of different rhythmic events, and the cross-talk of rhythms in different brain structures are all fundamental to advance the functional understanding of these rhythms. Importantly, light-triggered rhythms need to be compared in waveform and time course to their natural counterparts. The recruitment of cellular compartments, profiles of sinks and sources in laminated structures, synaptic inputs, and topological distribution are other elements to be explored using timed and reproducible oscillatory events initiated by light.

Analyses on the mechanics of sleep and wakefulness will provide clues to the roles of brain structures and rhythms in sleep functions related to recovery, homeostasis, and plasticity. The paper by Rolls et al. (2011), already discussed in this

chapter, is an exemplary case for identifying novel approaches on sleep's implication in learning and memory through selectively interfering with one hierarchically high brain area implied in wakefulness initiation. Optogenetic targeting of cellular kernels of rhythms will also help elucidate actions of somnogens on sleep, daytime cognition, and memory, which in turn can provide indications to designing more specific pharmacological strategies.

On the therapeutic side, optogenetic strategies to interfere or drive brain activity related to states of sleep and wakefulness are already ongoing. Closed-loop stimulation to trigger light stimuli in response to ongoing events, such as individual sleep rhythms, turns out to be extremely successful not only to probe their role (Miyamoto et al. 2016), but also to prevent pathological perversions of these, such as it occurs during certain forms of epilepsies (Paz et al. 2013). In this latter case, once slow rhythmic hyperactivity started, optogenetic closed-loop control was used to inhibit activity in key neurons, thereby effectively anticipating and preventing hypersynchrony. Thus, although optogenetic techniques are currently not amenable for therapeutic purposes, they will definitely inspire techniques and targets based on feedback to improve sleep or render it more efficient in terms of its beneficial effects.

6 Conclusive Remarks

Optogenetics have guided the way into a new area of neuroscience in general, and into identifying key brain structures and projections involved in vigilance state transition in particular. Still, the overall hierarchy of events needs to be elaborated. Many brain areas involved in NREM or REM sleep generation are now identified, but still it is not clear how they communicate with each other and how the transitions between these is regulated by bodily states, homeostatic control, and pathological conditions.

While unprecedented in specificity and dynamics, optogenetic tools do show limitations. Not only are they invasive techniques that require complex tools, but they are also limited in the specificity once targeted brain areas are really small or consist of highly intermixed neuronal populations for which there is no good genetic marker available yet. This holds true in particular for brainstem within which many of the key areas involved in vigilance state initiation and maintenance reside. Furthermore, optogenetic stimulation may lead to discharge patterns that do not reproduce those occurring naturally, questioning whether the amount and type of neurotransmitter released naturally could be reproduced (Arrigoni and Saper 2014). For example, the release of neuropeptides typically requires more intense, more prolonged, or burst discharge patterns. Clearly, unless optogenetics are controlled for in terms of the faithfulness of reproducing natural neuronal activity and synaptic transmission, they risk to provide false estimations of the roles of brain structures in vigilance state control.

Optogenetic tools are currently being complemented by genetical engineering techniques allowing to drive neuronal activity using pharmacological agents. Amongst these, Designer receptor exclusively activated by designer drugs

(DREADDs) can be virally transfected in a manner similar to ChR2 and lead to the expression of G-protein-coupled receptors activated by intraperitoneal injection of a selective ligand. These have already become a technological standard and turn out to be extremely useful for studies of brain states, as they allow for stable manipulation of neuronal activity over periods from minutes to hours (Fuller et al. 2015). However, DREADDs suffer from similar limitations as optogenetic tools in terms of the specificity limited by the genetic construct and the injection size. Moreover, their use has led to unexpected results in terms of the regulation of REM and NREM sleep states (Hayashi et al. 2015).

Notwithstanding these limitations, the advent of optogenetics and related methods had an extraordinary impact not only in experimental sleep research but also in system neurosciences in general. The future refinement of the optogenetics tools and targeting methods will undoubtedly shine light on the brain mechanisms underlying the states of sleep, wakefulness, and to a broader extend consciousness and reveal basic functions of sleep for the brain, the mind, and the body.

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References

Adamantidis AR, Zhang F, Aravanis AM, Deisseroth K, de Lecea L (2007) Neural substrates of awakening probed with optogenetic control of hypocretin neurons. Nature 450:420–424

Adamantidis A, Arber S, Bains JS, Bamberg E, Bonci A, Buzsáki G, Cardin JA, Costa RM, Dan Y, Goda Y et al (2015) Optogenetics: 10 years after ChR2 in neurons-views from the community. Nat Neurosci 18:1202–1212

Adesnik H, Scanziani M (2010) Lateral competition for cortical space by layer-specific horizontal circuits. Nature 464:1155–1160

Anaclet C, Lin J-S, Vetrivelan R, Krenzer M, Vong L, Fuller PM, Lu J (2012) Identification and characterization of a sleep-active cell group in the rostral medullary brainstem. J Neurosci 32:17970–17976

Anaclet C, Ferrari L, Arrigoni E, Bass CE, Saper CB, Lu J, Fuller PM (2014) The GABAergic parafacial zone is a medullary slow wave sleep-promoting center. Nat Neurosci 17:1217–1224

Arrigoni E, Saper CB (2014) What optogenetic stimulation is telling us (and failing to tell us) about fast neurotransmitters and neuromodulators in brain circuits for wake-sleep regulation. Curr Opin Neurobiol 29C:165–171

Aston-Jones G, Bloom FE (1981) Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. J Neurosci 1:876–886

Astori S, Wimmer RD, Lüthi A (2013) Manipulating sleep spindles – expanding views on sleep, memory, and disease. Trends Neurosci 36:738–748

Barthó P, Slézia A, Mátyás F, Faradzs-Zade L, Ulbert I, Harris KD, Acsády L (2014) Ongoing network state controls the length of sleep spindles via inhibitory activity. Neuron 82:1367–1379

Beierlein M (2014) Synaptic mechanisms underlying cholinergic control of thalamic reticular nucleus neurons. J Physiol Lond 592:4137–4145

Beltramo R, D'Urso G, Dal Maschio M, Farisello P, Bovetti S, Clovis Y, Lassi G, Tucci V, De Pietri Tonelli D, Fellin T (2013) Layer-specific excitatory circuits differentially control recurrent network dynamics in the neocortex. Nat Neurosci 16:227–234

Bender F, Gorbati M, Cadavieco MC, Denisova N, Gao X, Holman C, Korotkova T, Ponomarenko A (2015) Theta oscillations regulate the speed of locomotion via a hippocampus to lateral septum pathway. Nat Commun 6:8521

Borbély AA, Achermann P, Trachsel L, Tobler I (1989) Sleep initiation and initial sleep intensity: interactions of homeostatic and circadian mechanisms. J Biol Rhythm 4:149

Boucetta S, Cissé Y, Mainville L, Morales M, Jones BE (2014) Discharge profiles across the sleep-waking cycle of identified cholinergic, GABAergic, and glutamatergic neurons in the pontomesencephalic tegmentum of the rat. J Neurosci 34:4708–4727

Boulant JA (2000) Role of the preoptic-anterior hypothalamus in thermoregulation and fever. Clin Infect Dis 31(Suppl 5):S157–S161

Boyce R, Glasgow SD, Williams S, Adamantidis A (2016) Causal evidence for the role of REM sleep theta rhythm in contextual memory consolidation. Science 352:812–816

Boyden ES (2015) Optogenetics and the future of neuroscience. Nat Neurosci 18:1200–1201

Brown RE, McKenna JT (2015) Turning a negative into a positive: ascending GABAergic control of cortical activation and arousal. Front Neurol 6:135

Brown RE, Basheer R, McKenna JT, Strecker RE, McCarley RW (2012) Control of sleep and wakefulness. Physiol Rev 92:1087–1187

Burke PGR, Stephen B, Coates MB, Viar KE, Stornetta RL, Guyenet PG (2014) Optogenetic stimulation of adrenergic C1 neurons causes sleep state-dependent cardiorespiratory stimulation and arousal with sighs in rats. Am J Respir Crit Care Med 190:1301–1310

Butler JL, Mendonça PRF, Robinson HPC, Paulsen O (2016) Intrinsic cornu ammonis area 1 theta-nested gamma oscillations induced by optogenetic theta frequency stimulation. J Neurosci 36:4155–4169

Buzsáki G (2015) Hippocampal sharp wave-ripple: a cognitive biomarker for episodic memory and planning. Hippocampus 25:1073–1188

Carter ME, Adamantidis A, Ohtsu H, Deisseroth K, de Lecea L (2009) Sleep homeostasis modulates hypocretin-mediated sleep-to-wake transitions. J Neurosci 29:10939–10949

Carter ME, Yizhar O, Chikahisa S, Nguyen H, Adamantidis A, Nishino S, Deisseroth K, de Lecea L (2010) Tuning arousal with optogenetic modulation of locus coeruleus neurons. Nat Neurosci 13:1526–1533

Carter ME, de Lecea L, Adamantidis A (2013) Functional wiring of hypocretin and LC-NE neurons: implications for arousal. Front Behav Neurosci 7:43

Colgin LL (2016) Rhythms of the hippocampal network. Nat Rev Neurosci 17:239–249

Cox J, Pinto L, Dan Y (2016) Calcium imaging of sleep-wake related neuronal activity in the dorsal pons. Nat Commun 7:10763

Dahan L, Astier B, Vautrelle N, Urbain N, Kocsis B, Chouvet G (2007) Prominent burst firing of dopaminergic neurons in the ventral tegmental area during paradoxical sleep. Neuropsychopharmacology 32:1232–1241

Dauvilliers Y, Tafti M, Landolt HP (2015) Catechol-O-methyltransferase, dopamine, and sleep-wake regulation. Sleep Med Rev 22:47–53

David F, Schmiedt JT, Taylor HL, Orban G, Di Giovanni G, Uebele VN, Renger JJ, Lambert RC, Leresche N, Crunelli V (2013) Essential thalamic contribution to slow waves of natural sleep. J Neurosci 33:19599–19610

Deisseroth K (2015) Optogenetics: 10 years of microbial opsins in neuroscience. Nat Neurosci 18:1213–1225

Ding F, O'Donnell J, Xu Q, Kang N, Goldman N, Nedergaard M (2016) Changes in the composition of brain interstitial ions control the sleep-wake cycle. Science 352:550–555

Dulac C, O'Connell LA, Wu Z (2014) Neural control of maternal and paternal behaviors. Science 345:765–770

Eban-Rothschild A, Rothschild G, Giardino WJ, Jones JR, de Lecea L (2016) VTA dopaminergic neurons regulate ethologically relevant sleep-wake behaviors. Nat Neurosci 19:1356–1366
Ego-Stengel V, Wilson MA (2010) Disruption of ripple-associated hippocampal activity during rest impairs spatial learning in the rat. Hippocampus 20:1–10
Eriksson KS, Sergeeva O, Brown RE, Haas HL (2001) Orexin/hypocretin excites the histaminergic neurons of the tuberomammillary nucleus. J Neurosci 21:9273–9279
Fattinger S, Jenni OG, Schmitt B, Achermann P, Huber R (2014) Overnight changes in the slope of sleep slow waves during infancy. Sleep 37:245–253
Fort P, Bassetti CL, Luppi PH (2009) Alternating vigilance states: new insights regarding neuronal networks and mechanisms. Eur J Neurosci 29:1741–1753
Franken P, Chollet D, Tafti M (2001) The homeostatic regulation of sleep need is under genetic control. J Neurosci 21:2610–2621
Fuhrmann F, Justus D, Sosulina L, Kaneko H, Beutel T, Friedrichs D, Schoch S, Schwarz MK, Fuhrmann M, Remy S (2015) Locomotion, theta oscillations, and the speed-correlated firing of hippocampal neurons are controlled by a medial septal glutamatergic circuit. Neuron 86:1253–1264
Fuller PM, Yamanaka A, Lazarus M (2015) How genetically engineered systems are helping to define, and in some cases redefine, the neurobiological basis of sleep and wake. Temperature (Austin) 2:406–417
Giber K, Diana MA, Plattner V, Dugué GP, Bokor H, Rousseau CV, Maglóczky Z, Havas L, Hangya B, Wildner H et al (2015) A subcortical inhibitory signal for behavioral arrest in the thalamus. Nat Neurosci 18:562–568
Girardeau G, Benchenane K, Wiener SI, Buzsáki G, Zugaro MB (2009) Selective suppression of hippocampal ripples impairs spatial memory. Nat Neurosci 12:1222–1223
Greene RW, Frank MG (2010) Slow wave activity during sleep: functional and therapeutic implications. Neuroscientist 16(6):618–633
Halassa MM, Acsády L (2016) Thalamic inhibition: diverse sources, diverse scales. Trends Neurosci 39:680–693
Halassa MM, Siegle JH, Ritt JT, Ting JT, Feng G, Moore CI (2011) Selective optical drive of thalamic reticular nucleus generates thalamic bursts and cortical spindles. Nat Neurosci 14:1118–1120
Han Y, Shi Y-F, Xi W, Zhou R, Tan Z-B, Wang H, Li X-M, Chen Z, Feng G, Luo M et al (2014) Selective activation of cholinergic basal forebrain neurons induces immediate sleep-wake transitions. Curr Biol 24:693–698
Hassani OK, Lee MG, Jones BE (2009) Melanin-concentrating hormone neurons discharge in a reciprocal manner to orexin neurons across the sleep-wake cycle. Proc Natl Acad Sci U S A 106:2418–2422
Hassani OK, Henny P, Lee MG, Jones BE (2010) GABAergic neurons intermingled with orexin and MCH neurons in the lateral hypothalamus discharge maximally during sleep. Eur J Neurosci 32:448–457
Hayashi Y, Kashiwagi M, Yasuda K, Ando R, Kanuka M, Sakai K, Itohara S (2015) Cells of a common developmental origin regulate REM/non-REM sleep and wakefulness in mice. Science 350:957–961
Hegemann P, Nagel G (2013) From channelrhodopsins to optogenetics. EMBO Mol Med 5(2):173–176
Herrera CG, Cadavieco MC, Jego S, Ponomarenko A, Korotkova T, Adamantidis A (2015) Hypothalamic feedforward inhibition of thalamocortical network controls arousal and consciousness. Nat Neurosci 19(2):290–298
Irmak SO, de Lecea L (2014) Basal forebrain cholinergic modulation of sleep transitions. Sleep 37:1941–1951
Jego S, Glasgow SD, Herrera CG, Ekstrand M, Reed SJ, Boyce R, Friedman J, Burdakov D, Adamantidis AR (2013) Optogenetic identification of a rapid eye movement sleep modulatory circuit in the hypothalamus. Nat Neurosci 16:1637–1643

Kim A, Latchoumane C, Lee S, Kim GB, Cheong E, Augustine GJ, Shin H-S (2012) Optogenetically induced sleep spindle rhythms alter sleep architectures in mice. Proc Natl Acad Sci 109:20673–20678

Kim T, Thankachan S, McKenna JT, McNally JM, Yang C, Choi JH, Chen L, Kocsis B, Deisseroth K, Strecker RE et al (2015) Cortically projecting basal forebrain parvalbumin neurons regulate cortical gamma band oscillations. Proc Natl Acad Sci 112:3535–3540

Konadhode RR, Pelluru D, Blanco-Centurion C, Zayachkivsky A, Liu M, Uhde T, Glen WB, van den Pol AN, Mulholland PJ, Shiromani PJ (2013) Optogenetic stimulation of MCH neurons increases sleep. J Neurosci 33:10257–10263

Koyama Y, Takahashi K, Kodama T, Kayama Y (2003) State-dependent activity of neurons in the perifornical hypothalamic area during sleep and waking. Neuroscience 119:1209–1219

Krueger JM, Frank MG, Wisor JP, Roy S (2016) Sleep function: toward elucidating an enigma. Sleep Med Rev 28:46–54

Kuki T, Ohshiro T, Ito S, Ji Z-G, Fukazawa Y, Matsuzaka Y, Yawo H, Mushiake H (2013) Frequency-dependent entrainment of neocortical slow oscillation to repeated optogenetic stimulation in the anesthetized rat. Neurosci Res 75:35–45

Lee S-H, Dan Y (2012) Neuromodulation of brain states. Neuron 76:209–222

Lewis LD, Voigts J, Flores FJ, Schmitt LI, Wilson MA, Halassa MM, Brown EN (2015) Thalamic reticular nucleus induces fast and local modulation of arousal state. elife 4:e08760

Longordo F, Kopp C, Lüthi A (2009) Consequences of sleep deprivation on neurotransmitter receptor expression and function. Eur J Neurosci 29:1810–1819

Lu J, Greco MA, Shiromani P, Saper CB (2000) Effect of lesions of the ventrolateral preoptic nucleus on NREM and REM sleep. J Neurosci 20:3830–3842

Lüthi A (2014) Sleep spindles: where they come from, what they do. Neuroscientist 20:243–256

Mang GM, Franken P (2015) Genetic dissection of sleep homeostasis. Curr Top Behav Neurosci 25:25–63

McGinty DJ, Sterman MB (1968) Sleep suppression after basal forebrain lesions in the cat. Science 160:1253–1255

Miesenbock G (2009) The optogenetic catechism. Science 326:395–399

Miyamoto D, Hirai D, Fung CCA, Inutsuka A, Odagawa M, Suzuki T, Boehringer R, Adaikkan C, Matsubara C, Matsuki N et al (2016) Top-down cortical input during NREM sleep consolidates perceptual memory. Science 352:1315–1318

Modirrousta M, Mainville L, Jones BE (2004) Gabaergic neurons with alpha2-adrenergic receptors in basal forebrain and preoptic area express c-Fos during sleep. Neuroscience 129:803–810

Moruzzi G, Magoun HW (1949) Brain stem reticular formation and activation of the EEG. Electroencephalogr Clin Neurophysiol 1:455–473

Nauta WJH (1946) Hypothalamic regulation of sleep in rats; an experimental study. J Neurophysiol 9:285–316

Ni K-M, Hou X-J, Yang C-H, Dong P, Li Y, Zhang Y, Jiang P, Berg DK, Duan S, Li X-M (2016) Selectively driving cholinergic fibers optically in the thalamic reticular nucleus promotes sleep. elife 5:A97

Pace-Schott EF, Hobson JA (2002) The neurobiology of sleep: genetics, cellular physiology and subcortical networks. Nat Rev Neurosci 3:591–605

Paz JT, Davidson TJ, Frechette ES, Delord B, Parada I, Peng K, Deisseroth K, Huguenard JR (2013) Closed-loop optogenetic control of thalamus as a tool for interrupting seizures after cortical injury. Nat Neurosci 16:64–70

Rasch B, Born J (2013) About sleep's role in memory. Physiol Rev 93:681–766

Rechtschaffen A, Bergmann BM, Everson CA, Kushida CA, Gilliland MA (1989) Sleep deprivation in the rat: X. Integration and discussion of the findings. Sleep 12:68–87

Rolls A, Colas D, Adamantidis A, Carter M, Lanre-Amos T, Heller HC, de Lecea L (2011) Optogenetic disruption of sleep continuity impairs memory consolidation. Proc Natl Acad Sci 108:13305–13310

Sallanon M, Denoyer M, Kitahama K, Aubert C, Gay N, JOUVET M (1989) Long-lasting insomnia induced by preoptic neuron lesions and its transient reversal by muscimol injection into the posterior hypothalamus in the cat. Neuroscience 32:669–683

Sanchez-Vives MV, McCormick DA (2000) Cellular and network mechanisms of rhythmic recurrent activity in neocortex. Nat Neurosci 3:1027–1034

Saper CB, Fuller PM, Pedersen NP, Lu J, Scammell TE (2010) Sleep state switching. Neuron 68:1023–1042

Schlingloff D, Káli S, Freund TF, Hájos N, Gulyás AI (2014) Mechanisms of sharp wave initiation and ripple generation. J Neurosci 34:11385–11398

Schmidt MH (2014) The energy allocation function of sleep: a unifying theory of sleep, torpor, and continuous wakefulness. Neurosci Biobehav Rev 47C:122–153

Schöne C, Apergis-Schoute J, Sakurai T, Adamantidis A, Burdakov D (2014) Coreleased orexin and glutamate evoke nonredundant spike outputs and computations in histamine neurons. Cell Rep 7:697–704

Shi Y-F, Han Y, Su Y-T, Yang J-H, Yu Y-Q (2015) Silencing of cholinergic basal forebrain neurons using archaerhodopsin prolongs slow-wave sleep in mice. PLoS One 10:e0130130

Siegel JM (2008) Do all animals sleep? Trends Neurosci 31:208–213

Siegel JM (2009) Sleep viewed as a state of adaptive inactivity. Nat Rev Neurosci 10:747–753

Stroh A, Adelsberger H, Groh A, Rühlmann C, Fischer S, Schierloh A, Deisseroth K, Konnerth A (2013) Making waves: initiation and propagation of corticothalamic Ca2+ waves in vivo. Neuron 77:1136–1150

Sun Y-G, Pita-Almenar JD, Wu C-S, Renger JJ, Uebele VN, Lu H-C, Beierlein M (2013) Biphasic cholinergic synaptic transmission controls action potential activity in thalamic reticular nucleus neurons. J Neurosci 33:2048–2059

Szymusiak R, Alam N, Steininger TL, McGinty D (1998) Sleep-waking discharge patterns of ventrolateral preoptic/anterior hypothalamic neurons in rats. Brain Res 803:178–188

Takahashi K, Lin J-S, Sakai K (2008) Neuronal activity of orexin and non-orexin waking-active neurons during wake–sleep states in the mouse. Neuroscience 153:860–870

Tononi G, Cirelli C (2014) Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. Neuron 81:12–34

Tsunematsu T, Kilduff TS, Boyden ES, Takahashi S, Tominaga M, Yamanaka A (2011) Acute optogenetic silencing of orexin/hypocretin neurons induces slow-wave sleep in mice. J Neurosci 31:10529–10539

Tsunematsu T, Tabuchi S, Tanaka KF, Boyden ES, Tominaga M, Yamanaka A (2013) Long-lasting silencing of orexin/hypocretin neurons using archaerhodopsin induces slow-wave sleep in mice. Behav Brain Res 255:64–74

Tsunematsu T, Ueno T, Tabuchi S, Inutsuka A, Tanaka KF, Hasuwa H, Kilduff TS, Terao A, Yamanaka A (2014) Optogenetic manipulation of activity and temporally controlled cell-specific ablation reveal a role for MCH neurons in sleep/wake regulation. J Neurosci 34:6896–6909

Urry E, Landolt HP (2015) Adenosine, caffeine, and performance: from cognitive neuroscience of sleep to sleep pharmacogenetics. Curr Top Behav Neurosci 25:331–366

Valdés JL, McNaughton BL, Fellous JM (2015) Offline reactivation of experience-dependent neuronal firing patterns in the rat ventral tegmental area. J Neurophysiol 114:1183–1195

Van Dort CJ, Zachs DP, Kenny JD, Zheng S, Goldblum RR, Gelwan NA, Ramos DM, Nolan MA, Wang K, Weng F-J et al (2015) Optogenetic activation of cholinergic neurons in the PPT or LDT induces REM sleep. Proc Natl Acad Sci 112:584–589

Vandecasteele M, Varga V, Berényi A, Papp E, Barthó P, Venance L, Freund TF, Buzsáki G (2014) Optogenetic activation of septal cholinergic neurons suppresses sharp wave ripples and enhances theta oscillations in the hippocampus. Proc Natl Acad Sci U S A 111:13535–13540

Venner A, Anaclet C, Broadhurst RY, Saper CB, Fuller PM (2016) A novel population of wake-promoting GABAergic neurons in the ventral lateral hypothalamus. Curr Biol 26:2137–2143

Verret L, Goutagny R, Fort P, Cagnon L, Salvert D, Leger L, Boissard R, Salin P, Peyron C, Luppi P-H (2003) A role of melanin-concentrating hormone producing neurons in the central regulation of paradoxical sleep. BMC Neurosci 4:19

Von Economo C (1930) Sleep as a problem of localization. J Nerv Ment Dis 71:249–259

Wang DV, Yau H-J, Broker CJ, Tsou J-H, Bonci A, Ikemoto S (2015) Mesopontine median raphe regulates hippocampal ripple oscillation and memory consolidation. Nat Neurosci 18:728–735

Weber F, Chung S, Beier KT, Xu M, Luo L, Dan Y (2015) Control of REM sleep by ventral medulla GABAergic neurons. Nature 526:435–438

Williams RH, Chee MJS, Kroeger D, Ferrari LL, Maratos-Flier E, Scammell TE, Arrigoni E (2014) Optogenetic-mediated release of histamine reveals distal and autoregulatory mechanisms for controlling arousal. J Neurosci 34:6023–6029

Xie L, Kang H, Xu Q, Chen MJ, Liao Y, Thiyagarajan M, O'Donnell J, Christensen DJ, Nicholson C, Iliff JJ et al (2013) Sleep drives metabolite clearance from the adult brain. Science 342:373–377

Xu M, Chung S, Zhang S, Zhong P, Ma C, Chang W-C, Weissbourd B, Sakai N, Luo L, Nishino S et al (2015) Basal forebrain circuit for sleep-wake control. Nat Neurosci 18:1641–1647

Ye M, Hayar A, Strotman B, Garcia-Rill E (2010) Cholinergic modulation of fast inhibitory and excitatory transmission to pedunculopontine thalamic projecting neurons. J Neurophysiol 103:2417–2432

Yu X, Ye Z, Houston CM, Zecharia AY, Ma Y, Zhang Z, Uygun DS, Parker S, Vyssotski AL, Yustos R et al (2015) Wakefulness is governed by GABA and histamine cotransmission. Neuron 87:164–178

Zant JC, Kim T, Prokai L, Szarka S, McNally J, McKenna JT, Shukla C, Yang C, Kalinchuk AV, McCarley RW et al (2016) Cholinergic neurons in the basal forebrain promote wakefulness by actions on neighboring non-cholinergic neurons: an opto-dialysis study. J Neurosci 36:2057–2067

Zhang Z, Ferretti V, Güntan İ, Moro A, Steinberg EA, Ye Z, Zecharia AY, Yu X, Vyssotski AL, Brickley SG et al (2015) Neuronal ensembles sufficient for recovery sleep and the sedative actions of α2 adrenergic agonists. Nat Neurosci 18:553–561

Pharmacosynthetic Deconstruction of Sleep-Wake Circuits in the Brain

Christophe Varin and Patricia Bonnavion

Contents

Abstract

Over the past decade, basic sleep research investigating the circuitry controlling sleep and wakefulness has been boosted by pharmacosynthetic approaches, including chemogenetic techniques using designed receptors exclusively activated by designer drugs (DREADD). DREADD offers a series of tools that selectively control neuronal activity as a way to probe causal relationship between

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neuronal sub-populations and the regulation of the sleep-wake cycle. Following the path opened by optogenetics, DREADD tools applied to discrete neuronal sub-populations in numerous brain areas quickly made their contribution to the discovery and the expansion of our understanding of critical brain structures involved in a wide variety of behaviors and in the control of vigilance state architecture.

Keywords
Arousal · Chemogenetic · Designer Receptor Exclusively Activated by Designer Drugs (DREADD) · Non-REM sleep · Paradoxical sleep · Pharmacogenetic · REM sleep · Slow-wave sleep · Wakefulness

1 Introduction

The development of new hypnotic and wake-promoting medications relies on a solid understanding of the neurochemical mechanisms and brain circuits regulating sleep and wakefulness to improve specificity of action with enhanced safety and efficiency. Over the past decade, basic sleep research investigating the circuitry controlling sleep and wakefulness has been boosted by pharmacosynthetic approaches, some of which can be perceived as an ideal pharmacotherapeutic strategy involving G-protein-coupled receptor (GPCR) signaling. Indeed GPCRs represent the largest class of neuronal signal-transducing molecules capable of modulating neuronal firing (see for review Farrell and Roth 2013) and have proven to be the most highly favorable class of drug targets in modern pharmacology (Drews 2000; Wise et al. 2004). In this context, the development of *d*esigned *r*eceptors *e*xclusively *a*ctivated by *d*esigner *d*rugs (DREADDs) has already proven its relevance to molecular therapy implicating GPCR-linked pathways in the modulation of the circadian rhythm and sleep-wake cycle and in many behaviors and disorders including eating- and addictive-related behaviors or autonomic dysfunctions (see for review Urban and Roth 2015).

Similar to optogenetic approaches (see Adamantidis and Lüthi 2018), the primary goal of DREADD technique applied to systems neuroscience is to offer a series of tools that selectively control neuronal activity as a way to probe causal relationship between neuronal subpopulations and behavior. Its basic principle consists in introducing exogenous genes into neurons that enable them to respond to specific exogenous chemical ligands that have no interfering pharmacological effect. These tools provide a powerful combination of genetic specificity and spatial precision for a potential multiplexed control (multiple chemogenetic actuators controlling simultaneously or sequentially different neuronal pathways) that are critical for a systems-level understanding of brain functions. However, in contrast with optogenetics, DREADD technique lacks a temporal resolution to control the activation or silencing of neurons within milliseconds. On the other hand, it further mimics longer-term modulation of neuronal signaling associated with pharmacotherapeutic strategies targeting GPCRs (see for review Urban and Roth 2015) and could be better suited for large brain structures instead of volume-limited control through optic fibers. The

expansion of pharmacosynthetic approaches not only offers multiple ways to control neuronal excitability but also allows to study and control gene transcription and translation, protein-protein interactions, enzymatic function, and protein stability (see for review Sternson and Roth 2014).

This chapter first draws up the principles of the DREADD technology that has been the most widely used in sleep research, followed by a discussion on the potential limitations and concerns that were recently raised in the literature. This section intends to identify and clarify the risks associated with DREADDs in order to control and support future experiments and bring a better perspective at the reading of the findings emerging from DREADDs in sleep research. Next we will review the experiments deconstructing arousal- and sleep-promoting circuits and their resulting discoveries and discuss their significance in the face of past knowledge and future directions.

2 Description of Pharmacosynthetic Tools

Two techniques emerged over the past decade to remotely control neuronal activity in vivo: optogenetics based on engineered opsins (see Adamantidis and Lüthi 2018) and pharmacosynthetic approaches including DREADD-based chemogenetics utilizing engineered GPCRs and *p*harmacologically *s*elective *a*ctuator *m*olecules (PSAM) utilizing chimeric *l*igand-*g*ated *i*on *c*hannels (LGIC) that both selectively interact with synthetic small molecules (see for review Farrell and Roth 2013; Sternson and Roth 2014). In basic sleep research using rodent models, the DREADD technology referred to as the third generation of muscarinic GPCR-based chemogenetics (Armbruster et al. 2007) has been the most broadly adopted tool.

2.1 Principles of the DREADD Technology

The DREADDs were first generated from the human M3 muscarinic receptors subjected to random mutagenesis in the yeast and screened from absence of drug-dependent growth assays in the presence of clozapine N-oxide (CNO). Resulting surviving mutants (hM3DGq) evolve receptors that are activated only by CNO and lack activation by their native ligand, acetylcholine (ACh) (Dong et al. 2010). CNO is an inactive metabolite of the atypical antipsychotic clozapine. It was chosen for its ability to penetrate the central nervous system and for its favorable pharmacokinetics and inert pharmacology (Bender et al. 1994; Armbruster et al. 2007). However, few reports raised concerns that CNO could be interconverted to clozapine in various species (Loffler et al. 2012; Gomez et al. 2017). While a small fraction of interconverted CNO is established in humans, primates, and guinea pigs, the occurrence of this process in rodents is still debated and is further addressed in the next section. Still in vitro, CNO activation of the hM3DGq mutant in transfected neurons triggers Gq-coupled signaling leading to membrane depolarization through

phospholipase C/PIP2-mediated inhibition of voltage-activated KCNQ potassium channels (Armbruster et al. 2007; Alexander et al. 2009).

Using a similar design scheme, a second mutated human M4 receptor coupled to Gi proteins (hM4DGi) was generated leading to neuronal hyperpolarization mediated by G-protein-coupled inwardly rectifying potassium channels (GIRKs) upon CNO application (Armbruster et al. 2007; Zhu et al. 2014). In vivo validation of DREADD-driven neuronal activation or inhibition has been reported in numerous studies conjointly with major behavioral consequences (see for review Sternson and Roth 2014). However so far, only few groups combined CNO administration and direct in vivo unit recordings to examine and directly control CNO-driven DREADD effects on the spontaneous neuronal activity and firing discharge. Most of these studies confirmed modifications of neuronal activities by ex vivo electrophysiological recordings in brain slices or by indirect methods involving the labeling of the cFos immediate early gene, as a marker of neuronal activation.

A third DREADD construct was generated from a chimeric muscarinic-adrenergic receptor that selectively activates Gαs signaling pathways (rM3/β1Gs) promoting cAMP production through the activation of adenylate cyclase (Guettier et al. 2009; Farrell et al. 2013). Its first use was applied to pancreatic β-cells in vitro and in vivo to deconstruct insulin secretion signaling pathway (Guettier et al. 2009). Unlike hM3DGq or hM4DGi receptors, a small degree of constitutive activity from transfected cells has been reported with rM3/β1Gs leading to a modest basal phenotype (Guettier et al. 2009). Interestingly, the specific activation of the different G-coupled pathways using DREADD approaches was interrogated to deconstruct the intracellular control of circadian pacemaking activity of suprachiasmatic nucleus (SCN) neurons in vitro (Brancaccio et al. 2013). This study showed that, although CNO-mediated induction of Gs significantly increases calcium/cAMP-responsive elements in a larger degree than Gq, the activation of Gq-dependent pathway in a subset of SCN neurons, but not Gs or Gi, selectively reprograms the circadian oscillations of intracellular calcium and transcriptional/posttranslation feedback loop rhythms in the entire SCN (Brancaccio et al. 2013). This reorganization requires a VIPergic signaling revealing a Gq-intracellular calcium-VIP leitmotif that determines the intrinsic network encoding of SCN circadian time (Brancaccio et al. 2013). In addition to a broad remote control of neuronal activity, this work illustrates a major potential of DREADDs to interrogate the relationship between G-coupled pathways, cytosolic signaling, and the transcriptional feedback loops with neuronal activity.

The DREADD genes are expressed via transgenic or viral approaches for conditional gene targeting. The most commonly used method to obtain high-expression levels of DREADD consists in Cre-dependent viruses carrying the DREADD transgene under the control of strong ubiquitous promoters (such as EF1α, hSyn) injected in specific Cre-driver lines. The vast library of Cre-driver knock-in and bacterial artificial chromosomes (BAC) transgenic mice available today offers thousands of targeting possibilities. Still confirmation of the specificity of the Cre expression in the desired genetically defined cell population by the absence of ectopic expression at least in the region of interest when using viral vector approach is required. The

activation of GPCR signaling pathways using DREADDs has revealed remarkably specific behavioral and physiological influences for a variety of neuronal, but also non-neuronal, cell types that are often intermingled with populations having different or even opposite functions (see for review Sternson and Roth 2014; Roth 2016). Sleep studies using DREADDs are further detailed in the next sections (Sect. 3) and listed in Table 1. The main advantages of this technology are as follows: (1) it can be minimally invasive as CNO can be administered orally in drinking water; (2) CNO kinetics involve a prolonged modulation of neuronal activity (minutes to hours) with physiological relevance to canonical and conserved GPCR signaling pathways; (3) there is no specialized equipment required as all chemical actuators are commercially available; (4) it allows a large-scale control of a diffuse neuronal ensemble, which is challenging to illuminate with optogenetics, while it still allows subdomain specific targeting with local injections; and finally (5) it is a relatively inert intervention upon the CNO doses used. The main disadvantage of the DREADD system is the lack of precise temporal control in comparison with what is achieved with optogenetics. Compounds with enhanced pharmacokinetic properties and light-activated photocaged compounds allowing for more precise control over time of the DREADD receptor activation are still under development (see for review Sternson and Roth 2014). Other disadvantages related to CNO and inherent properties of GPCRs are further discussed in the following sections (Sects. 2.2 and 2.3).

2.2 What to Keep in Mind Before DREADD Use?

For a comprehensive understanding of the DREADD technology and a more effective use, inherent properties of GPCRs should be kept in mind. Many GPCRs show a considerable amount of basal, ligand-independent activity implying that a GPCR can activate its G protein in the absence of an agonist (see for review Kobilka and Deupi 2007). Therefore a major concern for chemogenetic approaches is the possibility that high levels of expression of an engineered protein might have effects in the absence of chemical activation (Conklin et al. 2008). This constitutive activity has been questioned and examined for DREADD receptors. To date, none of the studies using hM3DGq or hM4DGi have reported a basal phenotype implying no detectable constitutive activity. Lifelong and considerably high levels of expression that were attained using either a genetically encoded tetracycline-sensitive induction system or viral transduction of hM3DGq or hM4DGi were not reported to have any abnormality from basal electrophysiological, behavioral, or anatomical characterizations (Alexander et al. 2009; Zhu et al. 2014; see for review Roth 2016). However, the lack of report does not imply the absence of basal activity. A relatively low constitutive activity was found with the rM3/β1Gs construct looking at cAMP and inositol phosphate basal levels from transfected cells, which were not apparent with hM3DGq (Guettier et al. 2009). However, no detectable electrophysiological, behavioral, or anatomical phenotype was reported in a rM3/β1Gs transgenic mouse (Farrell et al. 2013). To counteract this issue if any basal activity is observed, cautions on titrating

Table 1 Chemogenetic control of brain regions/neurotransmitter systems and effects on sleep-wake behavior

Authors	Brain area	Animal model[a]	DREADD-containing vector[b]	CNO dose	Effects on vigilance states[c]
Medulla					
Chen et al. (2017) Experimental neurology	RVM	Sprague-Dawley rats	AAV_{10}-Cre + AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.2 mg/kg	↑ Wake; ↓ SWS; ↓ REM
	pSOM	Sprague-Dawley rats	AAV_{10}-Cre + AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.2 mg/kg	↔ Wake; ↔ SWS; ↓ REM
	pSOM (GABA)	*Vgat-IRES-Cre* mice	AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↔ Wake; ↔ SWS; ↓ REM
Anaclet et al. (2014) Nat Neurosci	PZ (GABA)	*Vgat-IRES-Cre* (129/B6/FVB) mice	AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↓ Wake; ↑ SWS; ↓ REM
			AAV_{10}-hSyn-DIO-hM4Di-mCherry	0.3 mg/kg	↑ Wake; ↓ SWS; ↓ REM
Pons					
Hayashi et al. (2015) Science	Medial Atoh1-E10.5 neurons	*Atoh1-CreER^{T2}*; *CAG-LSL-tTA* (B6) mice (tamoxifen @E10.5)	AAV_5-TRE-hM3Dq-mCherry	1.5 mg/kg	↔ Wake; ↑ SWS; ↓ REM
	Lateral Atoh1-E10.5 neurons	*Atoh1-CreER^{T2}*; *CAG-LSL-tTA* (B6) mice (tamoxifen @E10.5)	AAV_5-TRE-hM3Dq-mCherry	1.5 mg/kg	↑ Wake; ↓ SWS; ↓ REM
	Medial Atoh1-E10.5 neurons (glutamate)	*Atoh1-CreER^{T2}*; *Camk2a-LSL-tTA* (B6) mice (tamoxifen @E10.5)	AAV_5-TRE-hM3Dq-mCherry	1.5 mg/kg	↔ Wake; ↔ SWS; ↓ REM
		Atoh1-CreER^{T2}; *Vglut2-LSL-tTA* (B6) mice (tamoxifen @E10.5)	AAV_5-TRE-hM3Dq-mCherry	1.5 mg/kg	↔ Wake; ↔ SWS; ↓ REM
	Lateral Atoh1-E10.5 neurons (glutamate)	*Atoh1-CreER^{T2}*; *Camk2a-LSL-tTA* (B6) mice (tamoxifen @E10.5)	AAV_5-TRE-hM3Dq-mCherry	1.5 mg/kg	↑ Wake; ↓ SWS; ↓ REM

	dDpMe (GABA)	*Vgat-Cre* KI (B6) mice	AAV_8- EF1α-DIO-hM3Dq-mCherry	1.5 mg/kg	↔ Wake; ↑ SWS; ↓ REM
		Vgat-Cre Tg (B6) mice	AAV_8- EF1α-DIO-hM3Dq-mCherry	1.5 mg/kg	↔ Wake; ↑ SWS; ↓ REM
		Vgat-Cre KI (B6) mice	AAV_8- EF1α-DIO-hM4Di-mCherry	1.5 mg/kg	↓ Wake; ↔ SWS; ↑ REM
		Vgat-Cre Tg (B6) mice	AAV_8- EF1α-DIO-hM4Di-mCherry	1.5 mg/kg	↔ Wake; ↔ SWS; ↑ REM
Qiu et al. (2016a) Curr Biol	PB	Sprague-Dawley rats	AAV_{10}-EF1α-hM3dq-mCherry	0.2 mg/kg	↑ Wake (11 h long); ↓ SWS; ↓ REM
	PB projecting to LPO	Sprague-Dawley rats	$AAV_{2/6}$-CAG-Cre (in LPO) and AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.2 mg/kg	↑ Wake; ↓ SWS; ↓ REM
	PB projecting to LH	Sprague-Dawley rats	$AAV_{2/6}$-CAG-Cre (in LH) and AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.2 mg/kg	↑ Wake; ↓ SWS; ↓ REM
	PB projecting to thalamus	Sprague-Dawley rats	$AAV_{2/6}$-CAG-Cre (in thalamus) and AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.2 mg/kg	No effect
Kaur et al. (2017) Neuron	PBel (CGRP)	*CGRP-CreER* (B6) mice (tamoxifen treatment prior to AAV injections)	$AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.1–0.3 mg/kg	↑ Wake; ↓ SWS; ↓ REM
Kroeger et al. (2017) J Neurosci	PPT (acetylcholine)	*ChAT-IRES-Cre* (B6) mice	AAV_{10}-EF1α-DIO-hM3Dq-mCherry	0.3 mg/kg	↔ Wake; ↓ deep SWS; ↑ light SWS; ↔ REM
	PPT (glutamate)	*Vglut2-IRES-Cre* (B6) mice	AAV_8-EF1α-DIO-hM3Dq-mCherry (note different serotypes)	0.3 mg/kg	↑ Wake; ↓ SWS; ↓ REM
	PPT (GABA)	*Vgat-IRES-Cre* (B6) mice	AAV_8-EF1α-DIO-hM3Dq-mCherry	0.3 mg/kg	↑ REM

(continued)

Table 1 (continued)

Authors	Brain area	Animal model[a]	DREADD-containing vector[b]	CNO dose	Effects on vigilance states[c]
	PPT (glutamate)	*Vglut2-IRES-Cre* (B6) mice	AAV_8-EF1α-DIO-hM4Di-mCherry	1 mg/kg	↓ Wake; ↑ SWS; ↔ REM
Vazey and Aston-Jones (2014) Proc Natl Acad Sci U S A	LC (noradrenaline)	Long-Evans rats	$AAV_{2/9}$-PRSx8-HA-hM3Dq (PRSx8: adrenergic neurons promoter)	0.1, 1, 10 mg/kg/intracerebral 5 μM (30–60 nL)	↓ Emergence from general anesthesia; ↑ cortical arousal; ↓ Delta power; ↑ theta power during anesthesia
Hasegawa et al. (2014) J Clin Invest	DR (5HT) ↔ LH (orexin)	*Orexin/ataxin-3* (B6) mice (postnatal degeneration)	AAV_2-Pet1-HA-hM3Dq (Pet1: serotonergic-specific transcription factor)	5 mg/kg	↓ Cataplexy; ↑ REM latency; ↓ wake episode duration
	LC (noradrenaline) ↔ LH (orexin)	*Orexin/ataxin-3* (B6) mice	AAV_2-PRSx8-HA-hM3Dq (PRSx8: adrenergic neurons promoter)	5 mg/kg	↔ Cataplexy; ↑ wake episode duration; ↓ wake episode number
Midbrain					
Sun et al. (2017) Sleep Biol Rhythms	VTA	WT (129) mice	AAV_{10}-hSyn-hM3Dq-mCherry	1 mg/kg	↑ Wake; ↓ SWS; ↓ REM
			AAV_{10}-hSyn-hM4Di-mCherry	1 mg/kg	No effect
Eban-Rothschild et al. (2016) Nat Neurosci	VTA (dopamine)	*Th-IRES-Cre* KI (B6) mice	AAV_5-EF1α-DIO-hM4Di-mCherry	1 mg/kg	↓ Wake; ↑ SWS; ↑ REM
Oishi et al. (2017) Brain Struc Funct	VTA (dopamine)	*Dat-Cre* KI (B6) mice	AAV_{10}-hSyn-hM3Dq-mCherry	0.3 mg/kg	↑ Wake
				0.3 mg/kg + D_1R antago (SCH23390)	↑ Wake
				0.3 mg/kg + D_2R/D_3R	↔ Wake

				antago (racloprine)	
			AAV_{10}-hSyn-hM4Di-mCherry	0.3, 1 mg/kg	No effect
	SNc (dopamine)	*Dat-Cre* KI (B6) mice	AAV_{10}-hSyn-hM3Dq-mCherry	0.3 mg/kg	No effect
Hypothalamus					
Pedersen et al. (2017) Nat Commun	SUM (glutamate)	*Vglut2-IRES-Cre* (B6) mice	$AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↑ Wake (large)
		Vglut2$^{lox/lox}$ (B6) mice	AAV_{10}-hSyn-Cre-GFP and $AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↑ Wake (midl)
		Vglut2-IRES-Cre (B6) mice	$AAV_{2/10}$-hSyn-DIO-hM4Di-mCherry	0.3 mg/kg	↓ Wake; ↑ SWS; ↓ REM
	SUM (GABA)	*Vgat-IRES-Cre* (B6) mice	$AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↑ Wake
	SUM (glutamate/ NOS1)	*NOS1-IRES-Cre* (B6) mice	$AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↑ Wake
		NOS1-IRES-Cre (B6) mice	$AAV_{2/10}$-hSyn-DIO-hM4Di-mCherry	0.3 mg/kg	No effect on sleep/ wake; ↓ REM theta power
Venner et al. (2016) Curr Biol	LH (GABA)	*Vgat-IRES-Cre* (129/B6/FVB) mice	AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↑ Wake; ↓ SWS; ↓ REM
			AAV_{10}-hSyn-DIO-hM4Di-mCherry	0.3 mg/kg	↓ Wake; ↑ SWS; ↔ REM
Sasaki et al. (2011) PLoS One	LH (orexin)	*Orexin-cre* (B6) mice	AAV_{2}-EF1α-DIO-HA-hM3Dq	5 mg/kg	↑ Wake; ↓ SWS; ↓ REM
			AAV_{2}-EF1α-DIO-HA-hM4Di	5 mg/kg	↓ Wake; ↑ SWS; ↓ REM
Vetrivelan et al. (2016) Neuroscience	LH (MCH)	*MCH-cre* mice	AAV_{8}-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↑ REM

(continued)

Table 1 (continued)

Authors	Brain area	Animal model[a]	DREADD-containing vector[b]	CNO dose	Effects on vigilance states[c]
Varin et al. (2018) Sleep	LH (MCH)	*MCH-cre* (B6) mice	AAV_{10}-hSyn-DIO-mCherry	0.5–10 mg/kg	↑ SWS; ↓ REM
			AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.5–1 mg/kg	↓ SWS duration; ↑ SWS depth; ↑ REM
			AAV_{10}-hSyn-DIO-hM4Di-mCherry	1–5 mg/kg	↑ SWS duration; ↓ SWS depth; ↔ REM
Yu et al. (2015) Neuron	TMN (histamine)	*HDC-cre* (129/B6) mice	$AAV_{1/2}$-hSyn-DIO-hM3Dq-mCherry	5 mg/kg	↑ Motor activity
Kodani et al. (2017) J Neurosci	BNST (GABA)	*GAD67-Cre* (B6) mice	AAV_{10}-EF1α-DIO-hM3Dq-mCherry	5 mg/kg	↑ Wake; ↓ SWS; ↓ REM
Saito et al. (2013) Front Neural Circuits	Preoptic area (GABA)	*Gad67-Cre* (B6) mice	AAV_2-EF1α-DIO-HAhM3Dq	5 mg/kg	↓ Wake; ↑ SWS; ↔ REM
Zhang et al. (2015) Nat Neurosci	Preoptic area – LPO or MnPO	WT (B6) mice	$AAV_{1/2}$-ITR-P_{cFos}-tTA-WPRE-pA-ITR and $AAV_{1/2}$-ITR-$P_{TRE\text{-}tight}$-hM3Dq-mCherry (doxycycline removal before dexmedetomidine anesthesia or sleep-deprivation-induced hypersomnia)	5 mg/kg	↓ Locomotor activity; ↑ SWS
Basal forebrain					
Anaclet et al. (2015) Nat Commun	VP, SI, MCPO, HBD (acetylcholine)	*ChAT-IRES-Cre* mice	$AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	No effect on sleep/wake ↓ SWS EEG power
	VP, SI, MCPO, HBD (glutamate)	*Vglut2-IRES-Cre* mice	$AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	No effect on sleep/wake ↓ SWS EEG power
	VP, SI, MCPO, HBD (GABA)	*Vgat-IRES-Cre* mice	$AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	↑ Wake; ↓ SWS; ↓ REM ↑ Wake fast rhythms
			$AAV_{2/10}$-hSyn-DIO-hM4Di-mCherry	0.3 mg/kg	↓ Wake; ↑ SWS

	Thalamus (glutamate)	*Vglut2-IRES-Cre* mice	$AAV_{2/10}$-hSyn-DIO-hM3Dq-mCherry	0.3 mg/kg	No effect on sleep/wake ↑ Fast rhythms
Chen et al. (2016) Neuropsychopharmacology	MCPO, HDB, SI (acetylcholine)	*ChAT-IRES-Cre* (B6) mice	AAV-hSyn-DIO-hM3Dq-mCherry	0.1, 0.3, 1 mg/kg	↑ Wake; ↓ SWS; ↓ REM ↓ SWS delta power
			AAV-hSyn-DIO-hM4Di-mCherry	1 mg/kg	↓ Wake; ↑ SWS; ↓ REM ↑ SWS delta power
Subthalamus					
Liu et al. (2017) Nature	ZI (Lhx6)	*Lhx6-Cre* (B6) mice	AAV_9-EF1α-DIO-hM3Dq-mCherry	0.5 mg/kg	↓ Wake; ↑ SWS; ↑ REM
			AAV_9-EF1α-DIO-hM4Di-mCherry	0.5 mg/kg	↑ Wake; ↓ SWS; ↓ REM
Basal ganglia					
Yuan et al. (2017) eLife	Caudal STRd (iMSN)	*Adora2a-Cre* (B6) mice	AAV-hSyn-DIO-hM3Dq-mCherry	1 mg/kg	↓ Wake; ↑ SWS; ↔ REM
	Centromedial STRd (iMSN)	*Adora2a-Cre* (B6) mice	AAV-hSyn-DIO-hM3Dq-mCherry	1 mg/kg	↓ Wake; ↑ SWS; ↔ REM
	Centrolateral STRd (iMSN)	*Adora2a-Cre* (B6) mice	AAV-hSyn-DIO-hM3Dq-mCherry	1 mg/kg	↓ Wake; ↑ SWS; ↔ REM
	Caudal STRd (iMSN)	*Adora2a-Cre* (B6) mice	AAV-hSyn-DIO-hM3Dq-mCherry	1 mg/kg	No effect
	GPe (PV)	*Pvalb-Cre* (129) mice	AAV-hSyn-DIO-hM4Di-mCherry	1 mg/kg	↓ Wake; ↑ SWS; ↔ REM
	STRd (iMSN) → GPe (PV)	*Adora2a-Cre/Pvalb-Cre* (129/B6) mice	AAV-hSyn-DIO-hM3Dq-mCherry (in STRd) and	1 mg/kg	No effect

(continued)

Table 1 (continued)

Authors	Brain area	Animal model[a]	DREADD-containing vector[b]	CNO dose	Effects on vigilance states[c]
			AAV-EF1α-Flex-taCasp3-TEVp + AAV EF1α-DIO-eGFP (in GPe: ablation)		
	STRd (iMSN)	*Adora2a-Cre* (B6) mice	AAV-hSyn-DIO-hM4Di-mCherry	1 mg/kg	↑ Wake; ↓ SWS; ↓ REM
Oishi et al. (2017) Nat Commun	NAc (iMSN)	*Adora2a-Cre* (B6) mice	AAV_{10}-hSyn-DIO-hM3Dq-mCherry	0.01–1 mg/kg	↓ Locomotor activity; ↑ SWS
			AAV_{10}-hSyn-DIO-hM4Di-mCherry	0.03–0.3 mg/kg	↓ SWS
Limbic system					
Hasegawa et al. (2017) Proc Natl Acad Sci U S A	Amygdala (LA, BLA, CeA)	*Orexin/ataxin-3* (B6) mice	$AAV_{2/rh10}$-SynI-iCre + $AAV_{2/rh10}$-EF1α-DIO-hM3Dq-mCherry	5 mg/kg	↑ Cataplexy
			$AAV_{2/rh10}$-SynI-iCre + $AAV_{2/rh10}$-EF1α-DIO-hM4Di-mCherry	5 mg/kg	↓ Cataplexy

BLA basolateral amygdala, *BNST* bed nucleus of the stria terminalis, *CeA* central amygdala, *dDpMe* dorsal part of the deep mesencephalic nucleus, *GPe* globus pallidus, external part, *HDB* horizontal limb of the diagonal band, *LA* lateral amygdala, *LC* locus coeruleus, *LH* lateral hypothalamus, *LPO* lateral preoptic area, *MCH* melanin-concentrating hormone, *MCPO* magnocellular preoptic nucleus, *MnPO* median preoptic nucleus, *NAc* nucleus accumbens, *PB* parabrachial nuclei, *PBel* external lateral parabrachial nucleus, *PPT* pedunculopontine tegmentum, *PZ* parafacial zone, *RMV* rostral ventromedial medulla, *SI* substantia innominate, *SNc* substantia nigra, pars compacta, *pSOM* ventromedial medulla rostral to the inferior olive, *STRd* dorsal striatum, *SUM* supramammillary nucleus, *TMN* tuberomammillary nucleus, *VP* ventral pallidum, *VTA* ventral tegmental area, *ZI* zona incerta

[a]Genetic background or strain mentioned when available

[b]Serotype and promoter used for AAV constructs provided when available

[c]Main effects are reported, otherwise mentioned wake, SWS and REM refer to total wake SWS and REM sleep amounts

and lowering the level of DREADD expression using diluted viral preparations or using a weaker promoter should be considered.

Another conceptual issue relates to the fact the GPCRs can activate more than one G-protein isoform and can also signal through G-protein-independent pathways involving β-arrestin (see for review Roth 2016). Thus, it questions under which conditions β-arrestin signaling is activated and whether the effects observed on neuronal activity and behavior result from canonical or noncanonical (G-protein-independent) signaling. To date, there have been no reports suggesting that the actions of CNO-mediated neuronal silencing or excitation, through hM4DGi and hM3DGq, respectively, could be explained by other mechanisms than altered neuronal firing. Without excluding alternative pathways, the argument developed in favor of major canonical signaling is based on several studies applying optogenetics and DREADDs on the same neuronal populations that globally show equivalent effects in nature and magnitude. However, some differences between optogenetics and DREADD studies are still observed. This is notably the case when manipulating hypothalamic peptidergic systems such as hypocretin/orexin (Hcrt/Orx)- or melanin-concentrating hormone (MCH)-containing neurons and examining their impact on sleep macroarchitecture (see Sect. 3.5). While optogenetic stimulation of Hcrt/Orx neurons induces sleep fragmentation without affecting global sleep or wake amounts (Rolls et al. 2011; Bonnavion et al. 2015), DREADD activation results in prolonged wake and sleep deprivation over several hours (Sasaki et al. 2011). Many factors here can have an impact underlying such differences including viral transduction versus transgenic mice, or photostimulation paradigms versus CNO-driven activation, whose impact on Hcrt/Orx neuronal firing in vivo is unknown. Still an additional factor to consider could relate to the activation of differential downstream effector pathways and physiological changes that could favor multiple transmitters' co-release (see for review Bonnavion et al. 2016; Schöne and Burdakov 2017). Interestingly, a DREADD that signals exclusively via β-arrestin has been developed (Nakajima and Wess 2012) allowing to tackle in vivo the specific behaviors downstream of β-arrestin signaling.

One last concern with GPCR inherent properties relates to possible desensitization and subsequent DREADD receptor downregulation following repeated administration of CNO. As with many GPCRs undergoing significant downregulation only following hours of agonist treatment, chronic treatment with CNO might result in diminished responses or might induce a shift in the dose-response curve to higher concentrations due to DREADD receptor desensitization and internalization. However, the degree of desensitization greatly depends on the extent to which receptors are expressed. When DREADD expression is quite high, lower concentrations of the chemical actuator are needed to achieve a maximal response (see for review Roth 2016). Therefore, considering the very high levels and density of virally or transgenically induced DREADD expression, it is likely that the cellular responses will be less sensitive to repeated CNO than when the mutated receptors are expressed at lower levels. So far, no significant desensitization was seen or reported using viral or transgenic approaches.

2.3 The CNO Versus Clozapine Case

Before the expansion of the CNO/DREADD system use, former studies focusing on clozapine metabolism showed that clozapine is converted into its N-oxide metabolite CNO but that a reversible pathway exists for a small fraction of CNO back-metabolized to clozapine in guinea pigs and humans (Jann et al. 1994; Chang et al. 1998). From the start, this observation challenged the mechanisms of action of CNO in vivo and questioned whether this conversion could also occur in other species including rodents and nonhuman primates. From many reports, CNO appeared to be pharmacologically and behaviorally inert in rodents (Bender et al. 1994; Armbruster et al. 2007; Alexander et al. 2009; Guettier et al. 2009). However, recent studies performed in mice, rats, and monkeys further investigated this possibility by measuring the presence of clozapine following CNO administration through various assays (Hellman et al. 2016; Gomez et al. 2017; Raper et al. 2017). Additionally, the blood-brain permeability of CNO has been reexamined (Hellman et al. 2016; Ji et al. 2016; Nagai et al. 2016; Gomez et al. 2017; Raper et al. 2017). These recent papers emphasize three major points: (1) the affinity of clozapine to bind DREADDs is higher than CNO (Gomez et al. 2017); (2) CNO may not cross the blood-brain barrier (Hellman et al. 2016; Gomez et al. 2017; Raper et al. 2017); and (3) CNO can be converted to clozapine under certain doses in monkeys and rodents (Hellman et al. 2016; Gomez et al. 2017; Raper et al. 2017).

The first point was made based on binding data and suggests that clozapine could be more potent than CNO in activating DREADDs. Still, several studies showed that CNO efficiently and rapidly controls neuronal firing both in vitro and in vivo (Alexander et al. 2009; Krashes et al. 2011; Parnaudeau et al. 2013; Alcacer et al. 2017). Importantly, it is also possible that clozapine, even at very low doses, can activate endogenous receptors in addition to its action on DREADDs. Thus, to appropriately compare efficiencies and kinetics of clozapine and CNO on targeted neurons and neighbor cells, both drugs should be examined in parallel with in vitro and/or in vivo electrophysiological recordings. In addition, Gomez and colleagues performed locomotor behavioral tests to examine the effects of both CNO and "sub-threshold concentrations of clozapine in dopamine D1 receptor-Cre mice expressing hM4DGi transgene in the striatum and control GFP mice (Gomez et al. 2017). Their results suggest that 1 mg/kg CNO would be equivalent to 0.01 mg/kg clozapine to control locomotion in striatal D1-hM4Di-expressing mice without affecting locomotion in control animals. However, the lack of locomotor effects in control mice treated with sub-threshold concentrations of clozapine does not imply that it would not affect other behaviors or cognitive processes. Plus, this efficiency on DREADD-transfected neurons is limited to one neural system for now and should be further tested over DREADD-validated ones on various behavioral responses. The need for developing alternative compounds appears critical, but the switch to clozapine should be carefully considered.

To conclude the last two other points, Gomez and colleagues first employed PET imaging and autoradiography in rats and mice that shows low-to-no signal of radiolabeled CNO in brain tissue, which is contrasting with similar experiments

also performed in transgenic DREADD mice showing a highest contrast of radio signals for DREADD versus background with CNO than with clozapine (Ji et al. 2016). The major argument resides in the fact that the radiolabeled CNO signal could have been due to converted radiolabeled clozapine. Indeed, contrasting results from clozapine/CNO plasma measurements were reported in rodents and in monkeys detecting clozapine after CNO systemic injections (Hellman et al. 2016; Gomez et al. 2017; Raper et al. 2017) or not (Alexander et al. 2009; Guettier et al. 2009; Nagai et al. 2016). It still remains elusive to explain or reconcile these differences. Importantly, if such conversion happens, it primarily occurs in the liver through cytochrome P450 enzymes, which are also present in the brain but at very low levels (Mahler and Aston-Jones 2018). In addition, several studies demonstrated that CNO efficiently activates signaling pathways on cell cultures and on diverse mammalian and drosophila neuronal systems, in a shorter time frame than what is observed for CNO/clozapine conversion (see for review Roth 2016). Indeed, Gomez and colleagues reported that clozapine metabolized from CNO accumulates over time such that its effects may be strongest 2–3 h after CNO injection. Based on the short plasma half-life of CNO within 2 h (Alexander et al. 2009; Guettier et al. 2009), most studies so far examined its effects within this first 2-h period. However, late-onset effects on behavior were not necessarily analyzed outside this 2-h window and should be further monitored from now on.

It becomes clear that CNO is not an inert ligand upon the dose used. “Safe” doses to administer in a systemic manner range from 0.1 to a maximum of 3 mg/kg in order to avoid or minimize this interconversion. Also, in light of the low distribution in the central nervous system of CNO, its delivery route could be revised using local intracerebral (i.c.) injections (Stachniak et al. 2014; Vazey and Aston-Jones 2014) instead of systemic administration to enhance its actions locally but involves a more invasive approach.

Finally the critical point that these studies highlighted is the importance of controlling and comparing CNO effects in appropriate DREADD-free control animals, in addition to vehicle injections in the same DREADD-expressing subjects. Ultimately these comparisons within and between subjects will reinforce the significance and specificity of action of CNO. Indeed most studies limited their results comparing CNO to vehicle/saline injections, while growing evidences from mice, rats, and monkeys studies report endogenous effects of CNO at certain doses in control DREADD-free animals (Eldridge et al. 2015; MacLaren et al. 2016; Gomez et al. 2017; Varin et al. 2018). Taken the possible conversion of CNO and the sedative properties of clozapine (Hinze-Selch et al. 1997), these controls are crucial in sleep studies. Indeed, a recent study using DREADD approaches to control and modulate hypothalamic MCH neurons across the sleep-wake cycle showed that CNO at the doses of 5 and 10 mg/kg increases slow-wave sleep and inhibits REM sleep in control wild-type mice (Varin et al. 2018).

Altogether, these recent findings calling to re-evaluate the use of CNO at certain doses through a systemic delivery route stress the need to develop and test alternative compounds. Interestingly, non-CNO chemical actuators for DREADDs have been tested such as a newly developed actuator named compound 21, and the

FDA-approved hypnotic compound perlapine, both highly selective for hM3DGq (Chen et al. 2015). Importantly, compound 21 is not back-metabolized via normal routes to clozapine or related compound and thus represents a good alternative to CNO (Chen et al. 2015). Perlapine has a modest affinity for certain biogenic amine receptors such as 5-HT2A, 5-HT6, 5-HT7 and D4, but appears to have more than a 10,000-fold selectivity for activating hM3DGq versus muscarinic receptors (Chen et al. 2015). With careful titration to define sub-threshold doses, application of perlapine in conjunction with DREADD-targeted activation could still be quite relevant to study sleep-wake circuits notably in models of insomnia, and sedative processes.

3 CNO-DREADD Experiments in Sleep Studies

The following sections review the major findings identifying the role of defined cell populations in the regulation of the sleep-wake cycle that emerged from pharmacosynthetic approaches in rodents over the past 10 years (Fig. 1). So far, the techniques employed appear to be exclusive to the use of the CNO/DREADD system.

Following up on the later concerns about the use of CNO and its possible conversion to clozapine, most of the sleep studies presented here used CNO delivered systemically under the critical dose of 3 mg/kg. Although the majority of the literature compared CNO effects to vehicle injections on their main figures, most studies examined whether CNO at the dose employed had any endogenous effects on DREADD-free control animals. So far in sleep studies, only one study explicitly reported undesired sleep effects from CNO injections in wild-type mice at the dose of 5 and 10 mg/kg (Varin et al. 2018). To have a clear vision of the procedures employed, we drew up a table integrating all the studies presented here summarizing the neuronal system and brain structure targeted, viral or transgenic approaches, CNO dose used, and the major effects found on sleep-wake states (see Table 1) (Fig. 1).

3.1 GABA Systems

For the last decade, the use of both optogenetic and DREADD techniques highlighted the multifaceted role of the major inhibitory neurotransmitter GABA in sleep and wakefulness processes. Although the role of GABA in sleep induction and maintenance is well established since most of the hypnotic treatments targets $GABA_A$ receptors (Watson et al. 2012), the identification of the GABAergic populations involved remained elusive. Part of the reasons come from the fact that GABAergic neurons are often intertwined among other neurons utilizing different neurotransmitters but also that several neuronal populations previously thought to release only glutamate (Glu), ACh, dopamine (DA), or histamine (His) also release GABA (see for review Tritsch et al. 2016). These complex configurations prevented the access and possibility to question the respective function and physiological role of GABA neurotransmission until transgenic animals and gene targeting techniques

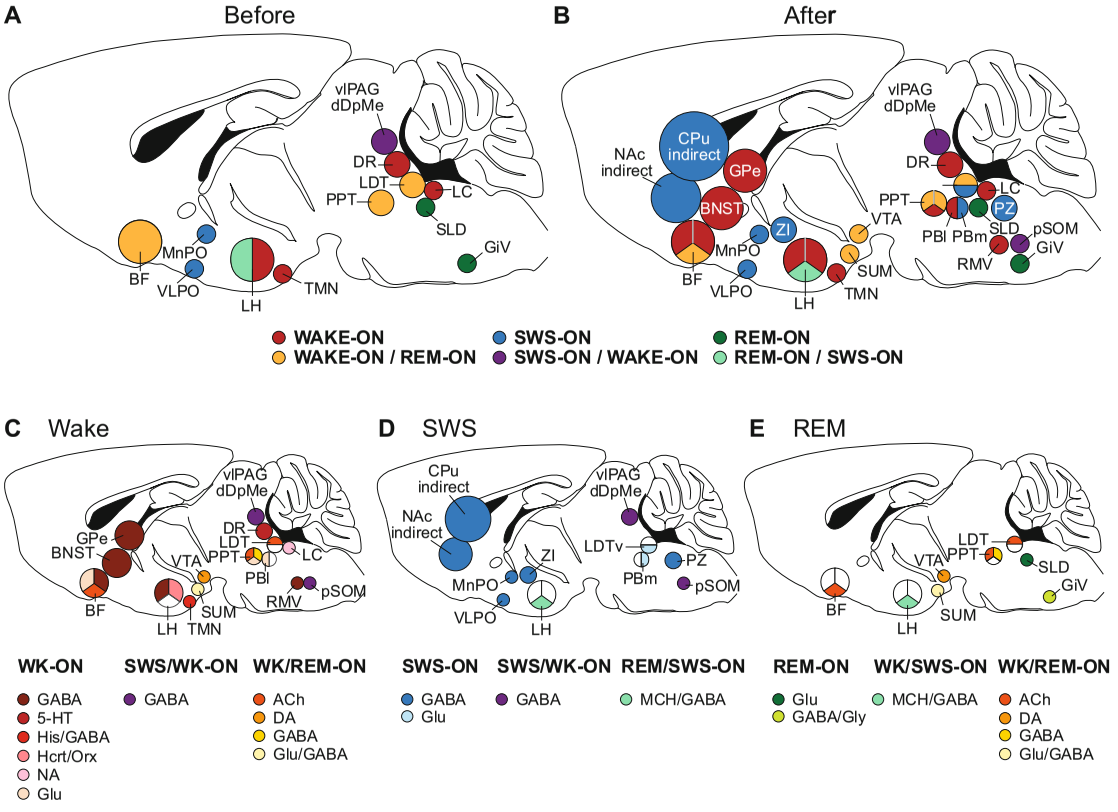

Fig. 1 Schematic summary of sleep-/wake-related structures evidenced by chemogenetics. Here are displayed most of the structures involved in brain state transitions between waking and sleeping states before (**a**) and after (**b**) chemogenetic applications. Wake-, SWS-, and REM sleep-promoting nuclei originating from the classical model of sleep/wake regulation (see for reviews Brown et al. 2012 and Luppi et al. 2017) and chemogenetically identified or refined structures are subdivided between specific state-promoting profiles (wake, red; SWS, blue; REM sleep, green) and multiple state-promoting profiles such as wake- and REM sleep-promoting systems (orange), wake- and SWS-associated nuclei (purple), and SWS- and REM sleep-related neurons (teal). In (**c**–**e**), nuclei associated with either wake (**c**), SWS (**d**), or REM sleep (**e**) are depicted in regard to their neurochemical nature labeled with individual color code

including optogenetic and DREADD were developed. Thanks to these approaches, our understanding of sleep circuits integrating multiple GABAergic systems has been considerably revised for a predominant role of various GABA neurons in the control of the sleep-wake cycle (see for reviews Brown and McKenna 2015; Luppi et al. 2017) (Fig. 1). We review here the different studies using DREADD approaches on various GABAergic populations that evidenced a role for GABA neurotransmission in the enhancement and maintenance of both sleep and wake states (Fig. 1).

3.1.1 GABAergic Sleep-Promoting Neurons of the Preoptic Area

A large body of evidence indicates that GABAergic neurons of the preoptic area (POA) of the anterior hypothalamus play a crucial role in slow-wave sleep (SWS) (Luppi et al. 2017). Indeed the CNO-induced excitation of GABAergic neurons in the whole preoptic area resulted in an increase in SWS amounts during both the diurnal inactive and the nocturnal active photoperiods (Saito et al. 2013). More specifically, a dense population of SWS-active GABAergic neurons were found in the median (MnPO) and the ventrolateral (VLPO) preoptic nuclei, evidenced by a high number of cFos-positive neurons (immediate early gene used as a marker of neuronal activation) correlating with time spent in SWS (Luppi et al. 2017). However, the causal role of these different populations in the MnPO and VLPO in the initiation and maintenance of SWS still needed to be confirmed. Besides, the POA is composed of a mixture of mostly GABAergic sleep-active, wake-active, and state-indifferent neurons (Takahashi et al. 2009). Interestingly, a group of researchers took advantage of this consistent cFos pattern in the POA elicited by sleep enhancement following either total sleep deprivation or sedation, to control the activity of these sleep-active neurons and test their causal role in sedation and sleep (Zhang et al. 2015). To do so, Zhang and colleagues employed a Fos-TRAP/TetTag-DREADD approach. This approach consists in the expression of a cFos-promoter inducible hM3DGq gene selectively in the POA, MnPO, or lateral POA (LPO), by TetTagging (Reijmers et al. 2007). More specifically, hM3DGq gene is only turned on following cFos expression induced by neuronal activation that in turn drives tTA expression. Temporal control over tTA-TRE recombination, and thus cFos-dependency, is achieved through doxycycline administration and removal. As a result, neurons that have been activated in vivo by a stimulus are tagged with hM3DGq and can be specifically reactivated later by systemic CNO injection. To examine the causal relationship between these neurons and sleep, TetTagging is performed during recovery sleep following total sleep deprivation or during sedation induced by the adrenergic α2 agonist, dexmedetomidine. Importantly, reactivating either sleep rebound-tagged or sedation-tagged neuronal populations produces sustained SWS, 20 min after CNO injections, together with a lower body temperature suggesting that α2 adrenergic-induced sedation and recovery sleep share homologous hypothalamic circuitry (Zhang et al. 2015). Single-cell RT-qPCR revealed that a majority of TetTagged POA neurons are GABAergic (84%) and the remaining cells glutamatergic. This study brings the proof of concept that some MnPO and LPO GABAergic neurons are not only passive sleep-active neurons but are indeed major effectors in SWS induction. In another study, downstream targets of POA

GABAergic neurons were further examined with optogenetics highlighting wake-active histaminergic (His) neurons of the tuberomammillary nucleus (TMN) as a major target to inhibit in order to elicit SWS (Chung et al. 2017). In agreement with this observation, acute optogenetic silencing of TMN His neurons rapidly induces SWS (Fujita et al. 2017).

The next three discoveries using DREADDs on GABAergic neurons allowed to identify three novel systems involved in the promotion or facilitation of sleep in mice.

3.1.2 GABAergic Sleep-Promoting Neurons in the Rostral Medulla

Early experiments 60 years ago suggested that the caudal brainstem contains SWS-promoting system (see for review Anaclet and Fuller 2017). While most studies focused on the rostral hypothalamus and in particular the POA as a major structure at the core of sleep onset and maintenance mechanisms since von Economo's first observations (Economo 1930), seminal work proposing the hypothesis of a brainstem sleep system was dismissed until recently and further examined with DREADDs. The newly identified neuronal ensemble located lateral and dorsal to the facial nerve in the rostral medullary brainstem, named the parafacial zone (PZ), was first evidenced by a state-specific cFos pattern related to sleep (Anaclet et al. 2012). Those sleep-active neurons express the vesicular GABA/glycine transporter (VGAT) also known as vesicular inhibitory amino acid transporter (VIAAT). Using transgenic *VGAT-cre* mice in combination with cre-dependent viral vector carrying DREADDs, Anaclet and colleagues challenged both the sufficiency and necessity of PZ GABAergic neurons to control sleep-wake states (Anaclet et al. 2014). Upon CNO-mediated activation of hM3DGq-expressing GABAergic neurons in the PZ, animals quickly express a predominance of SWS state within the first hour during their nocturnal active period, lasting for 3 h. This increase of SWS was observed at the expense of both wakefulness and REM sleep and is associated with increased cortical slow-wave activity (Anaclet et al. 2014). Reciprocally, CNO-induced inhibition of hM4DGi-expressing PZ neurons during the inactive diurnal period rapidly and strongly decreases SWS (Anaclet et al. 2014). One puzzling result harvested in this study is the consistent inhibition of REM sleep observed following both the activation and the inhibition of PZ GABAergic neurons. This effect remains unclear and could depend on parallel circuits impacting on REM sleep initiation and its EEG features. However this effect coupled to the absence of REM sleep rebound also questions the maintenance of a natural sleep physiological regulation after CNO-induced activation or inhibition of PZ neurons.

Using optogenetic-mediated circuit mapping, Anaclet and colleagues dissected the circuitry underlying PZ GABAergic promotion of SWS. They observed that neurons in the parabrachial nucleus (PB) projecting to the magnocellular basal forebrain (BF) receive monosynaptic inhibitory inputs from GABAergic PZ neurons and that BF neurons projecting to the dorsomedial prefrontal cortex (dmPFC) receive monosynaptic excitatory inputs from glutamatergic PB neurons (Anaclet et al. 2014). Previous work performed by the same group also demonstrated that the PB to BF pathway is critical for the desynchronization of the cortical EEG (Fuller

et al. 2011). This follow-up study shows a direct inhibitory control of PZ VGAT neurons onto PB neurons projecting to the BF (Anaclet et al. 2014). Thus, the authors hypothesize that the PB inhibition applied by PZ neurons would decrease the major excitatory inputs to dmPFC-projecting neurons in the BF that would in turn modulate cortical activity and result in disrupted arousal. This brainstem-forebrain-cortex pathway involving medullary release of GABA would act in parallel to the reticulo-thalamo-cortical pathway (Llinas and Steriade 2006) to control behavioral and electrocortical arousal. However the causal relationship of this series of inhibitions and activations of different brain structures and neuronal subpopulations occurring cooperatively to control behavioral arousal remains to be established.

3.1.3 Subthalamic GABAergic Control of Sleep

Another study investigating the organizational complexity of the diencephalon highlighted a subset of GABAergic neurons located in the ventral zona incerta (ZI) in the control of sleep (Liu et al. 2017). This subset of GABAergic neurons is characterized by the expression of a LIM homeodomain transcription factor gene, Lhx6, early in development. Using a Lhx6-cre transgenic mice, the researchers confirmed the presence of this marker in GABAergic neurons co-expressing GAD1 and VGAT, distinct from Hcrt/Orx and MCH neurons, in a zone extending from the ventral ZI through the dorsomedial (DMH) and lateral (LH) hypothalamus to the posterior hypothalamus (Liu et al. 2017). The first link associating ZI Lhx6/GABAergic neurons activity with sleep was shown by the circadian distribution of cFos staining in ZI Lhx6 neurons with increased cFos levels correlating with circadian times associated with high sleep pressure (Liu et al. 2017). However, puzzling results were obtained on cFos levels after a 6-h sleep deprivation alone or in combination with 1 h of recovery sleep. Both conditions elevate cFos in ZI Lhx6 neurons in a similar manner, which does not discriminate whether cFos pattern reflects purely wake- or sleep-active neurons or neurons integrating sleep pressure (Liu et al. 2017). Using DREADD, Liu and colleagues directly assessed whether the manipulation of ZI Lh6x GABAergic neuron signaling modulates sleep. In vivo, the selective activation of hM3DGq or hM4DGi receptors in ZI Lh6x neurons affects SWS amounts in a bidirectional manner, promoting or reducing SWS, respectively (Liu et al. 2017). However these effects are observed from 4 to 6 h after CNO injection during the diurnal inactive period. These late-onset effects are surprising and could be interpreted as unspecific or attributed to CNO conversion to clozapine (see Sect. 2.3). However, the CNO dose used here is among the lowest (0.5 mg/kg) which would dismiss any major effects of late interconversion of CNO to clozapine. In addition, CNO injection in control DREADD-free animals was reported to be inert. More immediate effects are observed when CNO is delivered during the active nocturnal period enhancing SWS with increased delta power. However, inconsistent results were found on REM sleep: no effect was observed when CNO was injected during the active phase, while a bimodal effect occurred when CNO was administered during the inactive period characterized by an immediate inhibition

followed by long-lasting enhancement of REM sleep. Further connectivity studies would be necessary to interpret these effects on REM sleep.

An alternative way to interpret these results would be that ZI Lh6x neuron activation is related to the integration of sleep pressure and sleep need. This would explain the late-onset SWS-promoting effects observed after neuronal excitation as well as the absence of increase in SWS amounts in the second half of the diurnal inactive period, after the inhibition of Lhx6 neurons. Experiments involving total sleep deprivation and inhibition of ZI Lh6x neurons during sleep deprivation or immediately in the beginning of the recovery period would better address this view. Moreover, mapping experiments show that ZI Lh6x neurons form an interconnected local network, which could partly explain slow-onset for SWS enhancement, implying that other ZI GABAergic neurons would be involved in either wake-promoting processes or REM suppression (Liu et al. 2017). However, another study showed that global DREADD activation of ZI VGAT-expressing neurons does not exhibit any increase in wakefulness following CNO injections at 0.3 mg/kg (Venner et al. 2016).

Tracing experiments indicated that ZI Lh6x neurons receive inputs from various brain structures both unrelated and related to the control of sleep-wake cycle (Liu et al. 2017). Among these structures, Liu and colleagues focused on reciprocal connections that ZI Lh6x neurons share with the lateral hypothalamus (LH). They observed that both GAD2-expressing GABAergic cells and Hcrt/Orx neurons of the LH are directly inhibited after ZI Lh6x optogenetic activation (Liu et al. 2017). As a result, they hypothesized that part of the mechanisms involved in ZI Lh6x-driven enhancement of sleep requires inhibition of the wake-promoting neurons of the LH. Consistently, direct inhibition of a subpopulation of GABAergic (VGAT-expressing) cells in the LH induces SWS, mainly through a disinhibition of the thalamic reticular nucleus (Herrera et al. 2015). Moreover, inhibition of LH GAD2-expressing neurons reduces physical activity and locomotion (Kosse et al. 2017). However, overall, modest effects result from ZI Lh6x DREADD activation considering that CNO-induced enhancement of sleep occurs with delay and is limited to increased amounts but unchanged SWS bout mean duration or wake-to-sleep transitions. Thus, it is unlikely that ZI Lh6x GABAergic neurons act as primary effectors in SWS induction as POA or PZ neurons do.

To apprehend the complexity of diencephalic and mesencephalic neuronal networks regulating sleep-wake cycles, modeling approaches could further inform on the hierarchical organization of these parallel circuits and their dynamics and could in particular better identify and position the role of ZI Lh6x GABAergic neurons and their relative involvement in sleep promotion of sleep pressure mechanisms. Besides, further investigations are necessary to track down functional diversity among ZI GABAergic neurons.

3.1.4 Sleep Control in the Basal Ganglia

At last, by investigating the mechanisms by which adenosine promotes sleep (Bjorness and Greene 2009), two groups of researchers recently identified alternative pathways in the basal ganglia (BG) circuitry necessary and sufficient to generate

slow-wave activity and a behavioral state resembling sleep using DREADDs (Oishi et al. 2017; Yuan et al. 2017). The BG is composed of four major nuclei including the striatum (caudate and putamen, CPu), which is the primary input nucleus, globus pallidus (GP), subthalamic nucleus (STN), and substantia nigra pars reticulata (SNr) as the major output nucleus. The BG is strongly interconnected with the cortex, thalamus, and midbrain structures including the DA-enriched ventral tegmental area (VTA) and substantia nigra pars compacta (SNc), as well as many other brain areas from the limbic system (Graybiel 2008). The striatum is divided into three territories, a dorsolateral (DLS), a dorsomedial (DMS), and a ventral domain also named nucleus accumbens (NAc), associated with distinct functions in regard to their cortical and limbic inputs (Graybiel 2008). These subdivisions, topographically connected with cortical and subcortical structures, carry information related to sensorimotor, associative, and limbic functions, respectively. The striatum is seen as a hub orchestrating BG functions including action control, skill learning, habit formation, incentive motivation, and goal-directed behaviors (Graybiel 2008; Balleine and O'Doherty 2010; Jin and Costa 2010). The BG activity during sleep-wake states has been largely underexplored, while dysfunction of the striatum is frequently associated with sleep disturbances (see for review Lazarus et al. 2012). Some studies indicated two major points: (1) several distinct firing patterns in BG nuclei across the sleep-wake cycle (Magill et al. 2000; Urbain et al. 2000) and (2) activity changes in striatal neurons from cyclic, fast active firing to highly irregular firing during sleep-to-wake transition (Mahon et al. 2006), suggesting distinct striatal processing depending on arousal state. In addition, the striatum is composed predominantly of GABAergic medium spiny output neurons (MSNs) divided into two populations: those expressing dopamine D_1-receptors, directly projecting to the globus pallidus pars interna (GPi)/SNr, and those expressing adenosine A_{2A} and dopamine D_2 receptors, projecting to the globus pallidus pars externa (GPe) involving multiple nodes down to the SNr (Durieux et al. 2011). These two neuronal subpopulations form the "direct" (dMSNs) and "indirect" (iMSNs) pathways of the BG thought to act concomitantly but antagonistically during action selection to execute appropriate motor and cognitive responses (see for reviews Balleine and O'Doherty 2010; Jin and Costa 2010). In this context, the recent studies using DREADDs to tackle the role of A_{2A}-expressing iMSNs, known as action suppressors, in the control of vigilance states further challenge the existence of parallel sleep-wake regulatory pathways and question the function of BG in sleep as well as the role of the hypnogenic factor adenosine (Oishi et al. 2017; Yuan et al. 2017).

Using hM3DGq and hM4DGi cre-dependent viral vectors targeting A2A-Cre-expressing iMSNs in the striatum, two groups of researchers observed that DREADD control of iMSNs located in the core region of the NAc (Oishi et al. 2017) and in the dorsal striatum in its rostral extension (Yuan et al. 2017) influences sleep in a bidirectional manner. DREADD hM3DGq activation of iMSNs in both striatal regions results in the promotion of a state resembling SWS, characterized by slow and high-voltage EEG. Reciprocally, hM4DGi-dependent inhibition of iMSNs reduces SWS amounts. Previous work examining the effects of local infusion of A_{2A}

agonist showed that adenosine-mediated activation in the ventral striatum elicits both SWS and REM sleep in rats (Satoh et al. 2006). However, here, DREADD hM3DGq activation of both ventral and dorsal iMSNs promotes SWS without affecting REM sleep amounts. Subtle differences are also observed between the two striatal subdivisions on SWS facilitation evoked by A_{2A}-iMSNs activation, which is underlined by an increase in the number of SWS episodes with NAc core activation (Oishi et al. 2017) and, on the other hand, by a lengthening of SWS episodes with rostro-dorsal activation (Yuan et al. 2017).

Both DREADD-driven effects on sleep increase are recapitulated by optogenetic stimulation of iMSNs in the NAc core (Oishi et al. 2017) or stimulation of dorsal iMSN terminals in the GPe (Yuan et al. 2017). However, kinetics of effects between optogenetics and DREADD activation importantly differ when targeting NAc core iMSNs (Oishi et al. 2017). The optogenetic-induced sleep-like state is almost immediate, while CNO-promoting effects appear only in the second hour after i.p. injection. In comparison, sleep induction resulting from hM3DGq activation of PZ neurons occurs rapidly after 15–20 min following injection for a similar dose of CNO (Anaclet et al. 2014; Oishi et al. 2017). This comparison cannot be made with DREADD activation of dorsal iMSNs as the dose of CNO employed was higher. Importantly, CNO-mediated activation of hM3DGq-expressing iMSNs was found to increase the response to depolarizing stimuli but does not induce any spiking per se (Alcacer et al. 2017). Unlike optogenetics (Oishi et al. 2017), DREADD activation of iMSNs through hM3DGq pathway likely increases the neuronal excitability but does not excite neurons, which still requires further in vivo confirmations. However, it might explain the differences in CNO versus optogenetic kinetics. In contrast, DREADD-dependent Gi inhibition of NAc core iMSNs seems to induce a reduction of SWS amounts more rapidly within the first hour following i.p. injection (Oishi et al. 2017). Interestingly in both regions, NAc core and rostro-dorsal striatum, presumed inhibition of hM4DGi-expressing iMSNs decreases SWS amounts for at least 4 h without inducing (Oishi et al. 2017; Yuan et al. 2017) or affecting homeostatic sleep rebound after sleep deprivation (Oishi et al. 2017), suggesting that rebound mechanisms may involve different pathways.

As a result, the involvement of the striatal indirect pathway in sleep induction is puzzling. Oishi and colleagues proposed that NAc iMSNs would act ahead of POA sleep-active neurons upon the influence of adenosine levels, by depressing the arousal circuitry in BF, which, in turn, would release POA sleep-promoting system. On the other hand, Yuan and colleagues were also able to promote SWS by directly inhibiting with DREADD the downstream target neurons of dorsal iMSNs in the GPe that are also inhibitory cells expressing parvalbumin (PV). Moreover, the selective lesion of PV neurons in the GPe abolished the increase in SWS caused by DREADD activation of iMSNs (Yuan et al. 2017), suggesting that the activation of A_{2A}R neurons in the striatum inhibits GPe PV neurons to promote SWS. These alternative circuits emerging from the striatum require further investigations notably including in vivo monitoring of iMSNs activity across the natural sleep-wake cycle in addition to tracing studies from the NAc core and GPe to further support their role in SWS induction. Interestingly, sleep-wake cycle is not altered by photostimulation

of iMSNs in the shell region of the NAc (Oishi et al. 2017) or by DREADD activation of the caudal part of the dorsal striatum (Yuan et al. 2017), suggesting specialized functions in the different striatal territories for the modulation of vigilance states.

It is important to keep in mind that the primary functions of the striatum are critical for behaving animals and thus arousal, in order to process multiple signals and execute motor control and action selection. Even if it does not necessarily imply that these neurons cannot have a role in sleep, the papers mentioned above focused their analysis on sleep-related parameters. However sleep and wake patterns are organized in a continuum and should not be considered as segregated events. The DREADD activation of NAc core iMSNs induces a concomitant reduction of locomotor activity (Oishi et al. 2017), but wakefulness amounts and waking EEG pattern have not been reported to further inform on mice levels of arousal or alertness. Reciprocally, DREADD inhibition of the NAc indirect pathway was found to increase ambulatory activity (Carvalho Poyraz et al. 2016), and DREADD inhibition of iMSNs in the dorsal striatum is only efficient in increasing wake during the active dark period (Yuan et al. 2017). However, activation of the latter neurons is also accompanied by decreased wake amounts with shortened mean duration (Yuan et al. 2017). This mixture of behaviors is critical when the dorsal striatopallidal pathway is involved as its activation through DREADD was also found to control general motor output (Alcacer et al. 2017). Therefore, it is still questionable whether the effects observed here reflect SWS induction or loss of motor control progressively driving the animals to enter into an immobile quiet state of wakefulness harder to distinguish from sleep. Alternatively, the effects described above could depend on an inhibition of wake maintenance mechanisms, which would ultimately and indirectly facilitate sleep onset. For future investigations, it is capital to examine closely iMSNs activity from distinct striatal subdivisions during spontaneous sleep-wake states to better characterize a potential role in sleep promotion and wake maintenance and challenge the influences of the direct pathway by opposition. In addition to the deconstruction of state transitions, it would be interesting to address the functions of BG in sleep that could be relevant to learning and memory processes.

3.1.5 GABA: Not Just a SWS Driver

GABA was first considered to act as the principle driver for SWS by silencing waking systems. However, recent findings using optogenetics and DREADD highlighted a more complex role for GABA neurotransmission in all vigilance states. DREADD approaches contributed to distinguish two types of GABA cells with the primary function to either promote waking or repress REM sleep, located in the hypothalamus, in the BF and cortex, or in the brainstem, respectively (see for review Luppi et al. 2017). The contributions of GABA from the BF in various features of waking will be addressed in a following section reviewing the role of ACh (see Sect. 3.3.2). Cortical GABAergic interneurons, principally PV neurons, have been shown to exert a critical role in the generation of gamma rhythm during waking, which was mainly investigated and recapitulated with optogenetics (see Adamantidis and Lüthi 2018). We will thus review here three papers using DREADDs highlighting the

diverse role of GABA in different brain regions (Fig. 1): promoting wake through the hypothalamus in the LH (Venner et al. 2016) and in the bed nucleus of the stria terminalis (BNST) (Kodani et al. 2017) and suppressing REM sleep in the ventromedial medulla (Chen et al. 2017).

Wake-Promoting GABAergic Neurons in the Hypothalamus

Optogenetics identified for the first time a subpopulation of GABAergic neurons in the LH, targeted by the expression of VGAT transporter in a *VGAT-IRES-Cre* line, responsible for a rapid switch from SWS to waking state involving a direct control of the thalamic reticular nucleus (TRN) as well as on the locus coeruleus and the periventricular thalamus (Herrera et al. 2015). Interestingly, using DREADDs in a similar transgenic *VGAT-IRES-Cre* mouse, Venner and colleagues described a GABAergic neuronal subpopulation in the LH qualitatively displaying a similar projection pattern in the POA and the pontine tegmentum but lacking axonal innervation of the TRN (Venner et al. 2016). Some slight differences in the localization of transfected neurons could explain such a difference: GABAergic cells in a more dorsal position within the LH and may be overflowing in the ZI could preferentially innervate the TRN. However an extensive description of the transfected area is missing to support this hypothesis or even formulate an alternative one. Despite these anatomical considerations, CNO-mediated activation of hM3DGq-expressing GABAergic neurons in the LH results in sustained waking, whereas the inhibition of the same neurons increases SWS amounts. The wake-facilitating effects of the stimulation of these GABAergic neurons could be mediated through their dense innervation of the POA and in particular the VLPO. However, previous work described that VGAT-expressing neurons in the LH fire maximally during SWS or REM sleep (Hassani et al. 2010) or increase their firing transiently after the transition from SWS to wake (Herrera et al. 2015). Moreover, a partially distinct population of GABAergic cells expressing GAD65 (GAD2) were found to be involved in stress-induced increase in locomotion downstream of Hcrt/Orx neurons (Kosse et al. 2017). All these results question the cellular complexity and heterogeneity of GABAergic neurons in the LH that serves as a major hub in multiple physiological responses and in particular where numerous allosteric factors affecting sleep-wake architecture could be integrated (see for reviews Bonnavion et al. 2016; Herrera et al. 2017).

One of these allosteric modulations could arise from the BNST, which is a constituent of the extended amygdala and plays a key role in stress responses and anxiety (Lebow and Chen 2016). Indeed, GABAergic GAD67 (GAD1)-expressing neurons of the BNST send dense axonal projections to the LH as well as to other brain areas involved in waking control such as the TMN, the locus coeruleus (LC), the dorsal raphe nucleus (DRN), or the dorsal deep mesencephalic reticular nucleus (dDpMe) (Kodani et al. 2017). The acute activation of these neurons using optogenetic stimulation during SWS episodes or a more sustained activation using DREADD tools resulted in increased wakefulness associated with an increased activation of Hcrt/Orx neurons in the LH and noradrenergic neurons in the LC quantified through cFos immunodetection. Interestingly, the arousal effects induced

by DREADD-mediated activation of GABAergic BNST neurons was reversed by the administration of the dual Hcrt/Orx receptor antagonist DORA-22, whereas it was not the case for the acute optogenetic activation of the same neurons (Kodani et al. 2017). Thus, GABAergic BNST neurons could play a key role in the SWS-to-wake transition without recruiting Hcrt/Orx neurons, while their sustained activation would involve Hcrt/Orx neurons to maintain waking. Additionally, the axonal projection of GABAergic BNST neurons to the LH was previously found to strongly drive food consummatory behaviors (Jennings et al. 2013). Thus, BNST neurons could provide a powerful allosteric modulation of LH neurons and potent other brain structures to drive and maintain arousal required for food-related behavior completion.

GABAergic Control of Waking in the Ventral Medulla

Multiple studies over the years highlighted the importance of GABAergic/glycinergic (GABA/Gly) neurons in the ventral medulla in the control of muscle atonia associated with REM sleep potentially acting as inhibitory premotor neurons through their descending projections to the spinal cord (Fraigne et al. 2015).

Recently, one paper using DREADD probed the role in the control of wakefulness and REM sleep atonia of GABA/Gly neurons in the ventromedial medulla at different levels in the rostrocaudal extent of the medulla (Chen et al. 2017). Chen and colleagues studied in parallel two portions of the ventromedial medulla: the ventromedial medulla rostral to the inferior olive (pSOM) and the rostral ventromedial medulla (RMV). Anatomically, RMV comprises the rostral raphe magnus (RMg), the raphe interpositus (RIP) nucleus, and the medial portion of the rostral gigantocellular reticular nucleus (Gi), whereas pSOM comprises the caudal part of the RMg, a centromedial portion and pars alpha of the Gi (GiA), and the rostral raphe obscurus nucleus (ROb). Diphtheria toxin-mediated ablation of either pSOM neurons or RMV neurons does not produce significant alteration in the sleep-wake architecture. However, lesions of the pSOM area cause an increase in phasic brief muscle activity during REM sleep recorded in the dorsal neck muscles without affecting tonic atonia. This suggests a role of pSOM neurons in the repression of twitches-like events during REM sleep. DREADD-mediated activation of RMV neurons whatever their neurochemical identity in rats induces increase in waking at the expense of both SWS and REM sleep, whereas activation of pSOM neurons unexpectedly induces a suppression of REM sleep compensated by a non-significant increase in waking and associated with a non-significant decrease in SWS. No effect was observed on REM sleep atonia or twitches-like events during REM sleep. The effect of pSOM neurons activation in rats was replicated in mice by specifically targeting DREADD expression in GABA/Gly neurons in the pSOM area. The above results reinforce the critical contribution of some GABA/Gly neuronal subpopulations in the control of REM sleep atonia and wake entry. It also contributes to highlight the complexity of neuronal networks controlling either REM sleep or REM sleep atonia in the ventral medulla. Indeed one population of GABA/Gly neurons in the ventral medulla located mostly in the lateral paragigantocellular nucleus (LPGi) and in the most ventral portion of the caudal Gi was found to be important in the promotion of REM sleep through ascending projections to the

ventrolateral periaqueductal gray (Weber et al. 2015). Conversely the suppression of GABA/Gly neurotransmission in adjacent neurons in the GiA, GiV and RMg causes a loss of muscle atonia during REM sleep associated with increased twitches mimicking symptoms of REM sleep behavior disorder (RBD) (Valencia Garcia et al. 2018). The upstream neurons of these systems controlling REM sleep onset or REM sleep atonia would be the glutamatergic neurons of the sublaterodorsal nucleus (SLD), the main REM sleep-promoting center, whose loss-of-function impedes REM sleep and produces similar RBD-like phenotype (Valencia Garcia et al. 2017).

3.2 Glutamatergic Systems

Multiple glutamatergic (Glu) neuronal populations were identified to contribute to the regulation of vigilance states. In comparison with the GABAergic systems that roughly facilitate sleep occurrence, Glu neurons hold a more versatile position (Fig. 1).

3.2.1 Glutamatergic Neurons in the Diencephalon

A first group of Glu neurons whose role in sleep-wake control has been evaluated using DREADD is located in the supramammillary nucleus (SUM) (Pedersen et al. 2017). Located in the caudal hypothalamus, the SUM exerts strong modulatory effects on the hippocampal formation and temporal cortex, in particular in relation to the control of theta rhythms (Thinschmidt et al. 1995). These neurons seem to play important roles during wakefulness in the control of anxiety-related behaviors, physiological stress, and defensive actions (see for review Pan and McNaughton 2004). In relation with sleep-wake regulation, SUM neurons strongly express the immediate early gene cFos after a REM sleep hypersomnia following REM sleep deprivation using the platform-over-water method and seem to be important for the activation of the dentate gyrus (DG) associated with REM sleep hypersomnia and theta rhythm during REM sleep (Renouard et al. 2015). Importantly, the majority of DG-projecting SUM neurons might be able to release both Glu and GABA (Soussi et al. 2010; Billwiller et al. 2017).

Chemogenetic interrogation of the role of SUM neurons in sleep-wake control was performed by injecting AAVs carrying cre-dependent DREADD constructs in the posterior hypothalamus of *VGlut2-cre* mice (Pedersen et al. 2017). The resulting transfected area indeed covered the entirety of the SUM but also extended more caudally in some portions of the posterior hypothalamus and the lateral hypothalamus. CNO-induced stimulation of hM3DGq-transfected neurons results in sustained wakefulness lasting from 6 to 10 h. This effect is almost completely abolished when knocking down VGlut2 in hM3DGq-expressing neurons. The remaining wake-promoting effects could be mediated through GABA release from the same neurons as CNO-induced stimulation of VGAT-expressing neurons produces similar effects. However, the size of transfection sites and the atypical absence of sleep rebound following prolonged wakefulness might raise the question about the specificity of the effects observed. Moreover, activation of DREADD-transfected Glu neurons

increases theta rhythms, which is consistent with previous reports (see for review Pan and McNaughton 2004), and promotes gamma oscillations. The latter observation should be interpreted with caution as EEG gamma rhythms can be easily contaminated by muscle activity (Buzsáki and Schomburg 2015). Conversely, CNO-induced inhibition of Glu neurons in a large area covering the SUM only produces slight effects with a small increase in sleep amounts and number of sleep episodes (Pedersen et al. 2017). Spectral analysis also indicates a reduction in theta oscillations during wake and a slowdown of theta rhythm during REM sleep that are consistent with previous observations during wake or REM sleep after deletion of SUM neurons (Pan and McNaughton 2004; Renouard et al. 2015).

Based on the contributions of SUM neurons in anxiety-related behaviors, stress, and defensive responses, the nature of the evoked-awakening and arousal state resulting from SUM DREADD-driven excitation remains unclear, whether it could actually be associated with high stress levels and anxiety. As with other arousal systems such as the Hcrt/Orx neurons (Bonnavion et al. 2015) and LC noradrenergic cells (McCall et al. 2015), sustained activation of such system with either semi-chronic optogenetic photostimulation or DREADD-driven activation might engage stress responses. However, considering the extension of the viral diffusion here, some of these neurons could also correspond to numerous posterior hypothalamic neurons, distinct from His neurons, displaying either a wake-active or a wake/REM sleep-active activity profile (Takahashi et al. 2006). Further investigations examining the activity profile of these neurons with particular regard to theta rhythm modulation during active wake and also during REM sleep would help in identifying the role of SUM in sleep/wake regulation. Considering the diversity of SUM neurons, anatomical studies of their respective connectivity would also further precise their position in sleep-wake regulatory networks.

3.2.2 Glutamatergic Control of Sleep-Wake Cycle in the Pontine Tegmentum

Glutamatergic neurons of the pontine reticular formation have a determinant role in sleep-wake state control (see for review Watson et al. 2011). Alongside the reticular formation, more defined nuclei containing REM-sleep-active Glu cells such as the laterodorsal (LDT) and pedunculopontine (PPT) tegmental nuclei and the sublaterodorsal nucleus (SLD) arose (see for review Brown et al. 2012). More recently, Hayashi and colleagues identified a subpopulation of neurons that derive from the cerebellar rhombic lip (a part of the developing hindbrain), transiently expressing the transcription factor Atoh1 at embryonic day 10.5 (E10.5), and that migrate to the pontine tegmentum (Hayashi et al. 2015). Atoh1 cells are either cholinergic or Glu neurons. A specific targeting of Atoh1 neurons was achieved by crossing *Atoh1-CreER*^{T2} mice with mice carrying a Cre-dependent tetracycline transactivator (tTA) transgene (*CAG-LSL-tTA*). Cre recombination and tTA expression in Atoh1 neurons were obtained with tamoxifen administration at E10.5 (Hayashi et al. 2015). AAVs carrying a tTA-dependent hM3DGq transgene were subsequently infused in either the medial or the lateral portion of the Atoh1 cell field in adult offspring. Thus, medial Atoh1 cells are localized in the ventral LDT and

medial parabrachial nucleus (PBm), and lateral Atoh1 cells are localized in the lateral portion of the PB (PBl). At the physiological level, in an extensively controlled experimental design, the authors observed that CNO-induced stimulation of medial or lateral Atoh1 cells increases SWS amounts and inhibits REM sleep or enhances wakefulness at the expense of both SWS and REM sleep, respectively (Hayashi et al. 2015). These effects seem to be mediated by Glu Atoh1 cells as the exact same effects were reproduced in transgenic mice obtained by crossing *Atoh-CreER*^{T2} mice with either *CaMkIIa-LSL-tTA* or *VGlut2-LSL-tTA* mice, in which tTA expression only occurs in Glu cells from the Atoh1 lineage (Hayashi et al. 2015). This nicely designed study led to the identification of two subpopulations of Glu cells that derive from the same developmental lineage and contribute to two distinct and yet complementary aspects of sleep regulation: lateral Atoh1 neurons that favor wake at the expense of sleep and medial Atoh1 neurons that facilitate SWS at the expense of REM sleep. These two subpopulations would act as gatekeepers of sleep and REM sleep, respectively.

Furthermore, Hayashi and colleagues dissected the neuronal pathway responsible for the REM sleep inhibiting effect of Glu medial Atoh1 neurons. They observed that these neurons densely innervate the dorsal deep mesencephalic reticular nucleus (dDpMe), which, alongside ventrolateral periaqueductal gray (vlPAG) GABAergic neurons, project and inhibit SLD neurons to suppress REM sleep (Fort et al. 2009; Sapin et al. 2009). CNO-mediated excitation of GABAergic dDpMe neurons expressing hM3DGq recapitulates the phenotype observed after the stimulation of medial Atoh1 cells, i.e., a decrease in REM sleep and an increase in SWS (Hayashi et al. 2015). Reciprocally, the inhibition of the same neurons expressing hM4DGi facilitates REM sleep onset. The latter effect is thus hypothesized to be mediated by a disinhibition of SLD REM sleep-promoting neurons.

Interestingly, wake-promoting lateral Atoh1 neurons partially overlap with neurons expressing Glu calcitonin gene-related peptide (CGRP) located in the external part of the lateral PB (PBel) (Bester et al. 1997; Yokota et al. 2015) As for Atoh1 lateral cells, the activation of CGRP positive cells using cre-dependent expression of hM3DGq in *CGRP-CreER* mice results in increasing wake at the expense of both SWS and REM sleep (Kaur et al. 2017). Moreover, optogenetic acute activation of these neurons during sleep reduces latency from sleep to wakefulness. PBel CGRP neurons, which are strongly activated in the context of hypercapnia (Yokota et al. 2015), appear to mediate their awakening signal through multiple excitatory projections into the central nucleus of the amygdala, the BF, and the LH. Altogether, these findings can be quite relevant to study circuit dysfunctions associated with obstructive sleep apnea or developmental defects that might occur in sudden infant death syndrome (SIDS) involving reduced CO_2 responses.

Finally, DREADD-mediated stimulation of a larger proportion of the PB complex was found to produce sustained wakefulness for more than 10 h (Qiu et al. 2016a). Similar wake-promoting effects are partially recapitulated by using a retrograde-driven expression of hM3DGq receptors in PB neurons projecting to the POA and the BF or projecting to the lateral and posterior hypothalamus. Conversely, no effect

is observed by stimulating PB neurons projecting to the midline and intralaminar thalamus (Qiu et al. 2016a). Strikingly, this sustained wakefulness is not followed by sleep rebound even after 4 days of daily CNO administration. In addition, cFos staining after this subchronic CNO treatment reveals a strong level of activation in many arousal-related centers including the LC, the TMN, the cholinergic BF, and the LH Hcrt/Orx neurons and in sleep-promoting areas, namely, the VLPO or the PZ. Indeed, the PB was found to be the main output structure of the sleep-promoting PZ (Anaclet et al. 2012, 2014) (Sect. 3.1.2). This cFos pattern that resembles both wake and sleep states is quite confusing and leaves open the question of the physiological relevance of this model and the multifaceted role of the PB complex in sleep/wake regulations.

3.3 Cholinergic Systems

Another major group of systems constitutive of the ascending arousal systems include cholinergic (ACh) neurons located in the pedunculopontine (PPT) and laterodorsal (LDT) tegmentum nuclei and in the basal forebrain (BF) (see for review Fort et al. 2009). These neurons are mostly active during both waking and REM sleep in association with cortical activation characterizing these two vigilance states. DREADD approaches brought significant revision on the role of ACh from both the BF and brainstem by highlighting the cell diversity including GABAergic and glutamatergic neurons in these brain areas and their respective contributions (Fig. 1).

3.3.1 Pontine Cholinergic Systems

Localized within LDT and PPT nuclei, ACh neurons in the mesencephalic tegmentum have long been associated with cortical desynchronization during waking and REM sleep (Steriade 2004; Boucetta et al. 2014) through projections to the forebrain and to the brainstem reticular formation. Moreover, acute optogenetic stimulation of ACh cells in either the PPT or the LDT during SWS leads to REM sleep onset within a minute (Van Dort et al. 2015). However ACh neurons are also intermingled with GABAergic and Glu cells that could contribute to sleep architecture, as they are maximally active during waking or REM sleep or both (Boucetta et al. 2014). Recently, one study tried to dissect the respective contributions of ACh, GABAergic, and Glu cells in the PPT using DREADD-mediated cell-specific modulation of their activity (Kroeger et al. 2017). CNO-mediated activation of VGlut2-expressing PPT cells results in increased waking for at least 4 h at the expense of SWS and a complete inhibition of REM sleep. This CNO-induced waking is mostly composed of quiet wake in the nest and is associated with increased levels of anxiety. Conversely, inhibition of the same neuronal group increases SWS, reduces waking, and lets REM sleep amounts unaffected (Kroeger et al. 2017). CNO-mediated excitation of hM3DGq-expressing ACh neurons of the PPT increases light SWS at the expense of deep SWS without affecting either wake or REM sleep amounts in sharp contrast with results harvested using optogenetic stimulation of the same neurons (Van Dort et al. 2015). This discrepancy could be due to a low transfection rate (about 20%) of

cholinergic cells obtained by Kroeger and colleagues. However, in an attempt to reconcile these findings, CNO-induced stimulation of PPT ACh cells could primarily facilitate the appearance of a transition sleep state between SWS and REM sleep, thus gating REM sleep onset (Mandile et al. 1996; Vescia et al. 1996). Eventually, the activation of local GABAergic neurons only mildly reduces REM sleep despite the fact that identified GABAergic cells in the PPT discharge maximally during REM sleep only or during both wake and REM sleep across the spontaneous sleep-wake cycle (Boucetta et al. 2014). Generally, as clearly stated by the authors, the overall results should be interpreted with caution as targeting precisely and specifically a small brain area in mice using viral vectors is challenging, especially in the pontine tegmentum that concentrate multiple nuclei antagonistically regulating the sleep-wake architecture.

3.3.2 Basal Forebrain Control of Sleep-Wake Cycle

Numerous studies pointed out a key role of BF, and especially BF ACh neurons, in the regulation of the sleep-wake cycle in particular in relation to cortical activation during waking or REM sleep (see for review Fort et al. 2009). However it remains unclear which BF neuronal subpopulations contribute to a given vigilance state and how they interact with each other and affect BF projection areas. In addition to ACh neurons, BF contains intermingled Glu and GABAergic cells that are also likely to play pivotal roles in sleep-wake regulation. Over the last few years, multiple studies tried to decipher the relative contributions of these subpopulations using either optogenetics or DREADDs.

In vivo recordings of channelrhodopsin-2-tagged neurons in the magnocellular preoptic area (MCPO) and the nucleus of the horizontal limb of the diagonal band (HDB) confirmed that ACh and Glu BF neurons display higher firing rates during wakefulness and REM sleep than during SWS (Xu et al. 2015). Interestingly, when considering GABAergic cells, two subpopulations with opposite modulation during spontaneous sleep-wake cycles emerged: parvalbumin (PV)-expressing are more active during wake and REM sleep, whereas some somatostatin (SOM)-containing cells would discharge maximally during SWS (Xu et al. 2015). The subsequent optogenetic activation of these subpopulations confirmed the above correlative characterization: stimulation of BF ACh neurons results in increased transition probability from SWS-to-wake or to a lesser extent from SWS-to-REM sleep; stimulation of either Glu or PV-expressing neurons facilitates wakefulness at the expense of SWS; and stimulation of SOM-containing neurons increases SWS at the expense of wakefulness (Han et al. 2014; Xu et al. 2015).

The same deconstruction of BF subpopulation contributions to sleep physiology was also conducted through DREADD-mediated modulation of their activity. CNO-mediated stimulation of ACh neurons in the HDB, the MCPO, the substantia innominata (SI), and the ventral pallidum (VP) does not modify the sleep-wake architecture but decreases slow-wave activity during SWS (Anaclet et al. 2015). Conversely, the same approach restricted to ACh cells in the HDB, the MCPO, and the SI results in increased wakefulness associated with decreased slow-wave activity during SWS (Chen et al. 2016). Moreover, the CNO-driven inhibition of the same

neurons produced the exact opposite phenotype (Chen et al. 2016). DREADD-mediated stimulation of Glu cells in the HDB, MCPO, SI, and VP is inefficient on sleep architecture and mildly decreases delta power during SWS (Anaclet et al. 2015). In contrast, CNO-induced stimulation of GABAergic neurons in the whole BF increases wake during more than 6 h at the expense of both SWS and REM sleep associated with an increase in EEG spectral power in the alpha and gamma bands (Anaclet et al. 2015). The latter effects on gamma rhythms would preferentially involve BF PV-expressing neurons (Kim et al. 2015).

The above studies provided additional clues into the respective contributions of various BF cellular subpopulations into the regulation of sleep-wake architecture as well as their contributions to brain oscillatory activities associated with either wake or SWS. However inconsistent observations were reported and could be dependent on the extent of the brain area considered: some restricted their targeting strategy to the HDB/MCPO region (Xu et al. 2015; Chen et al. 2016), whereas other included the VP (Han et al. 2014; Anaclet et al. 2015). The anatomical delineation of the sleep/wake-related BF may be a critical point, and the question to incorporate a given brain region into the "classical" ascending activating systems should be further addressed and discussed. Indeed, the VP is part of the basal ganglia, and thus its activation can directly influence motivation and locomotor behaviors through the limbic loop (see for review Root et al. 2015). An additional recruitment of VP could explain the long-lasting wake-promoting effect of GABAergic neuron stimulation obtained by Anaclet and colleagues (2015) in comparison with a more localized optogenetic stimulation of PV neurons in the HBD/MCPO (Anaclet et al. 2015; Xu et al. 2015). As a consequence, redefining the respective contributions of the different subregions (i.e., HDB, MCPO, SI, VP) would be a critical point to address. As the projection patterns of VP neurons differ from MCPO/HDB neurons, associating genetic tagging to retrograde mapping strategies would be of great use, in particular in mice in which targeting a small region with viral delivery is highly challenging.

3.4 Monoaminergic Systems

3.4.1 Serotonin

Serotonergic (5-HT) neurons located in the raphe nuclei belong to the ascending arousal system that triggers cortical activation during wake. Indeed, most of 5-HT neurons discharge maximally during wake, decrease their firing rate during SWS, and are silent during REM sleep (McGinty and Harper 1976; Rasmussen et al. 1984; Sakai 2011). As a result, extracellular 5-HT levels are higher during wake than during sleep both in the raphe nuclei and in brain areas targeted by 5-HT neurons (Portas et al. 2000). Nevertheless, 5-HT can also contribute to increase sleep propensity, as first observations showed that either lesions of 5-HT neurons of the raphe system or inhibition of 5-HT synthesis induce a severe insomnia which could be reversed by restoring 5-HT synthesis (Jouvet 1999). Importantly, enhancing 5-HT tone by systemic administration of selective serotonin reuptake inhibitors (SSRIs)

has been repeatedly reported to inhibit REM sleep across species (Slater et al. 1978; Sommerfelt and Ursin 1987; Maudhuit et al. 1994; Monaca et al. 2003). Thus, upon the different approaches used, while 5-HT was first believed to be a neuromodulator of sleep, it is now further thought to promote cortical activation while impeding REM sleep onset during wake. Still its role in sleep-wake regulation remains very unclear, and these differential effects involve complex modalities of action and multiple receptors in various brain structures (Ursin 2002) challenging investigations over 5-HT system.

Despite the development of novel techniques including DREADD and optogenetics, the reassessment of 5-HT role in sleep has been underexplored. Interestingly, the very first in vivo validation using inhibitory DREADD approach was implemented to the entire 5-HT system (Ray et al. 2011). Conditional intersectional genetics were used to switch on expression of the hM4DGi transgene in virtually all serotonergic neurons of *Slc6a4* (5-HT transporter)-*cre* or *Pet1-Flpe* mice (Ray et al. 2011). Acute inhibition of the entire 5-HT system, following CNO i.p. injection (10 mg/kg), induces a dramatic drop in body temperature and disrupts chemoreflex in response to CO_2 elevation. This first in vivo approach validating DREADD inhibitory control applied a unique dose of CNO that is now considered extremely high. However this study acutely shutting down the 5-HT system was aimed to model homeostatic dysfunctions involving significant 5-HT abnormalities occurring in fatal or life-threatening disorders such as in the sudden infant death syndrome (SIDS).

More recently, interesting findings using DREADD and optogenetic approaches have shown that 5-HT neurons of the dorsal raphe (DR) inhibit the pathological intrusion of REM sleep into wake in a mouse model of narcolepsy, highlighting a pharmacogenetic approach for the amelioration of narcolepsy (Hasegawa et al. 2014, 2017).

Narcolepsy, which is characterized by excessive daytime sleepiness and cataplexy, is associated with the loss of Hcrt/Orx neurons (Dauvilliers et al. 2003). Mice lacking Hcrt peptides, Hcrt neurons, or Hcrt receptors recapitulate human narcolepsy phenotypes (Taheri et al. 2002; Hasegawa et al. 2014). In particular, there are two features that these models share with human pathology: inability to maintain consolidated wakefulness and abrupt behavioral arrests with muscle atonia resembling cataplexy. In their first study, Hasegawa and colleagues investigated the primary wake-active target of Hcrt neurons that could mediate the suppression of narcoleptic symptoms and showed that 5-HT cells of the dorsal raphe (DR) and noradrenergic (NA) cells of the LC have differential roles (Hasegawa et al. 2014). First, in narcoleptic mice devoid of Hcrt/Orx receptors, the restoration of Hcrt2-R in DR 5-HT cells prevents cataplexy-like episodes (CLEs) and restores REM sleep amounts, whereas wake fragmentation persists. In contrast, restoration of Hcrt1-R in LC NA neurons corrects wake fragmentation by increasing mean duration of wake episodes and decreasing the number of wake episodes, without affecting CLEs or REM sleep hypersomnia (Hasegawa et al. 2014). Next, to further examine whether activation of 5-HT DR neurons and NA LC neurons could rescue narcoleptic symptoms, Hasegawa and colleagues implemented DREADD approach in another

model of narcoleptic mice in which Hcrt/Orx neurons are specifically ablated (*orexin/ataxin-3* mice). To combine this approach in their model, they used viral vectors stereotaxically injected into the DR or LC carrying DREADD transgene expression under the control of promoters specific to 5-HT or NA neurons (Pet-1 or PRSx8, respectively) of Hcrt/Orx-ablated mice. Importantly, DREADD activation of DR 5-HT neurons and LC NA neurons reverses the occurrence of CLEs and wake fragmentation, respectively, as seen when restoring Hcrt/Orx receptor expression (Hasegawa et al. 2014). This study allowed the identification of two pathways differentially regulating narcoleptic symptoms that highlights a critical role of DR 5-HT neurons in regulating REM sleep excess and CLE prevention, while LC NA system would rather control wakefulness maintenance.

In their latest work, Hasegawa and colleagues further investigated how DR 5-HT neurons mediate the suppression of CLEs (Hasegawa et al. 2017). By reproducing optogenetically the anti-cataplectic effects of DR 5-HT activation in orexin/ataxin-3 mouse model, they showed that stimulation of DR 5-HT fibers directly into the amygdala was sufficient to suppress the occurrence of CLEs, but did not correct REM sleep hypersomnia or wake fragmentation (Hasegawa et al. 2017). The photostimulation of 5-HT terminals also induced decreased expression of cFos in the amygdala in comparison with the non-stimulated condition in narcoleptic mice, suggesting that 5-HT may inhibit the amygdala to suppress CLEs. To further confirm the importance of the inhibition of amygdala activity to prevent CLEs, the authors employed direct DREADD inhibition of amygdala neurons by locally injecting a pair of AAV vectors: AAV-EF1α-DIO-hM4Di-mCherry and AAV-SynI-iCre in orexin/ataxin-3 narcoleptic mice. CNO treatment substantially reduces CLEs in comparison with saline treatment (Hasegawa et al. 2017). Consistently with 5-HT effects, DREADD inhibition of the amygdala does not improve wake fragmentation. In a complementary DREADD approach in these mice, Hasegawa and colleagues expressed the excitatory hM3DGq DREADD transgene in the amygdala and observed that CNO-driven activation of the amygdala significantly increases the occurrence of CLEs without affecting wake fragmentation (Hasegawa et al. 2017). Thus, chemogenetic manipulations of amygdala activity modulate specific symptoms of narcolepsy resembling cataplexy in a bidirectional manner in orexin/ataxin-3 narcoleptic mice. Finally, the authors designed a nice combination of molecular tools with optogenetics to demonstrate the specific involvement of DR 5-HT-amygdala pathway in the suppression of Hcrt-dependent CLEs showing that the anti-cataplectic effects of restoring Hcrt2-R in DR 5-HT neurons were blocked with the optogenetic inhibition of the amygdala in narcoleptic mice lacking both Hcrt receptors (Hasegawa et al. 2017).

In narcoleptic patients as well as in narcoleptic dogs and mice, cataplexy is most often triggered by positive emotions (Dauvilliers et al. 2003; Taheri et al. 2002). The DREADD results obtained in this study further link the amygdala, which is important for emotional processing, to the complex physiopathology of narcolepsy. It also suggested that the control of 5-HT on the excess of REM sleep and cataplexy in narcoleptic mice involves distinct pathways and mechanisms, as DREADD activation of DR 5-HT improved both excessive REM sleep duration and CLEs in

narcoleptic mice but optogenetic stimulation of 5-HT fibers in the amygdala or direct DREADD activation of amygdala neurons only modulated CLEs. It still poses certain questions on the nature of the anti-cataplectic actions of 5-HT in the amygdala whether it might be associated to fear or reward processing. The amygdala is a complex structure characterized by a central nucleus and a basolateral complex with diverse functions, responding to both aversive (fear) and positive (reward) signals (see for review Janak and Tye 2015). It remains discussed whether 5-HT neurotransmission in the amygdala participates to the acquisition of cued conditioned fear (see for review Bauer 2015), but it still questions whether 5-HT-mediated suppression of CLEs in the amygdala could be produced by fear. Further investigations on amygdala subnuclei and pathways involved in this model should help in shedding light on the circuit and mechanisms underlying these effects and better identify the role of 5-HT in narcolepsy.

3.4.2 Noradrenaline

The main source of noradrenaline (NA) in the central nervous system is supplied by the locus coeruleus (LC) through diverse and widespread efferent projections. Similarly to histamine and serotonergic systems, LC NA neurons are wake-active with the particularity that they start to fire before the onset of wake, suggesting a role in wake induction (Aston-Jones and Bloom 1981; Takahashi et al. 2010). During wakefulness, LC NA neurons show distinct tonic and phasic patterns of activity: tonic discharge rate is positively correlated to states of arousal with notable increase in firing when the animals encounter unexpected novelty or perform operant-discrimination tasks, while phasic activations characterized by brief excitatory component followed by a longer duration of inhibition are associated with discrete sensory stimuli (see for reviews Aston-Jones et al. 1999; Sara 2009). Other studies have also associated tonic and phasic activation of LC neurons in response to stressors (see for review Aston-Jones et al. 1999). LC NA neurons cease firing just before the onset of SWS and remain silent during both SWS and REM sleep (Takahashi et al. 2010). The robust wake-promoting actions of LC NA neurons were further evidenced with optogenetics (Carter et al. 2010). Stimulating LC NA neurons optogenetically at 5 Hz induces arousal and can trigger wakefulness from NREM sleep (Carter et al. 2010). However in contrast with histamine system (see Sect. 3.4.4) (Fujita et al. 2017), acute inhibition of LC NA neurons does not immediately alter the behavioral status of the mice by switching from wake to sleep (Carter et al. 2010). Indeed, only a 1-h-long semi-chronic inhibition progressively drives an increase of sleep amounts at the expense of wakefulness (Carter et al. 2010). To date, in our knowledge, pharmacogenetic manipulation of LC NA neurons in sleep studies has been used in one study in rats investigating the causal relationship between LC NA activity and general anesthetic state (Vazey and Aston-Jones 2014). By generating a vector allowing hM3DGq transgene expression in LC NA neurons using the synthetic dopamine-β-hydroxylase (DBH) promoter PRSx8 in rats, Vazey and Aston-Jones tested the role of LC NA activation during general anesthesia and in the emergence from it. To do so, CNO was first administered locally and unilaterally into the LC while monitoring the cortical EEG of deeply

anesthetized rats under continuous isoflurane (2%) exposure. The authors observed that CNO microinjection in LC-hM3DGq rats leads to cortical EEG activation with a significant decrease in delta band power and increase in theta band power, in comparison with either vehicle microinjections or LC-mCherry control rats (Vazey and Aston-Jones 2014). DREADD-driven activation of LC NA neurons during anesthesia also induces an increase in total EEG power consistent with less burst suppression in cortical EEG, which is a measure of anesthetic depth, indicating a transitioning state away from deep anesthesia. Consistently, systemic CNO activation of LC NA neurons accelerates emergence from isoflurane anesthesia measured by rapid return of righting reflex after discontinuation of isoflurane, and that was shown to be mediated through both β and α1 adrenergic receptors (Vazey and Aston-Jones 2014). These findings showed that selective activation of LC NA neurons is sufficient and powerful enough to overcome the general anesthetic effects of artificially strong GABAergic inhibition, and trigger arousal. LC NA neurons specific implication and efficiency to promote arousal has been also highlighted in mice models of narcolepsy in which DREADD activation of LC NA neurons corrects wake fragmentation associated with narcolepsy (Hasegawa et al. 2014) (see Sect. 3.4.1).

Application of DREADD technique to LC NA neurons has been also used to stimulate degenerating neurons and test beneficial effects of increasing brain NA levels on cognitive impairments in a mouse model of Down syndrome (Rorabaugh et al. 2017) and in a rat model of Alzheimer's disease (Fortress et al. 2015). In both models DREADD stimulation of LC NA neurons results in cognitive improvements. Interestingly, another recent study using DREADD control established a causal relationship between LC NA neurons activity and stress-induced anxiety showing that hM4DGi-driven inhibition of LC NA neurons during stress prevents subsequent anxiety-like behavior, and in contrast, increased tonic hM3DGq-driven activation of LC NA neurons is sufficient to promote anxiety-like and aversive behavior (McCall et al. 2015). Interestingly, the authors found that the effects of LC stimulation on acute anxiogenic responses are blocked by the β-adrenoreceptor antagonist, whereas the aversive effects require α1-adrenoceptor activity, suggesting that acute stress-induced anxiety and aversive behaviors are driven by distinct circuits. Indeed, NA has simultaneously excitatory and inhibitory signaling through α1/β receptors and α2 receptors, respectively. While NA system produces arousal and deepens cognition, or takes part in stress-induced responses, selective pharmacological activation of α2 receptors produces deep SWS. This class of α2 receptor selective agonists belongs to prominent sedative drugs used for long-term sedation in hospital intensive care units (dexmedetomidine) or in veterinary clinics to sedate animals (xylazine) (see for review Yu et al. 2018). Thus, apart from arousal-promoting functions covering various physiological and cognitive processes or emotional responding, NA can also have sleep-promoting contributions (Grivel et al. 2005). All together these studies highlight the diversity of functions of NA that should be further addressed in light of the complex input/output organization of the LC (Schwarz et al. 2015) but should also integrate arousal-enhancing actions originating

from other groups of NA-releasing cells including brainstem A1 and A2 nuclei (Berridge 2008).

3.4.3 Dopamine

Among monoamines, dopamine (DA) seems to hold a notable position, being excluded from the "classical" ascending wake-promoting monoaminergic systems. This might be the consequence of the initial demonstration of an absence of change in neuronal activity of VTA DA cells across the natural sleep-wake cycle (Miller et al. 1983; Steinfels et al. 1983).

One recent paper using DREADD among other methods contributed to reintegrate DA neurons in sleep-wake regulating networks (Eban-Rothschild et al. 2016). Using fiber photometry on GCaMP-expressing DA neurons, the authors observed that, at a population level, DA neurons of the ventral tegmental area (VTA) are more active during wake and REM sleep than during SWS. Interestingly, the change in fluorescence is significantly higher during REM sleep bouts than waking bouts. This might be linked to the actual discharge pattern of VTA DA neurons across the natural sleep-wake cycle and the bursting pattern they display during REM sleep or during wakefulness in relation to appetitive or rewarding events (Dahan et al. 2007). This observation is also consistent with microdialysis measurements of DA in the NAc or the mPFC that revealed increased concentration of DA during wake and REM sleep compared to SWS (Léna et al. 2005). Using DREADD techniques, Eban-Rothschild and colleagues found that hM4DGi-driven inhibition of VTA DA neurons soon after the onset of the dark active photoperiod results in decreased wakefulness compensated by an increase in both SWS and REM sleep (Eban-Rothschild et al. 2016). These effects even persisted when animals are facing a salient stimulus such as palatable food, female mouse, or predator odor. Strikingly, the inhibition of VTA DA also facilitates the appearance of active nest-building behavior prior to fastened sleep onset only in the context of an absence of available nest. The authors suggested that these observations indicate that VTA DA neuron activity is necessary for preparatory sleep-related behaviors.

Conversely, optogenetic stimulation of VTA DA neurons during diurnal inactive period results in short-onset awakening (Eban-Rothschild et al. 2016), and the same effect was observed after DREADD hM3DGq activation of the whole VTA (Sun et al. 2017). The above optogenetic effect was replicated by stimulating DA terminals specifically in the NAc, in the DLS, and in the central nucleus of the amygdala, but not in the mPFC (Eban-Rothschild et al. 2016). Similarly, a deletion of SNc DA neurons in rats increases waking, whereas the optogenetic stimulation of DA terminals in the dorsal striatum inhibits wake in favor of SWS (Qiu et al. 2016b). One of the main targets of DA cells in the context of sleep-wake regulation would thus lie in the striatum. As a consequence, when VTA/SNc DA neurons are active, D_1-expressing dMSNs in the striatum would be activated, whereas D_2/A_{2A}-expressing iMSNs facilitating sleep onset (Oishi et al. 2017; Yuan et al. 2017) would be inhibited (Sect. 3.1.4). These results thus provide evidence for a role of midbrain DA neurons in facilitating waking maintenance, in particular in the context of aversive or rewarding stimuli requiring an alerting response.

Midbrain DA cells are also strongly active during REM sleep and display a characteristic bursting firing pattern (Dahan et al. 2007) associated with large DA release (Léna et al. 2005) whose function remains unresolved. One possibility would be an involvement of DA transmission in memory consolidation processes. Indeed, the activity of VTA DA neurons seems to be associated with theta rhythm (see for review Orzeł-Gryglewska et al. 2015) that is preeminent during REM sleep, and multiple evidences highlighted a causal role of REM sleep in episodic memory consolidation (Datta et al. 2004; Inostroza et al. 2013; Ravassard et al. 2016) with a particular focus on theta rhythm (Nishida et al. 2009; Popa et al. 2010; Boyce et al. 2016). Moreover theta rhythm during REM sleep could putatively contribute to implicit memory formation (Santos et al. 2008). Midbrain DA neurons could thus play a significant role in memory consolidation, especially with respect to the sequential hypothesis (Ambrosini and Giuditta 2001; Giuditta 2014; Sara 2017), which proposes that "irrelevant" information would be downgraded during SWS, whereas "relevant" information would be tagged maybe through cell ensemble reactivations and then, sequentially, integrated into preexisting memories during REM sleep, thanks to fast theta oscillations and the expression of transcription factors that both seem to be under the influence of DA signal (Orzeł-Gryglewska et al. 2015; Rioult-Pedotti et al. 2015; Wieland et al. 2015).

3.4.4 Histamine

Until recently, the brain histaminergic (His) system was critically understudied in comparison with other arousal monoaminergic circuits. The use of optogenetics, DREADD approaches, and genetic models revealed important findings on the His neurotransmission and function (Fig. 1).

His neurons of the hypothalamic tuberomammillary nucleus (TMN) display unique electrophysiological signatures characterized by a slow pacemaking activity (1–5 Hz) (Reiner and McGeer 1987) that is tightly coupled to behavioral arousal and is the most wake-specific firing pattern of any cell type identified in the brain to date (Vanni-Mercier et al. 2003; Takahashi et al. 2006; Sakai et al. 2010). His neurons start to fire after waking onset, increase firing during attentive waking, and cease their activity before sleep onset to remain silent during all stages of sleep (Vanni-Mercier et al. 2003; Takahashi et al. 2006; Sakai et al. 2010). These data suggested that His neurons would play a role in the maintenance of wakefulness and in adapting optimal levels of arousal necessary for cognitive processes (Lin et al. 2011b). It also suggested that the cessation of His activity may be necessary for sleep induction, which was recently evidenced by optogenetic approaches (Chung et al. 2017; Fujita et al. 2017). Genetic loss-of-function manipulations and pharmacological data provide a consistent picture because impairment to the His neurotransmission results in sleepiness and behavioral deficits (Lin 2000; Parmentier et al. 2002; Anaclet et al. 2009), whereas its enhancement promotes wakefulness and displays pro-cognitive effects (see for review Haas et al. 2008; Schwartz 2011).

Recently, novel insights on His control of arousal and synaptic mechanisms associated with His neurotransmission were achieved in a complete study combining DREADD technique, optogenetics, and genetic models (Yu et al. 2015). First, Yu and colleagues observed that a sustained activation of the His system, using conditional Cre-mediated expression of hM3DGq in histidine decarboxylase (HDC)-expressing neurons (*HDC-Cre*), induces a strong behavioral change assessed by hyperlocomotion in an open field (Yu et al. 2015). The CNO dose used in this study was quite high (5 mg/kg), and effects were compared with saline i.p. injections. In light of the recent studies questioning CNO endogenous effects, further controls should be examined in future studies. However, if there were any endogenous effects of CNO through clozapine conversion, we could expect opposite behavioral responses as shown by Gomez and colleagues in a similar open field test (Gomez et al. 2017). The main question of this approach was to test the ability of the His system to evoke excitatory behavioral responses with a large activation of the His neurotransmission. As a result, DREADD activation of His neurons under high dose of CNO leads to long-lasting hyperactivity (Yu et al. 2015). Unfortunately, effects on sleep-wake cycle and EEG remain unknown.

Most importantly, by questioning the functional role of the presence of GABA in the vast majority of His neurons (Takeda et al. 1984; Senba et al. 1985; Trottier et al. 2002), Yu and colleagues showed that most His neurons use VGAT to release GABA and highlighted the importance of GABA co-release in controlling behavioral arousal (Yu et al. 2015). They showed that conditional knockdown and knockout mice with disrupted GABA function in His neurons are hyperactive, exhibiting hyperlocomotion and decreased amounts of sleep confined to the night in comparison with control mice (Yu et al. 2015). Using optogenetic stimulation of HDC-ChR2 fibers, they further demonstrated ex vivo that GABA is being directly released from His fibers in the neocortex and striatum. Interestingly in the VLPO, His axons do not release GABA when stimulated optogenetically but instead activate GABA interneurons by His release to suppress the activity of long-range projecting sleep-promoting neurons (Williams et al. 2014). Apart from technical differences, Yu and colleagues suggest that these contrasting results with neocortical neurons might be due to a subpopulation of His neurons projecting to the VLPO devoid of GABA (about ~20%), which is intriguing to test by further histological and tracing investigations considering the growing evidences of diversity among His neurons (Blandina et al. 2012; Fujita et al. 2017). Moreover, the striatum is also composed of a diversity of cells including two types of MSNs and various interneurons (Durieux et al. 2011). Thus, additional recordings in the distinct striatal neuronal populations should be conducted to precisely define the nature and mechanisms of His actions in the BG circuitry (Bolam and Ellender 2016).

The study led by Yu and colleagues emphasized a major phenomenon observed in other monoaminergic systems as subsets of 5-HT and DA neurons also have the ability to release Glu or GABA fast neurotransmitters through different vesicular transporters (see for review Lőrincz and Adamantidis 2017). As a consequence, it highlights the need to dissect in vivo effects with a circuit-level approach combining various techniques monitoring neuronal activity and/or release, notably when using DREADD or optogenetic approaches. Even if neurochemical specificity can be

achieved through genetic targeting, certain effects attributed to monoamine release can be in fact mediated or counteracted by fast neurotransmission. Why His-GABA neurons use contradictory signals remains an open question that is well-discussed by Yu and colleagues proposing that it could either serve to stop networks getting too excited by an overactive His system, which may have deleterious consequences on sleep and mental health as seen in their models. Conversely, it could actually better shape cognitive responses together with His, notably at the level of the cortex in adjusting the balance between synaptic excitation and inhibition to enhance processing (see discussion Yu et al. 2015).

Finally, the His system has emerged as an attractive therapeutic target for the treatment of neuropsychiatric and neurodegenerative diseases. His regulates its own release through autoinhibitory H3 receptors (H3R) (Lin et al. 2011a). Selective H3R blockers enhance both the firing rate of His neurons and His release (Lin et al. 2011a). Interestingly, administration of H3R blockers dramatically enhances the high-frequency component of neocortical EEG rhythms in cats, mice, and humans and improves attention and facilitates learning in rodent models (see for review Schwartz 2011), lending support to the role of His in higher cognitive processes. Selective blockers are now gaining entrance in clinical trials showing tremendous promise as a therapeutic intervention in schizophrenia, dementias, sleep disorders, and other conditions for its wake-promoting and pro-cognitive effects (Schwartz 2011). Thus, it is timely to further our understanding on the role and actions of TMN His neurons and His-GABA neurons in arousal and cognition.

3.5 Peptidergic Systems

3.5.1 Hypocretin/Orexin

Localized in the lateral hypothalamus, hypocretin/orexin (Hcrt/Orx)-secreting neurons are key contributors to wakefulness stabilization (Bonnavion and De Lecea 2010), and dysfunction of the Hcrt/Orx system is linked to narcolepsy (see Sects. 3.4.1 and 3.4.2). Pharmacosynthetic modulation of Hcrt/Orx neurons activity revealed that the CNO-elicited activation of hM3Dq-expressing Hcrt/Orx neurons increases wake at the expense of both SWS and REM sleep, whereas their inhibition facilitates SWS and decreases wake and REM sleep amounts (Sasaki et al. 2011). However, a more detailed description of the sleep-wake architecture after CNO/DREADD-driven control is still missing as acute optogenetic activation of Hcrt/Orx neurons during SWS produces Hcrt peptide-dependent awakening within a minute, whereas acute inhibition of the same neurons during wake accelerates SWS onset (Adamantidis et al. 2007; Tsunematsu et al. 2011). Hcrt/Orx neurons seem of particular importance for wake consolidation and proper control of sleep-wake dynamics without strongly affecting daily wake amounts (Hara et al. 2001; Diniz Behn et al. 2008, 2010; Kantor et al. 2009; De Lecea 2012). During wakefulness, Hcrt/Orx cells are not constantly firing and become stimulated in association with signals that require increased wakefulness (Yamanaka et al. 2003; Mileykovskiy et al. 2005; Williams et al. 2007; Bonnavion et al. 2015; González et al. 2016).

Altogether these findings reinforce the unique position of Hcrt/Orx neurons among wake-active systems that may thus be regarded as computational comparators generating an error signal to recalibrate arousal levels in comparison to the difference between the actual state and required arousal (Kosse and Burdakov 2014).

3.5.2 Melanin-Concentrating Hormone

Neurons expressing melanin-concentrating hormone (MCH) are a molecularly defined neuronal subpopulation largely distributed throughout the extent of the LH and ZI. Several experiments pointed out a pivotal contribution of MCH-secreting neurons in the control of sleep and in particular REM sleep. Indeed both optogenetic (Jego et al. 2013; Konadhode et al. 2013; Tsunematsu et al. 2014) and DREADD-mediated stimulations (Vetrivelan et al. 2016; Varin et al. 2018) of MCH neurons unfailingly result in facilitated REM sleep onset and increased REM sleep amounts. However, inconsistent and puzzling results on SWS were harvested by these studies, probably due to heterogeneous experimental and technical procedures. Indeed, the semi-chronic optogenetic stimulation of MCH neurons during 24 h extends SWS only during the nocturnal active phase by increasing the number of SWS bouts (Konadhode et al. 2013). Their acute stimulation at the onset of a given SWS episode lets the duration of the ongoing episode unaffected (Jego et al. 2013), whereas SWS amounts and the mean duration of SWS episodes are decreased upon continuous optogenetic stimulation of MCH neurons for 3 h during the light inactive photoperiod (Tsunematsu et al. 2014). In an attempt to solve these discrepancies, two groups recently examined the effects on sleep of the DREADD-mediated modulation of MCH neuron activity (Vetrivelan et al. 2016; Varin et al. 2018). The first study (Vetrivelan et al. 2016) analyzed the effects on sleep of CNO-induced activation of hM3DGq-expressing MCH neurons and confirmed previous evidences indicating that the stimulation of MCH neurons favors REM sleep onset and consolidation. The second one (Varin et al. 2018) combined the utilization of both hM3DGq and hM4DGi DREADD effectors. Varin and colleagues consistently confirmed the REM sleep-facilitating effect of MCH neuron activation and additionally observed that CNO-mediated stimulation of MCH neurons results in decreased SWS amounts, whereas the inhibition of MCH neurons resulted in the opposite phenotype. These effects were accompanied with modifications in SWS delta rhythmic activities suggesting that MCH neurons could participate in increasing SWS depth (Varin et al. 2018). Thanks to their observations using both excitatory and inhibitory DREADD tools and a multiple CNO doses design, the authors consequently proposed a novel formulation of the role of MCH neurons in sleep physiology, that is, MCH neurons would be involved in basic mechanisms occurring primarily during SWS aimed at deepening SWS to pave the way for SWS-to-REM sleep transition to occur.

4 Conclusion and Perspectives

Following the path opened by optogenetics more than a decade ago, the development and use of pharmacosynthetic tools quickly made their contribution to the discovery and the expansion of our understanding of critical brain structures and neuronal subpopulation involved in a wide variety of behaviors and in particular in the control of vigilance state architecture.

We now end up with a large – but probably not exhaustive – set of brain structures that are key modulators or serve executive functions in the control of the sleep-wake cycle (Fig. 1). This leads to a complex network of deeply interconnected and heterogeneous neuronal subpopulations (Fig. 2). Deciphering this complexity and unscrambling the hierarchical organization of the sleep-wake regulating network constitute a challenging task that sleep scientists are now facing. Indeed this challenge is particularly prominent when attempting to describe and quantify the series of activations and inhibitions of different brain structures and neuronal subpopulations occurring cooperatively and concurring to sharp and complete transitions between vigilance states.

In front of this demanding task, multiple novel tools offer new possibilities to move a step forward in our understanding of sleep physiology. Recent advances in in vivo electrophysiology provide opportunities to record multiple cellular subpopulations that can additionally be identified by optogenetic tagging at the same time in different brain regions in order to draw a finer picture of neuronal dynamics during vigilance states and transitions between states (Vyazovskiy et al. 2011; Herrera et al. 2015; Weber et al. 2015; Xu et al. 2015).

The hierarchical organization of sleep-wake systems could be probed by multiplexing various genetically encoded actuators or sensors. For instance, DREADD-mediated strategy can be expanded by coupling "classical" muscarinic receptor mutants with the recently developed inhibitory kappa-opioid DREADD that is activated by salvinorin B instead of CNO (Vardy et al. 2015). With this strategy two different neuronal populations can be inhibited or activated and inhibited at the same time or independently (Rapanelli et al. 2017). A similar approach could rely on the use of "PSAM-PSEM" actuators (see for review Sternson and Roth 2014). Moreover a similar experimental design can be drawn with optogenetics using the combination of both inhibitory and excitatory channels (Kleinlogel et al. 2011; Carter et al. 2012) and the use of red-shifted mutants of channelrhodopsin or halorhodopsin (Chow et al. 2010; Chuong et al. 2014; Klapoetke et al. 2014). On top of this profusion of various actuators, elegant genetic tagging of multiple neuronal subpopulations can be achieved through the use of intersectional strategies in transgenic animals expressing multiple recombination enzymes such as cre and flippase, under the control of different promoters (Fenno et al. 2014; He et al. 2016). Although challenging, this approach could be even expanded by adding an extra layer of control with the use of tetracycline-controlled transcriptional activation systems, which can be reversibly turned on and off in the presence of doxycycline (Zhang et al. 2015). And obviously, genetically encoded actuators can be

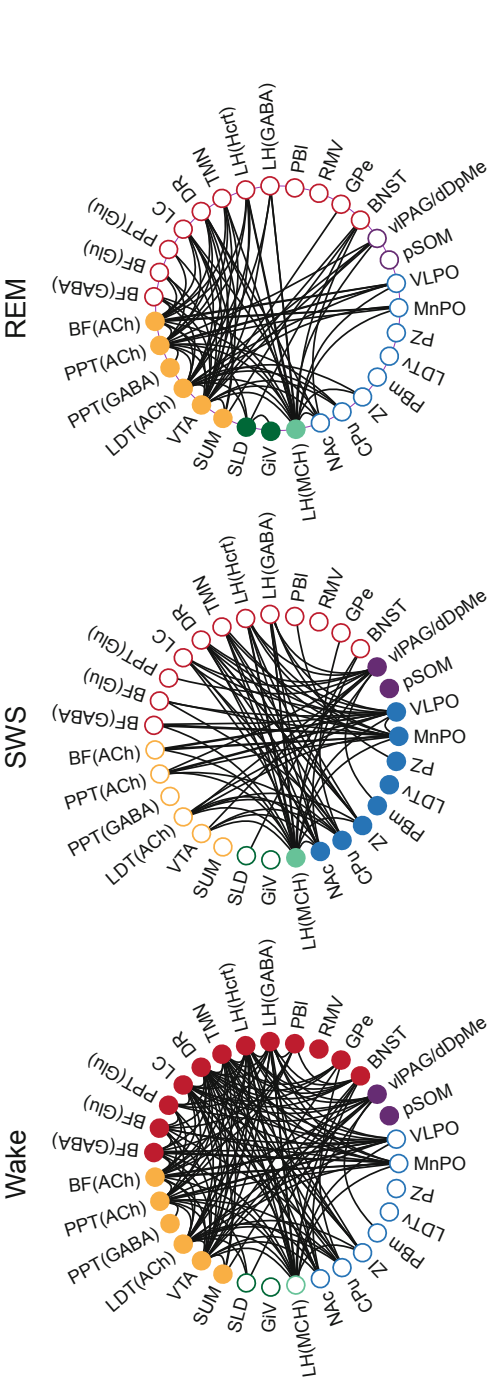

Fig. 2 Circular node diagrams of sleep/wake network connectivity including chemogenetic findings. Note that wake-related nuclei show high level of connectivity with sleeping state-related structures and a strong interconnectivity, which highlights their interdependency as well as their conjoint and reinforcing arousing actions. In contrast, formerly identified sleep-promoting nuclei and newly chemogenetically identified structures associated with either SWS or REM sleep both display important connections with the wake-promoting systems but barely share connections among them. At our current state of knowledge, the latter characteristics would imply multiple and independent nodes promoting and regulating sleep states. Alternatively, future investigations should further focus on identifying direct links between sleep-regulating structures

multiplexed with genetically encoded sensors such as calcium indicators or voltage-sensitive fluorescent proteins (Kim et al. 2016).

Finally, the complexity of sleep physiology and the complexity of neuronal networks on which it depends should inspire the use of more and more sophisticated metrics to acutely and precisely dissect sleep-wake architecture and dynamics, together with subtle regulatory mechanisms. Among these metrics can be mentioned Markov's chain analysis (Kim et al. 2009; Bianchi et al. 2012; Stephenson et al. 2013), survival analysis of sleep and wake episode duration (Lo et al. 2002, 2004; Blumberg et al. 2005; Diniz Behn et al. 2008; Varin et al. 2016), or EEG or LFP analysis using two dimensional map of behavioral state (Gervasoni et al. 2004; Dzirasa et al. 2006; Diniz Behn et al. 2010). All together, the above methods should provide new ways to analyze and decipher the precise temporal, hierarchical, and dynamical organization of vigilance states.

References

Adamantidis A, Lüthi A (2018) Optogenetic dissection of sleep-wake states in vitro and in vivo. In: Handbook of experimental pharmacology. Springer, Berlin/Heidelberg. https://doi.org/10.1007/164_2018_94

Adamantidis AR, Zhang F, Aravanis AM, Deisseroth K, de Lecea L (2007) Neural substrates of awakening probed with optogenetic control of hypocretin neurons. Nature 450:420–424

Alcacer C, Andreoli L, Sebastianutto I, Jakobsson J, Fieblinger T, Cenci MA (2017) Chemogenetic stimulation of striatal projection neurons modulates responses to Parkinson's disease therapy. J Clin Invest 127:720–734

Alexander GM, Rogan SC, Abbas AI, Armbruster BN, Pei Y, Allen JA, Nonneman RJ, Hartmann J, Moy SS, Nicolelis MA, McNamara JO, Roth BL (2009) Remote control of neuronal activity in transgenic mice expressing evolved G protein-coupled receptors. Neuron 63:27–39

Ambrosini MV, Giuditta A (2001) Learning and sleep: the sequential hypothesis. Sleep Med Rev 5:477–490

Anaclet C, Fuller PM (2017) Brainstem regulation of slow-wave-sleep. Curr Opin Neurobiol 44:139–143

Anaclet C, Parmentier R, Ouk K, Guidon G, Buda C, Sastre J-P, Akaoka H, Sergeeva OA, Yanagisawa M, Ohtsu H, Franco P, Haas HL, Lin JS (2009) Orexin/hypocretin and histamine: distinct roles in the control. J Neurosci 29:14423–14438

Anaclet C, Lin J-S, Vetrivelan R, Krenzer M, Vong L, Fuller PM, Lu J (2012) Identification and characterization of a sleep-active cell group in the rostral medullary brainstem. J Neurosci 32:17970–17976

Anaclet C, Ferrari L, Arrigoni E, Bass CE, Saper CB, Lu J, Fuller PM (2014) The GABAergic parafacial zone is a medullary slow wave sleep-promoting center. Nat Neurosci 17:1217–1224

Anaclet C, Pedersen NP, Ferrari LL, Venner A, Bass CE, Arrigoni E, Fuller PM (2015) Basal forebrain control of wakefulness and cortical rhythms. Nat Commun 6:1–14

Armbruster BN, Li X, Pausch MH, Herlitze S, Roth BL (2007) Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. Proc Natl Acad Sci U S A 104:5163–5168

Aston-Jones G, Bloom FE (1981) Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. J Neurosci 1:876–886

Aston-Jones G, Rajkowski J, Cohen J (1999) Role of locus coeruleus in attention and behavioral flexibility. Biol Psychiatry 46:1309–1320

Balleine BW, O'Doherty JP (2010) Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action. Neuropsychopharmacology 35:48–69
Bauer EP (2015) Serotonin in fear conditioning processes. Behav Brain Res 277:68–77
Bender D, Holschbach M, Stöcklin G (1994) Synthesis of n.c.a. carbon-11 labelled clozapine and its major metabolite clozapine-N-oxide and comparison of their biodistribution in mice. Nucl Med Biol 21:921–925
Berridge CW (2008) Noradrenergic modulation of arousal. Brain Res Rev 58:1–17
Bester H, Besson JM, Bernard JF (1997) Organization of efferent projections from the parabrachial area to the hypothalamus: a Phaseolus vulgaris-leucoagglutinin study in the rat. J Comp Neurol 383:245–281
Bianchi MT, Eiseman NA, Cash SS, Mietus J, Peng CK, Thomas RJ (2012) Probabilistic sleep architecture models in patients with and without sleep apnea. J Sleep Res 21:330–341
Billwiller F, Renouard L, Clement O, Fort P, Luppi PH (2017) Differential origin of the activation of dorsal and ventral dentate gyrus granule cells during paradoxical (REM) sleep in the rat. Brain Struct Funct 222:1495–1507
Bjorness TE, Greene RW (2009) Adenosine and sleep. Curr Neuropharmacol 7:238–245
Blandina P, Munari L, Provensi G, Passani MB (2012) Histamine neurons in the tuberomamillary nucleus: a whole center or distinct subpopulations? Front Syst Neurosci 6:33
Blumberg MS, Seelke AMH, Lowen SB, Karlsson KAE (2005) Dynamics of sleep-wake cyclicity in developing rats. Proc Natl Acad Sci U S A 102:14860–14864
Bolam JP, Ellender TJ (2016) Histamine and the striatum. Neuropharmacology 106:74–84
Bonnavion P, De Lecea L (2010) Hypocretins in the control of sleep and wakefulness. Curr Neurol Neurosci Rep 10:174–179
Bonnavion P, Jackson AC, Carter ME, de Lecea L (2015) Antagonistic interplay between hypocretin and leptin in the lateral hypothalamus regulates stress responses. Nat Commun 6:6266
Bonnavion P, Mickelsen LE, Fujita A, de Lecea L, Jackson AC (2016) Hubs and spokes of the lateral hypothalamus: cell types, circuits and behaviour. J Physiol 594:6443–6462
Boucetta S, Cisse Y, Mainville L, Morales M, Jones BE (2014) Discharge profiles across the sleep-waking cycle of identified cholinergic, GABAergic, and glutamatergic neurons in the pontomesencephalic tegmentum of the rat. J Neurosci 34:4708–4727
Boyce R, Glasgow SD, Williams S, Adamantidis A (2016) Sleep research: causal evidence for the role of REM sleep theta rhythm in contextual memory consolidation. Science 352:812–816
Brancaccio M, Maywood ES, Chesham JE, Loudon ASI, Hastings MH (2013) A Gq-Ca2+ axis controls circuit-level encoding of circadian time in the suprachiasmatic nucleus. Neuron 78:714–728
Brown RE, McKenna JT (2015) Turning a negative into a positive: ascending GABAergic control of cortical activation and arousal. Front Neurol 6:135
Brown RE, Basheer R, McKenna JT, Strecker RE, McCarley RW (2012) Control of sleep and wakefulness. Physiol Rev 92:1087–1187
Buzsáki G, Schomburg EW (2015) What does gamma coherence tell us about inter-regional neural communication? Nat Neurosci 18:484–489
Carter ME, Yizhar O, Chikahisa S, Nguyen H, Adamantidis A, Nishino S, Deisseroth K, de Lecea L (2010) Tuning arousal with optogenetic modulation of locus coeruleus neurons. Nat Neurosci 13:1526–1533
Carter ME, Brill J, Bonnavion P, Huguenard JR, Huerta R, de Lecea L (2012) Mechanism for hypocretin-mediated sleep-to-wake transitions. Proc Natl Acad Sci U S A 109:E2635–E2644
Carvalho Poyraz F, Holzner E, Bailey MR, Meszaros J, Kenney L, Kheirbek MA, Balsam PD, Kellendonk C (2016) Decreasing striatopallidal pathway function enhances motivation by energizing the initiation of goal-directed action. J Neurosci 36:5988–6001
Chang WH, Lin SK, Lane HY, Wei FC, Hu WH, Lam YWF, Jann MW (1998) Reversible metabolism of clozapine and clozapine N-oxide in schizophrenic patients. Prog Neuropsychopharmacol Biol Psychiatry 22:723–739

Chen X, Choo H, Huang XP, Yang X, Stone O, Roth BL, Jin J (2015) The first structure-activity relationship studies for designer receptors exclusively activated by designer drugs. ACS Chem Nerosci 6:476–484

Chen L, Yin D, Wang TX, Guo W, Dong H, Xu Q, Luo YJ, Cherasse Y, Lazarus M, Qiu ZL, Lu J, Qu WM, Huang ZL (2016) Basal forebrain cholinergic neurons primarily contribute to inhibition of electroencephalogram delta activity, rather than inducing behavioral wakefulness in mice. Neuropsychopharmacology 41:2133–2146

Chen MC, Vetrivelan R, Guo CN, Chang C, Fuller PM, Lu J (2017) Ventral medullary control of rapid eye movement sleep and atonia. Exp Neurol 290:53–62

Chow BY, Han X, Dobry AS, Qian X, Chuong AS, Li M, Henninger MA, Belfort GM, Lin Y, Monahan PE, Boyden ES (2010) High-performance genetically targetable optical neural silencing by light-driven proton pumps. Nature 463:98–102

Chung S, Weber F, Zhong P, Tan CL, Nguyen TN, Beier KT, Hörmann N, Chang WC, Zhang Z, Do JP, Yao S, Krashes MJ, Tasic B, Cetin A, Zeng H, Knight ZA, Luo L, Dan Y (2017) Identification of preoptic sleep neurons using retrograde labelling and gene profiling. Nature 545:477–481

Chuong AS, Miri ML, Busskamp V, Matthews GA, Acker LC, Sorensen AT, Young A, Klapoetke NC, Henninger MA, Kodandaramaiah SB, Ogawa M, Ramanial SB, Bandler RC, Allen BD, Forest CR, Chow BY, Han X, Lin Y, Tye KM, Roska B, Cardin JA, Boyden ES (2014) Noninvasive optical inhibition with a red-shifted microbial rhodopsin. Nat Neurosci 17:1123–1129

Conklin BR, Hsiao EC, Claeysen S, Dumuis A, Srinivasan S, Forsayeth JR, Guettier JM, Chang WC, Pei Y, McCarthy KD, Nissenson RA, Wess J, Bockaert J, Roth BL (2008) Engineering GPCR signaling pathways with RASSLs. Nat Methods 5:673–678

Dahan L, Astier B, Vautrelle N, Urbain N, Kocsis B, Chouvet G (2007) Prominent burst firing of dopaminergic neurons in the ventral tegmental area during paradoxical sleep. Neuropsychopharmacology 32:1232–1241

Datta S, Mavanji V, Ulloor J, Patterson EH (2004) Activation of phasic pontine-wave generator prevents rapid eye movement sleep deprivation-induced learning impairment in the rat: a mechanism for sleep-dependent plasticity. J Neurosci 24:1416–1427

Dauvilliers Y, Billiard M, Montplaisir J (2003) Clinical aspects and pathophysiology of narcolepsy. Clin Neurophysiol 114:2000–2017

De Lecea L (2012) Hypocretins and the neurobiology of sleep-wake mechanisms. Prog Brain Res 198:15–24

Diniz Behn CG, Kopell N, Brown EN, Mochizuki T, Scammell TE (2008) Delayed orexin signaling consolidates wakefulness and sleep: physiology and modeling. J Neurophysiol 99:3090–3103

Diniz Behn CG, Klerman EB, Mochizuki T, Lin SC, Scammell TE (2010) Abnormal sleep/wake dynamics in orexin knockout mice. Sleep 33:297–306

Dong S, Rogan SC, Roth BL (2010) Directed molecular evolution of DREADDs: a generic approach to creating next-generation RASSLs. Nat Protoc 5:561–573

Drews J (2000) Drug discovery: a historical perspective. Science 287:1960–1964

Durieux PF, Schiffmann SN, de Kerchove d'Exaerde A (2011) Targeting neuronal populations of the striatum. Front Neuroanat 5:40

Dzirasa K, Ribeiro S, Costa R, Santos LM, Lin S-C, Grosmark A, Sotnikova TD, Gainetdinov RR, Caron MG, Nicolelis MAL (2006) Dopaminergic control of sleep-wake states. J Neurosci 26:10577–10589

Eban-Rothschild A, Rothschild G, Giardino WJ, Jones JR, de Lecea L (2016) VTA dopaminergic neurons regulate ethologically relevant sleep-wake behaviors. Nat Neurosci 19(10):1356–1366

Economo C (1930) Sleep as a problem of localization. J Nerv Ment Dis 71:249–259

Eldridge MAG, Lerchner W, Saunders RC, Kaneko H, Krausz KW, Gonzalez FJ, Ji B, Higuchi M, Minamimoto T, Richmond BJ (2015) Chemogenetic disconnection of monkey orbitofrontal and rhinal cortex reversibly disrupts reward value. Nat Neurosci 19:37–39

Farrell MS, Roth BL (2013) Pharmacosynthetics: reimagining the pharmacogenetic approach. Brain Res 1511:6–20

Farrell MS, Pei Y, Wan Y, Yadav PN, Daigle TL, Urban DJ, Lee H-M, Sciaky N, Simmons A, Nonneman RJ, Huang X-P, Hufeisen SJ, Guettier J-M, Moy SS, Wess J, Caron MG, Calakos N, Roth BL (2013) A Gαs DREADD mouse for selective modulation of cAMP production in striatopallidal neurons. Neuropsychopharmacology 38:854–862

Fenno LE, Mattis J, Ramakrishnan C, Hyun M, Lee SY, He M, Tucciarone J, Selimbeyoglu A, Berndt A, Grosenick L, Zalocusky KA, Bernstein H, Swanson H, Perry C, Diester I, Boyce FM, Bass CE, Neve R, Huang ZJ, Deisseroth K (2014) Targeting cells with single vectors using multiple-feature Boolean logic. Nat Methods 11:763–772

Fort P, Bassetti CL, Luppi PH (2009) Alternating vigilance states: new insights regarding neuronal networks and mechanisms. Eur J Neurosci 29:1741–1753

Fortress AM, Hamlett ED, Vazey EM, Aston-Jones G, Cass WA, Boger HA, Granholm A-CE (2015) Designer receptors enhance memory in a mouse model of down syndrome. J Neurosci 35:1343–1353

Fraigne JJ, Torontali ZA, Snow MB, Peever JH (2015) REM sleep at its core-circuits, neurotransmitters, and pathophysiology. Front Neurol 6:123

Fujita A, Bonnavion P, Wilson MH, Mickelsen LE, Bloit J, de Lecea L, Jackson AC (2017) Hypothalamic tuberomammillary nucleus neurons: electrophysiological diversity and essential role in arousal stability. J Neurosci 37:9574–9592

Fuller P, Sherman D, Pedersen NP, Saper CB, Lu J (2011) Reassessment of the structural basis of the ascending arousal system. J Comp Neurol 519:933–956

Gervasoni D, Lin SC, Ribeiro S, Soares ES, Pantoja J, Nicolelis MA (2004) Global forebrain dynamics predict rat behavioral states and their transitions. J Neurosci 24:11137–11147

Giuditta A (2014) Sleep memory processing: the sequential hypothesis. Front Syst Neurosci 8:219

Gomez JL, Bonaventura J, Lesniak W, Mathews WB, Sysa-Shah P, Rodriguez LA, Ellis RJ, Richie CT, Harvey BK, Dannals RF, Pomper MG, Bonci A, Michaelides M (2017) Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. Science 357:503–507

González JA, Iordanidou P, Strom M, Adamantidis A, Burdakov D (2016) Awake dynamics and brain-wide direct inputs of hypothalamic MCH and orexin networks. Nat Commun 7:11395

Graybiel AM (2008) Habits, rituals, and the evaluative brain. Annu Rev Neurosci 31:359–387

Grivel J, Cvetkovic V, Bayer L, Machard D, Tobler I, Muhlethaler M, Serafin M (2005) The wake-promoting hypocretin/orexin neurons change their response to noradrenaline after sleep deprivation. J Neurosci 25:4127–4130

Guettier J-M, Gautam D, Scarselli M, Ruiz de Azua I, Li JH, Rosemond E, Ma X, Gonzalez FJ, Armbruster BN, Lu H, Roth BL, Wess J (2009) A chemical-genetic approach to study G protein regulation of beta cell function in vivo. Proc Natl Acad Sci U S A 106:19197–19202

Haas HL, Sergeeva OA, Selbach O (2008) Histamine in the nervous system. Physiol Rev 88:1183–1241

Han Y, Shi YF, Xi W, Zhou R, Tan ZB, Wang H, Li XM, Chen Z, Feng G, Luo M, Huang ZL, Duan S, Yu YQ (2014) Selective activation of cholinergic basal forebrain neurons induces immediate sleep-wake transitions. Curr Biol 24:693–698

Hara J, Beuckmann CT, Nambu T, Willie JT, Chemelli RM, Sinton CM, Sugiyama F, Yagami KI, Goto K, Yanagisawa M, Sakurai T (2001) Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. Neuron 30:345–354

Hasegawa E, Yanagisawa M, Sakurai T, Mieda M (2014) Orexin neurons suppress narcolepsy via 2 distinct efferent pathways. J Clin Invest 124:604–616

Hasegawa E, Maejima T, Yoshida T, Masseck OA, Herlitze S, Yoshioka M, Sakurai T, Mieda M (2017) Serotonin neurons in the dorsal raphe mediate the anticataplectic action of orexin neurons by reducing amygdala activity. Proc Natl Acad Sci U S A 114:E3526–E3535

Hassani OK, Henny P, Lee MG, Jones BE (2010) GABAergic neurons intermingled with orexin and MCH neurons in the lateral hypothalamus discharge maximally during sleep. Eur J Neurosci 32:448–457

Hayashi Y, Kashiwagi M, Yasuda K, Ando R, Kanuka M, Sakai K, Itohara S (2015) Cells of a common developmental origin regulate REM/non-REM sleep and wakefulness in mice. Science 350:957–962

He M, Tucciarone J, Lee S, Nigro MJ, Kim Y, Levine JM, Kelly SM, Krugikov I, Wu P, Chen Y, Gong L, Hou Y, Osten P, Rudy B, Huang ZJ (2016) Strategies and tools for combinatorial targeting of GABAergic neurons in mouse cerebral cortex. Neuron 91:1228–1243

Hellman K, Aadal Nielsen P, Ek F, Olsson R (2016) An ex vivo model for evaluating blood-brain barrier permeability, efflux, and drug metabolism. ACS Chem Nerosci 7:668–680

Herrera CG, Cadavieco MC, Jego S, Ponomarenko A, Korotkova T, Adamantidis A (2015) Hypothalamic feedforward inhibition of thalamocortical network controls arousal and consciousness. Nat Neurosci 19:1–12

Herrera CG, Ponomarenko A, Korotkova T, Burdakov D, Adamantidis A (2017) Sleep and metabolism: the multitasking ability of lateral hypothalamic inhibitory circuitries. Front Neuroendocrinol 44:27–34

Hinze-Selch D, Mullington J, Orth A, Lauer CJ, Pollmächer T (1997) Effects of clozapine on sleep: a longitudinal study. Biol Psychiatry 42:260–266

Inostroza M, Binder S, Born J (2013) Sleep-dependency of episodic-like memory consolidation in rats. Behav Brain Res 237:15–22

Janak PH, Tye KM (2015) From circuits to behaviour in the amygdala. Nature 517:284–292

Jann MW, Lam YW, Chang WH (1994) Rapid formation of clozapine in guinea-pigs and man following clozapine-N-oxide administration. Arch Int Pharmacodyn Ther 328:243–250

Jego S, Glasgow SD, Herrera CG, Ekstrand M, Reed SJ, Boyce R, Friedman J, Burdakov D, Adamantidis AR (2013) Optogenetic identification of a rapid eye movement sleep modulatory circuit in the hypothalamus. Nat Neurosci 16:1637–1643

Jennings JH, Rizzi G, Stamatakis AM, Ung RL, Stuber GD (2013) The inhibitory circuit architecture of the lateral hypothalamus orchestrates feeding. Science 341:1517–1521

Ji B, Kaneko H, Minamimoto T, Inoue H, Takeuchi H, Kumata K, Zhang M-R, Aoki I, Seki C, Ono M, Tokunaga M, Tsukamoto S, Tanabe K, Shin R-M, Minamihisamatsu T, Kito S, Richmond BJ, Suhara T, Higuchi M (2016) Multimodal imaging for DREADD-expressing neurons in living brain and their application to implantation of iPSC-derived neural progenitors. J Neurosci 36:11544–11558

Jin X, Costa RM (2010) Start/stop signals emerge in nigrostriatal circuits during sequence learning. Nature 466:457–462

Jouvet M (1999) Sleep and serotonin: an unfinished story. Neuropsychopharmacology 21:24S–27S

Kantor S, Mochizuki T, Janisiewicz AM, Clark E, Nishino S, Scammell TE (2009) Orexin neurons are necessary for the circadian control of REM sleep. Sleep 32:1127–1134

Kaur S, Wang JL, Ferrari L, Thankachan S, Kroeger D, Venner A, Lazarus M, Wellman A, Arrigoni E, Fuller PM, Saper CB (2017) A genetically defined circuit for arousal from sleep during hypercapnia. Neuron 96:1153–1167.e5

Kim JW, Lee JS, Robinson PA, Jeong DU (2009) Markov analysis of sleep dynamics. Phys Rev Lett 102:178104

Kim T, Thankachan S, McKenna JT, McNally JM, Yang C, Choi JH, Chen L, Kocsis B, Deisseroth K, Strecker RE, Basheer R, Brown RE, McCarley RW (2015) Cortically projecting basal forebrain parvalbumin neurons regulate cortical gamma band oscillations. Proc Natl Acad Sci U S A 112:3535–3540

Kim CK, Yang SJ, Pichamoorthy N, Young NP, Kauvar I, Jennings JH, Lerner TN, Berndt A, Lee SY, Ramakrishnan C, Davidson TJ, Inoue M, Bito H, Deisseroth K (2016) Simultaneous fast measurement of circuit dynamics at multiple sites across the mammalian brain. Nat Methods 13:325–328

Klapoetke NC et al (2014) Independent optical excitation of distinct neural populations. Nat Methods 11:338–346

Kleinlogel S, Terpitz U, Legrum B, Gökbuget D, Boyden ES, Bamann C, Wood PG, Bamberg E (2011) A gene-fusion strategy for stoichiometric and co-localized expression of light-gated membrane proteins. Nat Methods 8:1083–1091

Kobilka BK, Deupi X (2007) Conformational complexity of G-protein-coupled receptors. Trends Pharmacol Sci 28:397–406

Kodani S, Soya S, Sakurai T (2017) Excitation of GABAergic neurons in the bed nucleus of the stria terminalis triggers immediate transition from non-rapid eye movement sleep to wakefulness in mice. J Neurosci 37:7164–7176

Konadhode RR, Pelluru D, Blanco-Centurion C, Zayachkivsky A, Liu M, Uhde T, Glen WB, van den Pol AN, Mulholland PJ, Shiromani PJ (2013) Optogenetic stimulation of MCH neurons increases sleep. J Neurosci 33:10257–10263

Kosse C, Burdakov D (2014) A unifying computational framework for stability and flexibility of arousal. Front Syst Neurosci 8:192

Kosse C, Schöne C, Bracey E, Burdakov D (2017) Orexin-driven GAD65 network of the lateral hypothalamus sets physical activity in mice. Proc Natl Acad Sci U S A 114:4525–4530

Krashes MJ, Koda S, Ye CP, Rogan SC, Adams AC, Cusher DS, Maratos-Flier E, Roth BL, Lowell BB (2011) Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. J Clin Invest 121:1424–1428

Kroeger D, Ferrari LL, Petit G, Mahoney CE, Fuller PM, Arrigoni E, Scammell TE (2017) Cholinergic, glutamatergic, and GABAergic neurons of the pedunculopontine tegmental nucleus have distinct effects on sleep/wake behavior in mice. J Neurosci 37:1352–1366

Lazarus M, Huang ZL, Lu J, Urade Y, Chen JF (2012) How do the basal ganglia regulate sleep-wake behavior? Trends Neurosci 35:723–732

Lebow MA, Chen A (2016) Overshadowed by the amygdala: the bed nucleus of the stria terminalis emerges as key to psychiatric disorders. Mol Psychiatry 21:450–463

Léna I, Parrot S, Deschaux O, Muffat-Joly S, Sauvinet V, Renaud B, Suaud-Chagny MF, Gottesmann C (2005) Variations in extracellular levels of dopamine, noradrenaline, glutamate, and aspartate across the sleep-wake cycle in the medial prefrontal cortex and nucleus accumbens of freely moving rats. J Neurosci Res 81:891–899

Lin JS (2000) Brain structures and mechanisms involved in the control of cortical activation and wakefulness, with emphasis on the posterior hypothalamus and histaminergic neurons. Sleep Med Rev 4:471–503

Lin JS, Sergeeva OA, Haas HL (2011a) Histamine H3 receptors and sleep-wake regulation. J Pharmacol Exp Ther 336:17–23

Lin JS, Anaclet C, Sergeeva OA, Haas HL (2011b) The waking brain: an update. Cell Mol Life Sci 68:2499–2512

Liu K, Kim J, Kim DW, Zhang YS, Bao H, Denaxa M, Lim SA, Kim E, Liu C, Wickersham IR, Pachinis V, Hattar S, Song J, Brown SP, Blackshaw S (2017) Lhx6-positive GABA-releasing neurons of the zona incerta promote sleep. Nature 548:582–587

Llinas RR, Steriade M (2006) Bursting of thalamic neurons and states of vigilance. J Neurophysiol 95:3297–3308

Lo CC, Amaral LAN, Havlin S, Ivanov PC, Penzel T, Peter JH, Stanley HE (2002) Dynamics of sleep-wake transitions during sleep. Europhys Lett 57:625–631

Lo C-C, Chou T, Penzel T, Scammell TE, Strecker RE, Stanley HE, Ivanov PC (2004) Common scale-invariant patterns of sleep-wake transitions across mammalian species. Proc Natl Acad Sci U S A 101:17545–17548

Loffler S, Korber J, Nubbemeyer U, Fehsel K (2012) Comment on "Impaired respiratory and body temperature control upon acute serotonergic neuron inhibition". Science 337:646–646

Lőrincz ML, Adamantidis AR (2017) Monoaminergic control of brain states and sensory processing: existing knowledge and recent insights obtained with optogenetics. Prog Neurobiol 151:237–253

Luppi PH, Peyron C, Fort P (2017) Not a single but multiple populations of GABAergic neurons control sleep. Sleep Med Rev 32:85–94

MacLaren DA, Browne RW, Shaw JK, Krishnan Radhakrishnan S, Khare P, Espana RA, Clark SD (2016) Clozapine N-oxide administration produces behavioral effects in long-evans rats: implications for designing DREADD experiments. eNeuro 3

Magill PJ, Bolam JP, Bevan MD (2000) Relationship of activity in the subthalamic nucleus-globus pallidus network to cortical electroencephalogram. J Neurosci 20:820–833

Mahler SV, Aston-Jones G (2018) CNO Evil? considerations for the use of DREADDs in behavioral neuroscience. Neuropsychopharmacology 43:934–936

Mahon S, Vautrelle N, Pezard L, Slaght SJ, Deniau JM, Chouvet G, Charpier S (2006) Distinct patterns of striatal medium spiny neuron activity during the natural sleep-wake cycle. J Neurosci 26:12587–12595

Mandile P, Vescia S, Montagnese P, Romano F, Giuditta A (1996) Characterization of transition sleep episodes in baseline EEG recordings of adult rats. Physiol Behav 60:1435–1439

Maudhuit C, Jolas T, Lainey E, Hamon M, Adrien J (1994) Effects of acute and chronic treatment with amoxapine and cericlamine on the sleep-wakefulness cycle in the rat. Neuropharmacology 33:1017–1025

McCall JG, Al-Hasani R, Siuda ER, Hong DY, Norris AJ, Ford CP, Bruchas MR (2015) CRH engagement of the locus coeruleus noradrenergic system mediates stress-induced anxiety. Neuron 87:606–621

McGinty DJ, Harper RM (1976) Dorsal raphe neurons: depression of firing during sleep in cats. Brain Res 101:569–575

Mileykovskiy BY, Kiyashchenko LI, Siegel JM (2005) Behavioral correlates of activity in identified hypocretin/orexin neurons. Neuron 46:787–798

Miller JD, Farber J, Gatz P, Roffwarg H, German DC (1983) Activity of mesencephalic dopamine and non-dopamine neurons across stages of sleep and waking in the rat. Brain Res 273:133–141

Monaca C, Boutrel B, Hen R, Hamon M, Adrien J (2003) 5-HT1A/1B receptor-mediated effects of the selective serotonin reuptake inhibitor, citalopram, on sleep: studies in 5-HT1A and 5-HT1B knockout mice. Neuropsychopharmacol 28:850–856

Nagai Y, Kikuchi E, Lerchner W, Inoue KI, Ji B, Eldridge MA, Kaneko H, Kimura Y, Oh-Nishi A, Hori Y, Kato Y, Hirabayashi T, Fujimoto A, Kumata K, Zhang MR, Aoki I, Suhara T, Higuchi M, Takada M, Richmond BJ, Minamimoto T (2016) PET imaging-guided chemogenetic silencing reveals a critical role of primate rostromedial caudate in reward evaluation. Nat Commun 7:13605

Nakajima K, Wess J (2012) Design and functional characterization of a novel, arrestin-biased designer G protein-coupled receptor. Mol Pharmacol 82:575–582

Nishida M, Pearsall J, Buckner RL, Walker MP (2009) REM sleep, prefrontal theta, and the consolidation of human emotional memory. Cereb Cortex 19:1158–1166

Oishi Y, Xu Q, Wang L, Zhang BJ, Takahashi K, Takata Y, Luo YJ, Cherasse Y, Schiffmann SN, De Kerchove D'Exaerde A, Urade Y, Qu WM, Huang ZL, Lazarus M (2017) Slow-wave sleep is controlled by a subset of nucleus accumbens core neurons in mice. Nat Commun 8:734

Orzeł-Gryglewska J, Matulewicz P, Jurkowlaniec E (2015) Brainstem system of hippocampal theta induction: the role of the ventral tegmental area. Synapse 69:553–575

Pan WX, McNaughton N (2004) The supramammillary area: its organization, functions and relationship to the hippocampus. Prog Neurobiol 74:127–166

Parmentier R, Ohtsu H, Djebbara-Hannas Z, Valatx J-L, Watanabe T, Lin J-S (2002) Anatomical, physiological, and pharmacological characteristics of histidine decarboxylase knock-out mice: evidence for the role of brain histamine in behavioral and sleep-wake control. J Neurosci 22:7695–7711

Parnaudeau S, O'Neill PK, Bolkan SS, Ward RD, Abbas AI, Roth BL, Balsam PD, Gordon JA, Kellendonk C (2013) Inhibition of mediodorsal thalamus disrupts thalamofrontal connectivity and cognition. Neuron 77:1151–1162

Pedersen NP, Ferrari L, Venner A, Wang JL, Abbott SBG, Vujovic N, Arrigoni E, Saper CB, Fuller PM (2017) Supramammillary glutamate neurons are a key node of the arousal system. Nat Commun 8:1405

Popa D, Duvarci S, Popescu AT, Lena C, Pare D (2010) Coherent amygdalocortical theta promotes fear memory consolidation during paradoxical sleep. Proc Natl Acad Sci U S A 107:6516–6519

Portas CM, Bjorvatn B, Ursin R (2000) Serotonin and the sleep/wake cycle: special emphasis on microdialysis studies. Prog Neurobiol 60:12–35

Qiu MH, Chen MC, Fuller PM, Lu J (2016a) Stimulation of the pontine parabrachial nucleus promotes wakefulness via extra-thalamic forebrain circuit nodes. Curr Biol 26:2301–2312

Qiu MH, Yao QL, Vetrivelan R, Chen MC, Lu J (2016b) Nigrostriatal dopamine acting on globus pallidus regulates sleep. Cereb Cortex 26:1430–1439

Rapanelli M, Frick L, Bito H, Pittenger C (2017) Histamine modulation of the basal ganglia circuitry in the development of pathological grooming. Proc Natl Acad Sci U S A 114:6599–6604

Raper J, Morrison RD, Daniels JS, Howell L, Bachevalier J, Wichmann T, Galvan A (2017) Metabolism and distribution of clozapine-N-oxide: implications for nonhuman primate chemogenetics. ACS Chem Nerosci 8:1570–1576

Rasmussen K, Heym J, Jacobs BL (1984) Activity of serotonin-containing neurons in nucleus centralis superior of freely moving cats. Exp Neurol 83:302–317

Ravassard P, Hamieh AM, Joseph MA, Fraize N, Libourel PA, Lebarillier L, Arthaud S, Meissirel C, Touret M, Malleret G, Salin PA (2016) REM sleep-dependent bidirectional regulation of hippocampal-based emotional memory and LTP. Cereb Cortex 26:1488–1500

Ray RS, Corcoran AE, Brust RD, Kim JC, Richerson GB, Nattie E, Dymecki SM (2011) Impaired respiratory and body temperature control upon acute serotonergic neuron inhibition. Science 333:637–642

Reijmers LG, Perkins BL, Matsuo N, Mayford M (2007) Localization of a stable neural correlate of associative memory. Science 317:1230–1233

Reiner PB, McGeer EG (1987) Electrophysiological properties of cortically projecting histamine neurons of the rat hypothalamus. Neurosci Lett 73:43–47

Renouard L, Billwiller F, Ogawa K, Clément O, Camargo N, Abdelkarim M, Gay N, Scoté-Blachon C, Touré R, Libourel PA, Ravassard P, Salvert D, Peyron C, Claustrat B, Léger L, Salin P, Malleret G, Fort P, Luppi PH (2015) The supramammillary nucleus and the claustrum activate the cortex during REM sleep. Sci Adv 1:e1400177

Rioult-Pedotti MS, Pekanovic A, Atiemo CO, Marshall J, Luft AR (2015) Dopamine promotes motor cortex plasticity and motor skill learning via PLC activation. PLoS One 10:e0124986

Rolls A, Colas D, Adamantidis A, Carter M, Lanre-Amos T, Heller HC, de Lecea L (2011) Optogenetic disruption of sleep continuity impairs memory consolidation. Proc Natl Acad Sci U S A 108:13305–13310

Root DH, Melendez RI, Zaborszky L, Napier TC (2015) The ventral pallidum: subregion-specific functional anatomy and roles in motivated behaviors. Prog Neurobiol 130:29–70

Rorabaugh JM, Chalermpalanupap T, Botz-Zapp CA, Fu VM, Lembeck NA, Cohen RM, Weinshenker D (2017) Chemogenetic locus coeruleus activation restores reversal learning in a rat model of Alzheimer's disease. Brain 140:3023–3038

Roth BL (2016) DREADDs for neuroscientists. Neuron 89:683–694

Saito YC, Tsujino N, Hasegawa E, Akashi K, Abe M, Mieda M, Sakimura K, Sakurai T (2013) GABAergic neurons in the preoptic area send direct inhibitory projections to orexin neurons. Front Neural Circuits 7:192

Sakai K (2011) Sleep-waking discharge profiles of dorsal raphe nucleus neurons in mice. Neuroscience 197:200–224

Sakai K, Takahashi K, Anaclet C, Lin J-S (2010) Sleep-waking discharge of ventral tuberomammillary neurons in wild-type and histidine decarboxylase knock-out mice. Front Behav Neurosci 4:53

Santos LM, Dzirasa K, Kubo R, Silva MTA, Ribeiro S, Sameshima K, Valle AC, Timo-Iaria C (2008) Baseline hippocampal theta oscillation speeds correlate with rate of operant task acquisition. Behav Brain Res 190:152–155

Sapin E, Lapray D, Bérod A, Goutagny R, Léger L, Ravassard P, Clément O, Hanriot L, Fort P, Luppi PH (2009) Localization of the brainstem GABAergic neurons controlling paradoxical (REM) sleep. PLoS One 4:e4272

Sara SJ (2009) The locus coeruleus and noradrenergic modulation of cognition. Nat Rev Neurosci 10:211–223

Sara SJ (2017) Sleep to remember dual perspectives. J Neurosci 37:457–463

Sasaki K, Suzuki M, Mieda M, Tsujino N, Roth B, Sakurai T (2011) Pharmacogenetic modulation of orexin neurons alters sleep/wakefulness states in mice. PLoS One 6:e20360

Satoh S, Matsumura H, Kanbayashi T, Yoshida Y, Urakami T, Nakajima T, Kimura N, Nishino S, Yoneda H (2006) Expression pattern of FOS in orexin neurons during sleep induced by an adenosine A2Areceptor agonist. Behav Brain Res 170:277–286

Schöne C, Burdakov D (2017) Orexin/hypocretin and organizing principles for a diversity of wake-promoting neurons in the brain. Curr Top Behav Neurosci 33:51–74

Schwartz JC (2011) The histamine H3 receptor: from discovery to clinical trials with pitolisant. Br J Pharmacol 163:713–721

Schwarz LA, Miyamichi K, Gao XJ, Beier KT, Weissbourd B, DeLoach KE, Ren J, Ibanes S, Malenka RC, Kremer EJ, Luo L (2015) Viral-genetic tracing of the input–output organization of a central noradrenaline circuit. Nature 524:88–92

Senba E, Daddona PE, Watanabe T, Wu JY, Nagy JI (1985) Coexistence of adenosine deaminase, histidine decarboxylase, and glutamate decarboxylase in hypothalamic neurons of the rat. J Neurosci 5:3393–3402

Slater IH, Jones GT, Moore RA (1978) Inhibition of REM sleep by fluoxetine, a specific inhibitor of serotonin uptake. Neuropharmacol 17:383–389

Sommerfelt L, Ursin R (1987) The effects of zimeldine and alaproclate combined with a small dose of 5-HTP on waking and sleep stages in cats. Behav Brain Res 24:1–10

Soussi R, Zhang N, Tahtakran S, Houser CR, Esclapez M (2010) Heterogeneity of the supramammillary-hippocampal pathways: evidence for a unique GABAergic neurotransmitter phenotype and regional differences. Eur J Neurosci 32:771–785

Stachniak TJ, Ghosh A, Sternson SM (2014) Chemogenetic synaptic silencing of neural circuits localizes a hypothalamus→midbrain pathway for feeding behavior. Neuron 82:797–808

Steinfels GF, Heym J, Strecker RE, Jacobs BL (1983) Behavioral correlates of dopaminergic unit activity in freely moving cats. Brain Res 258:217–228

Stephenson R, Famina S, Caron AM, Lim J (2013) Statistical properties of sleep-wake behavior in the rat and their relation to circadian and ultradian phases. Sleep 36:1377–1390

Steriade M (2004) Acetylcholine systems and rhythmic activities during the waking-sleep cycle. Prog Brain Res 145:179–196

Sternson SM, Roth BL (2014) Chemogenetic tools to interrogate brain functions. Annu Rev Neurosci 37:387–407

Sun HX, Wang DR, Ye CB, Hu ZZ, Wang CY, Huang ZL, Yang SR (2017) Activation of the ventral tegmental area increased wakefulness in mice. Sleep Biol Rhythms 15:107–115

Taheri S, Zeitzer JM, Mignot E (2002) The role of hypocretins (orexins) in sleep regulation and narcolepsy. Annu Rev Neurosci 25:283–313

Takahashi K, Lin J-S, Sakai K (2006) Neuronal activity of histaminergic tuberomammillary neurons during wake-sleep states in the mouse. J Neurosci 26:10292–10298

Takahashi K, Lin JS, Sakai K (2009) Characterization and mapping of sleep-waking specific neurons in the basal forebrain and preoptic hypothalamus in mice. Neuroscience 161:269–292

Takahashi K, Kayama Y, Lin JS, Sakai K (2010) Locus coeruleus neuronal activity during the sleep-waking cycle in mice. Neuroscience 169:1115–1126

Takeda N, Inagaki S, Shiosaka S, Taguchi Y, Oertel WH, Tohyama M, Watanabe T, Wada H (1984) Immunohistochemical evidence for the coexistence of histidine decarboxylase-like and glutamate decarboxylase-like immunoreactivities in nerve cells of the magnocellular nucleus of the posterior hypothalamus of rats. Proc Natl Acad Sci U S A 81:7647–7650

Thinschmidt JS, Kinney GG, Kocsis B (1995) The supramammillary nucleus: is it necessary for the mediation of hippocampal theta rhythm? Neuroscience 67:301–312

Tritsch NX, Granger AJ, Sabatini BL (2016) Mechanisms and functions of GABA co-release. Nat Rev Neurosci 17:139–145

Trottier S, Chotard C, Traiffort E, Unmehopa U, Fisser B, Swaab DF, Schwartz JC (2002) Co-localization of histamine with GABA but not with galanin in the human tuberomamillary nucleus. Brain Res 939:52–64

Tsunematsu T, Kilduff TS, Boyden ES, Takahashi S, Tominaga M, Yamanaka A (2011) Acute optogenetic silencing of orexin/hypocretin neurons induces slow-wave sleep in mice. J Neurosci 31:10529–10539

Tsunematsu T, Ueno T, Tabuchi S, Inutsuka A, Tanaka KF, Hasuwa H, Kilduff TS, Terao A, Yamanaka A (2014) Optogenetic manipulation of activity and temporally controlled cell-specific ablation reveal a role for MCH neurons in sleep/wake regulation. J Neurosci 34:6896–6909

Urbain N, Gervasoni D, Soulière F, Lobo L, Rentéro N, Windels F, Astier B, Savasta M, Fort P, Renaud B, Luppi PH, Chouvet G (2000) Unrelated course of subthalamic nucleus and globus pallidus neuronal activities across vigilance states in the rat. Eur J Neurosci 12:3361–3374

Urban DJ, Roth BL (2015) DREADDs (designer receptors exclusively activated by designer drugs): chemogenetic tools with therapeutic utility. Annu Rev Pharmacol Toxicol 55:399–417

Ursin R (2002) Serotonin and sleep. Sleep Med Rev 6:55–67

Valencia Garcia S, Libourel PA, Lazarus M, Grassi D, Luppi PH, Fort P (2017) Genetic inactivation of glutamate neurons in the rat sublaterodorsal tegmental nucleus recapitulates REM sleep behaviour disorder. Brain 140:414–428

Valencia Garcia S, Brischoux F, Clément O, Libourel PA, Arthaud S, Lazarus M, Luppi PH, Fort P (2018) Ventromedial medulla inhibitory neuron inactivation induces REM sleep without atonia and REM sleep behavior disorder. Nat Commun 9:504

Van Dort CJ, Zachs DP, Kenny JD, Zheng S, Goldblum RR, Gelwan NA, Ramos DM, Nolan MA, Wang K, Weng F-J, Lin Y, Wilson MA, Brown EN (2015) Optogenetic activation of cholinergic neurons in the PPT or LDT induces REM sleep. Proc Natl Acad Sci U S A 112:584–589

Vanni-Mercier G, Gigout S, Debilly G, Lin JS (2003) Waking selective neurons in the posterior hypothalamus and their response to histamine H3-receptor ligands: an electrophysiological study in freely moving cats. Behav Brain Res 144:227–241

Vardy E, Robinson JE, Li C, Olsen RHJ, DiBerto JF, Giguere PM, Sassano FM, Huang XP, Zhu H, Urban DJ, White KL, Rittiner JE, Crowley NA, Pleil KE, Mazzone CM, Mosier PD, Song J, Kash TL, Malanga CJ, Krashes MJ, Roth BL (2015) A new DREADD facilitates the multiplexed chemogenetic interrogation of behavior. Neuron 86:936–946

Varin C, Arthaud S, Salvert D, Gay N, Libourel PA, Luppi PH, Léger L, Fort P (2016) Sleep architecture and homeostasis in mice with partial ablation of melanin-concentrating hormone neurons. Behav Brain Res 298:100–110

Varin C, Luppi PH, Fort P (2018) Melanin-concentrating hormone-expressing neurons adjust slow-wave sleep dynamics to catalyze paradoxical (REM) sleep. Sleep 41(6). https://doi.org/10.1093/sleep/zsy068

Vazey EM, Aston-Jones G (2014) Designer receptor manipulations reveal a role of the locus coeruleus noradrenergic system in isoflurane general anesthesia. Proc Natl Acad Sci U S A 111:3859–3864

Venner A, Anaclet C, Broadhurst RY, Saper CB, Fuller PM (2016) A novel population of wake-promoting GABAergic neurons in the ventral lateral hypothalamus. Curr Biol 26:2137–2143

Vescia S, Mandile P, Montagnese P, Romano F, Cataldo G, Cotugno M, Giuditta A (1996) Baseline transition sleep and associated sleep episodes are related to the learning ability of rats. Physiol Behav 60:1513–1525

Vetrivelan R, Kong D, Ferrari LL, Madara JC, Bandaru S, Lowell BB (2016) Melanin-concentrating hormone neurons specifically promote rapid eye movement sleep in mice. Neuroscience 336:102–113

Vyazovskiy VV, Olcese U, Hanlon EC, Nir Y, Cirelli C, Tononi G (2011) Local sleep in awake rats. Nature 472:443–447

Watson CJ, Lydic R, Baghdoyan HA (2011) Sleep duration varies as a function of glutamate and GABA in rat pontine reticular formation. J Neurochem 118:571–580

Watson CJ, Baghdoyan HA, Lydic R (2012) Neuropharmacology of sleep and wakefulness: 2012 update. Sleep Med Clin 7:469–486

Weber F, Chung S, Beier KT, Xu M, Luo L, Dan Y (2015) Control of REM sleep by ventral medulla GABAergic neurons. Nature 526:435–438

Wieland S, Schindler S, Huber C, Kohr G, Oswald MJ, Kelsch W (2015) Phasic dopamine modifies sensory-driven output of striatal neurons through synaptic plasticity. J Neurosci 35:9946–9956

Williams RH, Jensen LT, Verkhratsky A, Fugger L, Burdakov D (2007) Control of hypothalamic orexin neurons by acid and CO2. Proc Natl Acad Sci U S A 104:10685–10690

Williams RH, Chee MJS, Kroeger D, Ferrari LL, Maratos-Flier E, Scammell TE, Arrigoni E (2014) Optogenetic-mediated release of histamine reveals distal and autoregulatory mechanisms for controlling arousal. J Neurosci 34:6023–6029

Wise A, Jupe SC, Rees S (2004) The identification of ligands at orphan G-protein coupled receptors. Annu Rev Pharmacol Toxicol 44:43–66

Xu M, Chung S, Zhang S, Zhong P, Ma C, Chang WC, Weissbourd B, Sakai N, Luo L, Nishino S, Dan Y (2015) Basal forebrain circuit for sleep-wake control. Nat Neurosci 18:1641–1647

Yamanaka A, Beuckmann CT, Willie JT, Hara J, Tsujino N, Mieda M, Tominaga M, Yagami KI, Sugiyama F, Goto K, Yanagisawa M, Sakurai T (2003) Hypothalamic orexin neurons regulate arousal according to energy balance in mice. Neuron 38:701–713

Yokota S, Kaur S, Vanderhorst VG, Saper CB, Chamberlin NL (2015) Respiratory-related outputs of glutamatergic, hypercapnia-responsive parabrachial neurons in mice. J Comp Neurol 523:907–920

Yu X, Ye Z, Houston CM, Zecharia AY, Ma Y, Zhang Z, Uygun DS, Parker S, Vyssotski AL, Yustos R, Franks NP, Brickley SG, Wisden W (2015) Wakefulness is governed by GABA and histamine cotransmission. Neuron 87:164–178

Yu X, Franks NP, Wisden W (2018) Sleep and sedative states induced by targeting the histamine and noradrenergic systems. Front Neural Circuits 12:4

Yuan XS, Wang L, Dong H, Qu WM, Yang SR, Cherasse Y, Lazarus M, Schiffmann SN, De Kerchove D'Exaerde A, Li RX, Huang ZL (2017) Striatal adenosine A2Areceptor neurons control active-period sleep via parvalbumin neurons in external globus pallidus. Elife 6:e29055

Zhang Z, Ferretti V, Güntan I, Moro A, Steinberg EA, Ye Z, Zecharia AY, Yu X, Vyssotski AL, Brickley SG, Yustos R, Pillidge ZE, Harding EC, Wisden W, Franks NP (2015) Neuronal ensembles sufficient for recovery sleep and the sedative actions of α2 adrenergic agonists. Nat Neurosci 18:553–561

Zhu H, Pleil KE, Urban DJ, Moy SS, Kash TL, Roth BL (2014) Chemogenetic inactivation of ventral hippocampal glutamatergic neurons disrupts consolidation of contextual fear memory. Neuropsychopharmacology 39:1880–1892

Clinical and Experimental Human Sleep-Wake Pharmacogenetics

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Abstract

Sleep and wakefulness are highly complex processes that are elegantly orchestrated by fine-tuned neurochemical changes among neuronal and non-neuronal ensembles, nuclei, and networks of the brain. Important neurotransmitters and neuromodulators regulating the circadian and homeostatic facets of sleep-wake physiology include melatonin, γ-aminobutyric acid, hypocretin, histamine, norepinephrine, serotonin, dopamine, and adenosine. Dysregulation of these neurochemical systems may cause sleep-wake disorders, which are commonly classified into insomnia disorder, parasomnias, circadian rhythm sleep-wake disorders, central disorders of hypersomnolence, sleep-related movement disorders, and sleep-related breathing disorders. Sleep-wake disorders can have far-reaching consequences on physical, mental, and social well-being and health and, thus, need be treated with effective and rational therapies. Apart from behavioral (e.g., cognitive behavioral therapy for insomnia), physiological (e.g., chronotherapy with bright light), and mechanical (e.g., continuous positive airway pressure treatment of obstructive sleep apnea) interventions, pharmacological treatments often are the first-line clinical option to improve disturbed sleep and wake states. Nevertheless, not all patients respond to pharmacotherapy in uniform and beneficial fashion, partly due to genetic differences. The improved understanding of the neurochemical mechanisms regulating sleep and wakefulness and the mode of action of sleep-wake therapeutics has provided a conceptual framework, to search for functional genetic variants modifying individual drug response phenotypes. This article will summarize the currently known genetic polymorphisms that modulate drug sensitivity and exposure, to partly determine individual responses to sleep-wake pharmacotherapy. In addition, a pharmacogenetic strategy will be outlined how based upon classical and opto-/chemogenetic strategies in animals, as well as human genetic associations, circuit mechanisms regulating sleep-wake functions in humans can be identified. As such, experimental human sleep-wake pharmacogenetics forms a bridge spanning basic research and clinical medicine and constitutes an essential step for the search and development of novel sleep-wake targets and therapeutics.

Keywords
Adenosine · Circadian · Dopamine · GABA · H_3 receptor · Melatonin · Orexin · Pharmacodynamics · Pharmacokinetics · Polymorphism

1 Introduction

Wakefulness and sleep are complex behavioral processes, which are elegantly orchestrated by fine-tuned interactions of neurotransmitter and neuromodulator systems within defined wake-sleep circuits of the brain. Over the past 100 years, clinical observations in patients suffering from sleep-wake disorders and basic research spanning from in vitro preparations and animal models to healthy human volunteers have led to a widely accepted neurobiological model underlying the physiological alterations between wakefulness and distinct sleep states (for review, see Holst et al. 2016). More specifically, wakefulness and cortical arousal are thought to be governed primarily by concerted activity in upper brain stem and hypothalamic nuclei, which produce acetylcholine (ACh), norepinephrine (NE), serotonin (5-HT; 5-hydroxy-tryptamine), dopamine (DA), histamine (His), and hypocretin (Hcrt; aka orexin) as their neurotransmitters. These nuclei activate thalamus, cortex, and spinal cord and inhibit the sleep-promoting ventrolateral preoptic (VLPO) area of the hypothalamus.

The regulation of sleep differs substantially between the non-rapid eye movement (NREM) and rapid eye movement (REM) sleep states. NREM sleep is supposed to be promoted by GABA (γ-aminobutyric acid)- and galanin-containing neurons originating in VLPO and median preoptic nuclei of the hypothalamus. These nuclei inhibit the wake-promoting arousal systems described above. On the other hand, REM sleep exhibits both "wake-like" and "sleep-like" characteristics. "Wake-on"-"REM-on" basal forebrain (BF) and brain stem nuclei containing ACh and glutamate activate the BF and cortex, induce muscle atonia, and promote rapid eye movements. In addition, hypothalamic neurons containing melanin-concentrating hormone suppress the activity of "wake-on"-"REM-off" cell groups, which primarily include the upper brainstem monoaminergic nuclei.

Powerful new tools to interrogate sleep-wake regulating circuits, including sophisticated optogenetic and chemogenetic experiments, have revealed new molecular and cellular markers, as well as novel network mechanisms and pathways in sleep-wake regulation. These recent insights may necessitate an adaptation of the basic sleep-wake neurochemistry described above. They have been discussed in comprehensive reviews and other chapters of this book (Adamantidis and Luthi 2018; Saper and Fuller 2017; Tyree and de Lecea 2017; Eban-Rothschild et al. 2018; Luppi and Fort 2018; Varin and Bonnavion 2018) and are not the focus of this chapter. In this article, firstly, genetic aspects of the current pharmacotherapy of sleep-wake disorders will be reviewed. Secondly, an experimental pharmacogenetic strategy to identify circuit mechanisms underlying sleep-wake regulation in humans will be outlined.

2 Current Sleep-Wake Pharmacotherapy in a Nutshell

Based on the "standard model" of sleep-wake circuitry, subtle changes in important neurotransmitters and neuromodulators (including NE, 5-HT, DA, His, Hcrt, melatonin, glutamate, ACh, GABA, and adenosine) regulate not only the transitions among the behavioral states wakefulness, REM sleep, and NREM sleep but also their maintenance (Holst and Landolt 2018). Dysregulation of these fine-tuned neurochemical systems can lead to sleep-wake disturbances. According to the third edition of the *International Classification of Sleep Disorders*, sleep-wake disturbances are subdivided into the following major diagnostic sections: insomnia disorder, circadian rhythm sleep-wake disorders, sleep-related breathing disorders, sleep-related movement disorders, central disorders of hypersomnolence, parasomnias, and other sleep disorders (American Academy of Sleep Medicine 2014). Some of these pathologies are typically treated with pharmacological agents as summarized and reviewed in Part III of this book (insomnia and circadian rhythm sleep-wake disorder, Spiegelhalder et al. (2017); hypersomnias and sleep-related movement disorders, Baumann (2018)). All drugs presently used to treat the symptoms of sleep-wake disorders interfere with the neurochemical and neuromodulatory systems mentioned above (Idzikowski 2014; Holst et al. 2016). Thus, although the "standard model" of the neuroanatomical and neurochemical regulation of wakefulness and sleep will have to be adapted, it provides a useful conceptual framework for understanding the effects of the medications currently used to improve sleep and wake states (Fig. 1).

2.1 Insomnia Disorder

Drug treatment can be useful for short-term alleviation of insomnia symptoms. When combined with behavioral therapy, pharmacological treatments show durable improvements in sleep patterns (Riemann and Perlis 2009). Conceptually, disturbed sleep can be pharmacologically improved by compounds that either promote sleep or dampen the wake-promoting systems.

Agonistic modulators of $GABA_A$ receptors such as benzodiazepines and Z-drugs (e.g., zolpidem) enhance the inhibitory inputs of GABA-containing neurons that inhibit the ascending arousal pathways (Finelli et al. 2000). These compounds act as positive allosteric modulators and, at higher doses, as agonists of $GABA_A$ receptors. They are prescribed for the short-term pharmacological treatment of insomnia disorder and show demonstrated efficacy in shortening sleep latency and improving sleep maintenance (Dresler et al. 2014). Absolute effect sizes, however, may be rather small when compared to placebo (Wilt et al. 2016). In standard clinical practice of pharmacologically treating insomnia, benzodiazepines and Z-drugs have largely replaced the previously used barbiturates.

The dual orexin receptor antagonist (DORA), suvorexant, was recently approved by the US Food and Drug Administration (FDA) for treating insomnia disorder in adults (Wilt et al. 2016). Suvorexant acts by antagonizing wake-promoting Hcrt neurons located in the lateral hypothalamus. Some authors claim that suvorexant

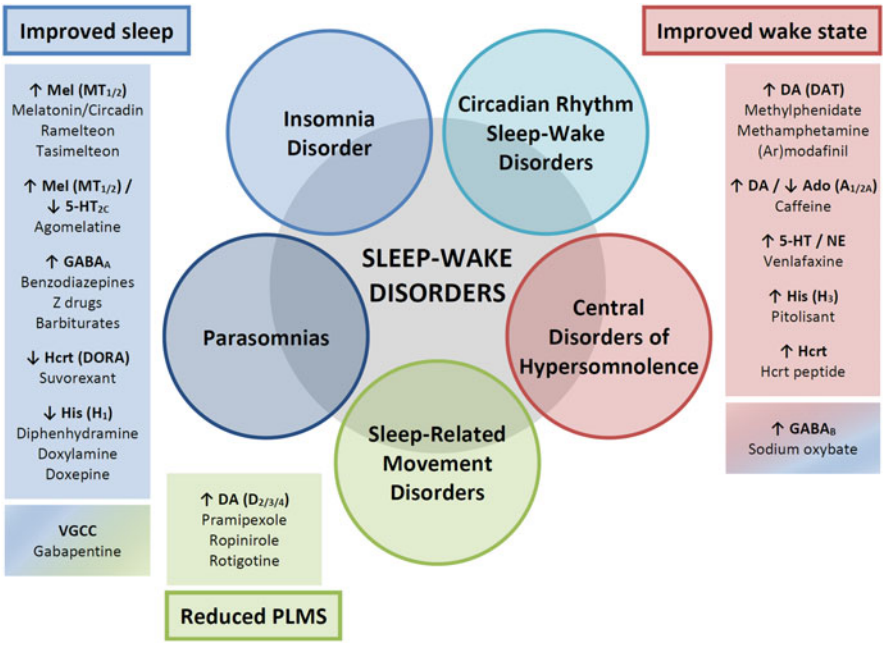

Fig. 1 Schematic representation of sleep-wake disorders according to the International Classification of Sleep Disorders (ICSD) currently amenable to pharmacotherapy for improved sleep and wake state (thus excluding sleep-related breathing disorders). Blue shading: drugs used to improve sleep in insomnia disorder, parasomnias, and circadian rhythm sleep-wake disorders. Red shading: drugs to improve wake state in central disorders of hypersomnolence. Green shading: drugs to reduce periodic limb movements in sleep (PLMS) in sleep-related movement disorders. *Mel* melatonin, *$MT_{1/2}$* melatonin MT_1 and MT_2 receptors, *5-HT* serotonin, *$5\text{-}HT_{2C}$* serotonin-2C receptor, *GABA* γ-aminobutyric acid, *$GABA_A$* $GABA_A$ receptor, *$GABA_B$* $GABA_B$ receptor, *His* histamine, *H_1* histamine H_1 receptor, *H_3* histamine H_3 receptor, *VGCC* voltage-gated calcium channel, *DA* dopamine, *DAT* dopamine transporter, *$D_{2/3/4}$* dopamine D_2, D_3 and D_4 receptors, *Ado* adenosine, *$A_{1/2A}$* adenosine A_1 and A_{2A} receptors, *NE* norepinephrine, *Hcrt* hypocretin. ↑ amplification of neurotransmission; ↓ inhibition of neurotransmission

elicits more physiological sleep than benzodiazepines (Patel et al. 2015). Nevertheless, the compound seems to mainly prolong REM sleep and stage 2 sleep, with marginal effect on deep slow-wave sleep (Hoyer and Jacobson 2013).

Drugs antagonizing key monoaminergic nuclei of the ascending arousal pathways commonly cause sleepiness. One example of such a mode of action is the blockade of H_1 receptors to antagonize wake promotion by His. "Antihistamines" such as diphenhydramine and doxylamine have long been available over the counter and are sometimes used to treat insomnia. Similarly, tricyclic and heterocyclic antidepressants and antipsychotics are often associated with sleepiness, probably related to their antihistamine and possibly other "off-target" receptor interactions (such as anticholinergic effects). Low doses of doxepin are FDA-approved to treat insomnia by promoting sleep onset (Goforth 2009). Other sedating antidepressant

and antipsychotic medications are more and more commonly prescribed "off-label" as sleeping pills (Proctor and Bianchi 2012; Krystal et al. 2013). Apart from H_1 and muscarinic ACh receptors, some of these agents (e.g., mirtazapine) have high affinity for 5-HT_{2A} and adrenergic α_1 receptors. Blockade of these receptors facilitates sleep. Conversely, these compounds often also inhibit 5-HT and NE reuptake transporters and antagonize 5-HT_{2C} and α_2 receptors and thus promote wakefulness, which detracts from the sleep-promoting effects of blocking the H_1 receptors (Krystal et al. 2013).

2.2 Parasomnias

The most commonly used drugs for NREM sleep parasomnias are intermediate- and long-acting benzodiazepines and antidepressants (Castelnovo et al. 2018). The pharmacologic treatment of REM sleep behavior disorder presently relies primarily on the benzodiazepine, clonazepam, whereas melatonin and melatonin receptor agonists may be equally efficacious with less unwanted effects (McGrane et al. 2015; Castelnovo et al. 2018).

2.3 Circadian Rhythm Sleep-Wake Disorders

The circadian resetting effects of melatonin are well documented. Interference with both melatonin MT_1 and MT_2 receptor subtypes contributes to the distinct phase-response profile of melatonin (Burgess and Emens 2018). Melatonin alone or in combination, as well as different melatonin analogues, are available either without prescription or as regulatory-approved drugs. There is increasing interest in the potential of melatonin-based therapies in the treatment of disturbed sleep (Alston et al. 2018). These therapies offer potential to treat sleep and mood disorders, especially when circadian rhythm disturbances are also present. Such disorders include insomnia induced by shifted sleep-wake cycles in healthy individuals, insomnia in older adults, delayed sleep-wake phase disorder, non-24-hour sleep-wake disorder (N24HSWD), seasonal affective disorder, and major depressive disorder (Alston et al. 2018; Burgess and Emens 2018). Prolonged-release melatonin (Circadin®) has been approved for chronic insomnia in patients older than 55 years. In combination with antihistamines, GABA-ergic hypnotics, herbal extracts, or bright light therapy, prolonged-release melatonin may improve hypnotic efficacy (Saxvig et al. 2014; Rivara et al. 2015). Apart from N24HSWD, the $MT_{1/2}$ receptor agonist, tasimelteon, may also be useful in certain patients with insomnia, advanced sleep phase syndrome, and delayed sleep phase syndrome (Lankford 2011). Ramelteon received FDA approval for treatment of insomnia in older adults, and the $MT_{1/2}$ agonist/5-HT_{2C} receptor antagonist, agomelatine, has EMA and TGA (Therapeutic Goods Administration, Australia) approval for major depressive

disorder. Agomelatine phase shifts the circadian system and improves sleep in depressed patients, although its usefulness for primary sleep disorders is debated (De Berardis et al. 2015).

2.4 Central Disorders of Hypersomnolence

Central disorders of hypersomnolence are characterized by excessive daytime sleepiness (EDS), often despite apparently normal quality, duration, and timing of nocturnal sleep. These disorders include narcolepsy type-1 (characterized by deficient Hcrt in cerebrospinal fluid), narcolepsy type-2, and idiopathic hypersomnia. Their pharmacological treatment focuses on the management of EDS, with additional treatment of cataplexy, which is typically present in patients with narcolepsy type-1 (Baumann 2018).

Modafinil and its (R)-enantiomer, armodafinil, are FDA-approved first-line treatments of EDS in narcolepsy (Mignot 2012; Abad and Guilleminault 2017). While the mode of action of (ar)modafinil is complex and may involve NE, 5-HT, His, Hcrt, glutamate, and GABA, it increases DA-ergic neurotransmission and blocks the DA reuptake transporter (DAT) (Wisor et al. 2001; Volkow et al. 2009; Wisor 2018).

Amphetamine and its derivatives are treatments of second choice against EDS in narcolepsy (Abad and Guilleminault 2017). These compounds actively promote the presynaptic release of DA and dampen DAT function (Mignot 2012). They cause autonomic arousal and psychomotor agitation and have strong addictive properties. Stimulants such as methylphenidate and mazindol are specific DAT inhibitors. Methylphenidate is FDA-approved for narcolepsy. Preliminary evidence also suggests therapeutic efficacy for mazindol (Nittur et al. 2013).

Certain antidepressants such as bupropion, venlafaxine, and clomipramine that block the reuptake transporters for DA (DAT), NE (NET), and/or 5-HT (SERT) are typically used against cataplexy in narcolepsy type 1 (Mignot 2012). Tricyclic antidepressants strongly reduce REM sleep and cataplectic attacks without affecting EDS (Houghton et al. 2004). They have undesired anticholinergic and antihistaminic properties and prolonged use can cause tolerance. Dual NET-SERT inhibitors or selective NE and 5-HT reuptake inhibitors (SSRIs) may provide alternative options to treat cataplexy (Houghton et al. 2004). Finally, monoamine oxidase (MAO) inhibitors such as selegiline are used "off-label" against cataplexy and EDS (Thorpy and Dauvilliers 2015).

The adenosine A_1 and A_{2A} receptor antagonist, caffeine, is the most widely used stimulant worldwide. Caffeine can improve vigilance in susceptible, sleep-deprived individuals (Gottselig et al. 2006; Rétey et al. 2007) yet probably has insufficient potency in patients with clinical EDS (Banerjee et al. 2004). Sleepy people may use caffeine excessively to improve alertness (Urry et al. 2017), and a positive correlation between disease severity and habitual caffeine use was observed in patients with narcolepsy-cataplexy (Mitler et al. 1998). Intake of 200–300 mg per day, which is

typical in Western societies, is generally well tolerated and does not posit a known health risk (Loftfield et al. 2018).

The safe improvement of wakefulness in hypersomnolent patients has so far proved difficult, despite the fact that the abovementioned compounds are potent. Based on the rationale that sleep and wakefulness form a functional continuum and that improved sleep also improves the quality of wakefulness, γ-hydroxybutyrate (GHB or the sodium salt of γ-hydroxybutyric acid, sodium oxybate) is currently the first-line, FDA-approved agent for cataplexy and EDS in patients with narcolepsy (Thorpy and Dauvilliers 2015; Abad and Guilleminault 2017). This rapid sedative may also alleviate sleep disruption in narcolepsy. The exact mode of action of GHB is unknown. GHB is an endogenous short-chain fatty acid that exhibits high affinity for GHB receptors ($K_D \approx$ 30–580 nM) and low affinity for $GABA_B$ receptors ($K_D \approx$ 2.6–16 μM) (Mamelak 2009). The stabilization of sleep continuity by supraphysiological doses of GHB may be predominantly mediated by agonistic stimulation of $GABA_B$ receptors. Thus, the $GABA_B$ receptor agonist, baclofen, may also be useful in treating patients with narcolepsy (Huang and Guilleminault 2009; Black et al. 2014).

Histamine H_3 receptor inverse agonists such as pitolisant provide a promising new class of wake-promoting medications with possible pro-cognitive properties (Stocking and Letavic 2008). The H_3 receptor is an inhibitory autoreceptor on presynaptic neurons, which attenuates the release of His and other excitatory neurotransmitters when activated (Lin et al. 2011).

It has been postulated that Hcrt neurons continuously integrate information from multiple processes, to modulate the propensity to stay awake or to fall asleep (Eban-Rothschild et al. 2018). The firing of Hcrt neurons in the lateral/posterior hypothalamus promotes wakefulness. Intranasal delivery of Hcrt peptide may reduce wake-REM sleep transitions and slightly improve attention after sleep in patients with narcolepsy-cataplexy (Weinhold et al. 2014).

2.5 Sleep-Related Movement Disorders

The pharmacologic treatment of restless legs syndrome (RLS) and periodic limb movement disorder during sleep traditionally relies on the dopamine D_2 receptor agonists, pramipexole, ropinirole and rotigotine (Baumann 2018). These dopaminergic agents, and in particular L-dopa, carry the risk of augmentation, i.e., aggravated RLS symptoms with continued medication use (Trenkwalder and Paulus 2010). Iron supplementation in case of iron deficiency, benzodiazepines, and opiates may also be effective and are often used as secondary treatments (Winkelmann et al. 2018). Prolonged-release oxycodone-naloxone, a combined opioid analgesic and opioid receptor antagonist, has recently provided an additional option for refractory RLS (Trenkwalder et al. 2013).

Because of the frequently observed augmentation with dopaminergic agents, an international task force recently recommended α2β receptor ligands of voltage-gated calcium channels, such as gabapentin and pregabalin, as first-line option to treat

restless legs (Garcia-Borreguero et al. 2016). Gabapentin and pregabalin are structurally related to GABA, increase GABA concentration the human brain (Cai et al. 2012), and inhibit calcium currents. The derivative gabapentin enacarbil was recently approved by FDA for RLS. In contrast to the dopaminergic drugs, gabapentin enacarbil does not lead to augmentation.

Based on the hypothesis that brain iron deficiency in RLS is associated with a hypoadenosinergic state, a preliminary study recently suggested that the nonselective ENT1/ENT2 (equilibrative nucleoside transporter) inhibitor, dipyridamole, has significant therapeutic effects on sensory and motor symptoms, as well as sleep (Garcia-Borreguero et al. 2018).

2.6 Sleep-Related Breathing Disorders

Sleep-related breathing disorders include upper airway obstructions (obstructive sleep apnea [OSA]) and ceased or decreased ventilatory effort (central sleep apnea) during sleep. While OSA is most commonly treated with continuous positive airway pressure (CPAP) therapy, the therapeutic success of CPAP and other available therapies is often hampered by limited compliance or efficacy (Hedner and Zou 2018). These and other reasons warrant the scientific search for pharmacological remedies of OSA. Nevertheless, currently no pharmacologic alternatives to CPAP are available. Promising research has recently characterized different phenotypes of OSA, which may unearth novel strategies and targets for drug development (Hedner and Zou 2018).

3 Variable Drug Response Genetics

3.1 Gene Polymorphisms Modulating Drug Sensitivity and Exposure Can Determine Individual Pharmacotherapeutic Responses

It has long been recognized that not all patients respond to pharmacotherapy in a uniform and beneficial fashion. How a therapeutic compound affects the organism and its physiology (pharmacodynamics) and how the organism acts on such a compound (pharmacokinetics) are highly individual processes, which are, in part, genetically determined. Pharmacogenetic approaches aim at elucidating genetically determined interindividual differences in drug responses, with the goal to discriminate responders from nonresponders and to minimize toxicity and adverse drug reactions. Such studies have shown that single nucleotide polymorphisms (SNPs), variable-number-tandem-repeat (VNTR) polymorphisms, copy number variations, and insertions/deletions in genes regulating the pharmacodynamics (e.g., receptors, ion channels, enzymes, immune molecules) of medications and/or their pharmacokinetics (e.g., metabolizing enzymes, transporters, plasma protein binding) contribute to wanted and adverse actions of pharmacotherapeutic agents (Evans and Relling 1999; Sadee and Dai 2005; Roden et al. 2011; Holst et al. 2016).

Genes modulating the pharmacodynamic response to a pharmacological treatment may code for the direct targets of drugs (e.g., receptors) or for proteins that synthesize, clear, or degrade the endogenous ligands of these targets (e.g., neurotransmitters). Functional genetic variation in these genes, thus, possibly affects drug sensitivity.

The field of pharmacogenetics has historically focused on genetically determined differences in the activity of drug-metabolizing enzymes. Functional variants of genes encoding such enzymes can modulate exposure to therapeutic agents and, thus, their efficacy and toxicity. For example, more than 80% of all clinically used drugs are metabolized by cytochrome-P450 (CYP) isoenzymes that are encoded in humans by 57 genes of 3 major families (Ingelman-Sundberg 2004). Functional variants of the *CYP1A2*, *CYP2D6*, *CYP2C8/9*, *CYP2C19*, and *CYP3A4/5* genes are of particular interest because they have been associated with altered clinical outcomes (Evans and Relling 1999; Ingelman-Sundberg 2004). Other known genetic variants affecting drug exposure include conjugation enzymes, such as uridine-5′-diphospho-glucuronosyltransferase (UGT) and *N*-acetyltransferase 2 (NAT2), and drug transporters, including solute carrier (SLC) and ATP-binding cassette (ABC) transporter families (Kerb 2006).

The main targets for the wanted effects, as well as the main metabolism and excretion pathways of the sleep-wake therapeutics introduced in the previous paragraphs, are summarized in Table 1. In the following section, known genetic differences in pharmacodynamics and pharmacokinetics of these drugs and drug classes will be reviewed. The contents of this section have been elaborated and updated based on a recent comprehensive overview (Holst et al. 2016). For that purpose, PubMed searches were performed between March 2015 and July 2018, including as search terms all generic drug names mentioned below AND ("pharmacogenetics" OR "polymorphism" OR "CYP" OR "therapy"). A total of 79 references was identified, yet those articles whose content was unrelated to the present review are not cited here.

3.2 Pharmacotherapy of Insomnia Disorder and Parasomnias

3.2.1 Benzodiazepines, Z-Drugs, and Barbiturates

Genetic variants of the α subunits of $GABA_A$ receptors alter the sensitivity to benzodiazepines and Z-drugs in transgenic animals (Tobler et al. 2001; Cope et al. 2004; Kopp et al. 2004), the treatment responses to antiepileptic drugs (Hung et al. 2013), and the sensitivity toward diazepam in alcoholic patients (Iwata et al. 1999). No studies on the relevance of $GABA_A$ receptor polymorphisms for variable hypnotic effects of benzodiazepines and Z-drugs in insomnia patients are currently available.

Most benzodiazepines and Z-drugs are degraded by CYP3A4/5 and CYP2C19 isoenzymes (Hohmann et al. 2016). Genetic variants of these enzymes affect the pharmacokinetics of various members of these drug classes (Park et al. 2006; Shen et al. 2013). Individual CYP isoenzyme metabolizer status is important for

Table 1 Currently available pharmacological treatments of sleep-wake disorders

Drug class	Approved compound	Sleep-wake indication(s)	Wanted sleep-wake effect(s)	Main mode of action for sleep-wake effect	Main metabolism/excretion pathways
Benzodiazepines	Quazepam Estazolam Flurazepam Triazolam Temazepam	Insomnia	Improved sleep	Positive agonistic modulation of $GABA_A$ receptors	CYP3A4/5, CYP2C19, UGT isoenzymes, NAT2
	Clonazepam	Parasomnias	Improved sleep	Positive agonistic modulation of $GABA_A$ receptors	CYP3A4/5, NAT2
Z-drugs	Zolpidem (Es)Zopiclone Zaleplon	Insomnia	Improved sleep	Positive agonistic modulation of $GABA_A$ receptors	CYP3A4, aldehyde oxidase
Barbiturates	Butabarbital Secobarbital	Insomnia (rarely used)	Improved sleep	$GABA_A$ receptor agonism	CYP1A2, CYP2C9/10, CYP3A4
GHB/$BABA_B$ receptor agonist	Sodium oxybate	Narcolepsy	Reduced cataplexy and improved wake state (EDS)	GHB/$GABA_B$ receptor agonism	GHB dehydrogenase → succinic acid → Krebs cycle → CO_2 exhalation
DORA	Suvorexant	Insomnia	Improved sleep	Hcrt-1/2 receptor antagonism	CYP3A4/5, CYP2C19
Antihistamines	Diphenhydramine Doxylamine	(Insomnia)	Improved sleep	H_1 receptor antagonism	CYP2D6, CYP1A2, CYP2C9
TCA	Doxepine	Insomnia	Improved sleep	H_1 receptor antagonism	CYP2D6, CYP2C19
TeCA	Mirtazapine	Insomnia	Improved sleep	H_1 receptor antagonism, 5-HT_{2A} antagonism	CYP2D6, CYP1A2, CYP3A4

(continued)

Table 1 (continued)

Drug class	Approved compound	Sleep-wake indication(s)	Wanted sleep-wake effect(s)	Main mode of action for sleep-wake effect	Main metabolism/excretion pathways
Melatonin	Melatonin/ Circadin® (prolonged release)	Adult insomnia ≥ 55 year; N24HSWD; DSPD; transient insomnia; SAD	Improved sleep and wake state	$MT_{1/2}$ receptor agonism	CYP1A2 → Sulphamethoxymelatonin (urine), CYP3A4
Melatonin agonists	Ramelteon	Sleep onset insomnia in older adults	Improved sleep	$MT_{1/2}$ receptor agonism	CYP1A2, CYP3A4
	Tasimelteon	N24HSWD; transient insomnia	Improved sleep and wake state	$MT_{1/2}$ receptor agonism	CYP1A2, CYP3A4
	Agomelatine	MDD	Antidepressant/ anxiolytic, beneficial effect on sleep	5-HT_{2C} antagonism/$MT_{1/2}$ receptor agonism	CYP1A2, CYP3A4
Psychostimulants	Methylphenidate	Narcolepsy	Improved wake state (EDS)	NET-DAT inhibition	CES1
	Methamphetamine	Narcolepsy	Improved wake state (EDS)	NE, DA, and 5-HT release NET-DAT inhibition	Urinary excretion as parent drug and/or D-amphetamine
	Caffeine	n/a	Improved wake state (EDS)	$A_{1/2A}$ receptor antagonism	CYP1A2
Wake promoting agents	(Ar)Modafinil	Narcolepsy, shiftwork sleep disorder, OSA	Improved wake state (EDS)	Unknown, DAT inhibition	Amide hydrolysis, CYP-mediated oxygenation
5-HT/NE re-uptake inhibitor	Venlafaxine	MDD, narcolepsy	Reduced cataplexy	SERT/NET inhibition	CYP2D6

H_3 inverse agonist	Pitolisant	Narcolepsy	Improved wake state (EDS) and reduced cataplexy	H_3 receptor inverse agonism	CYP3A4, CYP2D6
$D_{2/3}$ agonists	Pramipexole	PD, RLS	Reduction of PLMS	$D_{2/3/4}$ receptor agonism	Urinary excretion of unchanged drug (>90%)
	Ropinirole	PD, RLS	Reduction of PLMS	$D_{2/3/4}$ receptor agonism	CYP1A2
	Rotigotine	PD, RLS	Reduction of PLMS	$D_{2/3/4}$ receptor agonism	CYP2C19, UGT enzymes
$\alpha_2\delta$ receptor ligand	Gabapentin	RLS, insomnia	Reduction of PLMS, improved sleep	Unknown, binding to $\alpha_2\delta$ subunit of VGCC	Urinary excretion of unchanged drug

DORA dual orexin receptor antagonists, *TCA* tricyclic antidepressants, *TeCA* tetracyclic antidepressants, *5-HT* 5-hydroxytryptamine, serotonin, *NE* norepinephrine, *H_3* histamine H_3 receptor, *Hcrt* hypocretin, *D_2* dopamine D_2 receptor, *N24HSWD* non-24-hour sleep-wake disorder, *DSPD* delayed sleep phase disorder, *SAD* seasonal affective disorder, *MDD* major depressive disorder, *OSA* obstructive sleep apnea, *PD* Parkinson's disease, *RLS* restless legs syndrome, *EDS* excessive daytime sleepiness, *PLMS* periodic limb movements in sleep, *GHB* γ-hydroxybutyrate, *H_1* histamine H_1 receptor, *$MT_{1/2}$ receptors* melatonin MT_1 and MT_2 receptors, *5-HT_{2A}* serotonin-2A receptors, *5-HT_{2C}* serotonin-2C receptors, *DAT* dopamine transporter, *SERT* serotonin transporter, *NET* norepinephrine transporter, *$A_{1/2A}$ receptors* adenosine A_1 and A_{2A} receptors, *VGCC* voltage-gated calcium channels, *CYP* cytochrome-P450 isoenzymes, *UGT* uridine-5′-diphospho-glucuronyltransferase, *NAT2* *N*-acetyltransferase 2, *CES1* carboxylesterase 1, *COMT* catechol-O-methyltransferase, *MAOB* monoaminoxidase type-B

the correct dosing of these hypnotics, to optimize efficacy and avoid adverse actions, such as daytime impairment and next-morning sedation (Lozupone et al. 2017).

Some benzodiazepines (e.g., lorazepam, clonazepam, nitrazepam) are glucuronidated by UGT isoenzymes or acetylated by NAT2. Glucuronidation is a xenobiotic elimination reaction mainly occurring in the liver. Polymorphisms in some UGT genes affect lorazepam clearance. One case documented prolonged sedation after lorazepam, due to absence of the isoenzyme UGT2B7 (Siller et al. 2014). Furthermore, *NAT2* polymorphisms causing "slow acetylator" phenotypes impair the metabolism of clonazepam in vitro (Toth et al. 2016).

3.2.2 Dual Orexin Receptor Antagonists

While genetic variants of HCRTR1/2 receptors and Hcrt synthesis are frequent (Thompson et al. 2014), their functional and pharmacological significance are not well understood. The current development of Hcrt receptor antagonists as novel hypnotics should take these genetic variants into account, to better understand the variability in drug efficacy and side effects (Hoyer and Jacobson 2013; Thompson et al. 2014). For example, in vitro and in vivo characterization of the metabolism and disposition of suvorexant in humans determined that CYP3A4/5 are the predominant enzymes mediating oxidation of this compound (Cui et al. 2016).

3.2.3 Antihistamines

Large interindividual differences are observed in the sedative effects of diphenhydramine, which appear to be due to drug metabolism rather than genetic variants of His H_1 receptors. The isoenzymes *CYP2D6*, *CYP1A2*, *CYP2C9*, and *CYP2C19* contribute to diphenhydramine metabolism (Akutsu et al. 2007). Three CYP2D6 ultra-metabolizers reported paradoxical excitation rather than sedation on diphenhydramine treatment (de Leon and Nikoloff 2008), and the *CYP2D6*10* allele was identified as a risk factor for antihistamine-induced sleepiness in Japanese adults (Saruwatari et al. 2006).

3.2.4 Sedating Antidepressants and Antipsychotics

Pharmacogenetic studies of mirtazapine established several polymorphisms that modulate its hypnotic and antidepressant efficacy. For example, the c.-998G>A polymorphism (SNP-Id: rs6311) of the gene encoding the 5-HT_{2A} receptor (*HTR2A*) determined mirtazapine-induced sleep improvements in patients with major depressive disorder (MDD) (Kang et al. 2007). Mirtazapine also binds to DA, NE, and ACh receptors. Consistent with this pharmacological profile, also rs28363170 of the *DAT1* gene encoding the DAT (*aka* SLC6A3, solute carrier family 6 member 3) and distinct polymorphisms of *ARRB1* (β arrestin 1), *COMT* (catechol-O-methyltransferase), *MAOA,* and *MAOB* (monoamine oxidase isoenzymes A and B) genes affect responses to mirtazapine in patients with MDD (reviewed in Holst et al. 2016). These studies illustrate that sleep-promoting agents may act on multiple systems, and polymorphisms of genes regulating different neurotransmitters may mutually influence the efficacy of a given drug.

Polymorphisms of the *HTR2A* gene were also associated with treatment outcomes in depressed patients treated with SSRIs (Kato and Serretti 2010). The variants c.371T>C of *HTR1B* (5-HT_{1B} receptor; SNP-Id: rs130060) and c.102C>T of *HTR2A* (SNP-Id: rs6313) affect binding affinity of the 5-HT_{2A} receptor antagonist, ketanserin (Brüss et al. 1999; Holmes et al. 2007). These polymorphisms may modulate slow-wave sleep promotion by 5-HT_{2A} receptor antagonists that are currently in development.

Apart from genetic differences in drug targets, genetically determined metabolizer status modifies clearance of doxepin (metabolized by CYP2D6, CYP2C9, and CYP2C19) (Kirchheiner et al. 2002) and (es)mirtazapine (primarily metabolized by CYP2D6) (Brockmöller et al. 2007). Fatal poisoning with both drugs was associated with defective CYP2D6 enzymes (Neukamm et al. 2013). Nevertheless, systematic review of the available data indicates that the current evidence is insufficient to conclude that CYP2D6 poor metabolizers are more prone to adverse effects of doxepin than more extensive/rapid metabolizers (Haufroid and Hantson 2015). Driving performance in healthy volunteers was more impaired after esmirtazapine administration in poor rather than in rapid CYP2D6 metabolizers (Ramaekers et al. 2011).

3.3 Pharmacotherapy of Circadian Rhythm Sleep-Wake Disorders

3.3.1 Melatonin and Melatonin Analogues

Several polymorphisms in the melatonin receptor genes, *MTNR1A* and *MTNR1B*, were identified (Ekmekcioglu 2006), and *MT2* receptor gene variants were associated with type-2 diabetes (Karamitri et al. 2013). One study suggested a relevant association between polymorphism rs2119882 of *MTNR1A* (proposed to affect promoter activity) and insomnia symptoms in schizophrenia (Park et al. 2011). The development of melatonin receptor agonists for insomnia and circadian rhythm sleep-wake disorders would benefit from pharmacogenetic studies, taking into account functional polymorphisms of melatonin receptors.

Melatonin is synthesized from 5-HT, metabolized almost exclusively in the liver by CYP1A2, and excreted as sulphatoxymelatonin in urine (Claustrat et al. 2005). The plasma concentration of melatonin after oral ingestion was higher in individuals with *1A/*1A than with *1F/*1F genotypes of *CYP1A2* (Härtter et al. 2006). Furthermore, autistic patients with slow CYP1A2 metabolism due to *1F/*1A or *1A/*1A genotypes showed decreasing potency of melatonin to improve sleep problems (Braam et al. 2013). Finally, Chinese carriers of a *1A and other *CYP1A2* variants appear to metabolize agomelatine more slowly than carriers of the *1F allele (Song et al. 2014). Interestingly, sulphatoxymelatonin was recently proposed as a potential biomarker of brain 5-HT status in severe genetic disorders affecting 5-HT biosynthesis (Batllori et al. 2017).

3.4 Pharmacotherapy of Central Disorders of Hypersomnolence

3.4.1 (Ar)Modafinil and Stimulants

Individual effects of standard doses of (ar)modafinil and stimulants such as methylphenidate and amphetamines have been associated with genetic variants of proteins regulating endogenous neurotransmitter degradation and transmission. For example, the activity of the monoamine-metabolizing enzyme, COMT, depends on several known genetic variants, including the functional c.472G>A (p.Val158Met) polymorphism (SNP-Id: rs4680) (Chen et al. 2004). This common valine-to-methionine substitution drastically reduces enzymatic activity, leading to elevated prefrontal cortex (PFC) dopaminergic tone in Met/Met homozygotes when compared to Val/Val homozygotes (Tunbridge et al. 2006). The treatment response with modafinil in narcolepsy patients differs widely among COMT genotypes. To control EDS, patients (female and male) with the Val/Val genotype of rs4680 need almost 100 mg more modafinil per day than patients with the Met/Met genotype (Dauvilliers et al. 2002). Intriguingly, as will be outlined below, the impact of this polymorphism on modafinil's efficacy may be opposite in narcolepsy patients and sleep-deprived healthy volunteers.

Apart from genetic variance modulating monoaminergic neurotransmission, variants in the gene encoding the P-glycoprotein drug transporter ABCB1 (ATP binding cassette subfamily B member 1) may partly explain individual therapeutic response to modafinil treatment in narcolepsy (Moresco et al. 2016).

Both the Val-allele of rs4680 of *COMT* and the 9R-allele of rs28363170 of *DAT1* (see below) were associated with a pronounced response to methylphenidate (Kereszturi et al. 2008; Froehlich et al. 2011) and enhanced risk of methamphetamine-induced psychosis and abuse (Ujike et al. 2003; Li et al. 2004). Preliminary data further indicate that CYP2D6 extensive/ultra-rapid metabolizers could show an elevated risk for central nervous system risk adverse effects of methamphetamine (Haufroid and Hantson 2015). Yet, more work is needed to confirm this association.

3.4.2 Venlafaxine

Converging evidence from multiple studies indicates that CYP2D6 poor metabolizers are especially prone to adverse effects caused by venlafaxine (Haufroid and Hantson 2015), whereas no significant association with clinical response was found with polymorphisms of the *ABCB1* gene (Moresco et al. 2016).

3.4.3 Caffeine

Wake promotion and sleep disruption by the adenosine receptor antagonist, caffeine, are highly variable in patients and healthy people. The reasons for the pronounced interindividual differences in caffeine effects on sleep remain incompletely understood. Shared environmental factors contribute to poor sleep quality associated with sustained high caffeine consumption (Treur et al. 2018). In addition, a population-based survey in roughly 1,000 participants indicated that caffeine reduces sleep efficiency more potently in G/A allele carriers than in G/G homozygotes of

polymorphism rs73598374 of the gene encoding the adenosine metabolizing enzyme, adenosine deaminase (Mazzotti et al. 2011). This study, however, was not controlled and awaits replication.

Caffeine is mainly metabolized by CYP1A2, which shows 10- to 200-fold interindividual differences in enzymatic activity (Fredholm et al. 1999; Gunes and Dahl 2008). Consistent with the notion that the rate of caffeine metabolism is heritable (Rasmussen et al. 2002), a common C/A polymorphism in intron 1 of the *CYP1A2* gene (SNP-Id: rs762551) was found to affect caffeine half-life in blood plasma, such that A/A-allele carriers (*CYP1A2*1F*) metabolize caffeine faster than C-allele carriers (Sachse et al. 1999). Nevertheless, health status, gender, and age, as well as lifestyle factors (such as habitual smoking and caffeine consumption, both known to induce CYP1A2 enzymatic activity), also play important roles for the metabolism of caffeine to its primary metabolite, paraxanthine (e.g., Urry et al. 2016).

Disturbed sleep causes some people to voluntarily abstain from caffeine. Genome-wide association (GWA) studies examined genetic contributions to caffeine consumption in humans. In 47,341 participants of European descent, two polymorphisms regulating transcriptional activation of CYP1A2 determined habitual caffeine intake, one located in the bidirectional promoter region of the *CYP1A1-CYP1A2* locus (SNP-Id: rs2470893) and one located in the aryl hydrocarbon receptor (*AHR*) (SNP-Id: rs4410790) gene (Cornelis et al. 2011). Intriguingly, a second study with more than 10,000 participants of European ancestry, located two other polymorphisms in the same genes, rs2472297 (*CYP1A1-CYP1A2*) and rs6968865 (*AHR*) (Sulem et al. 2011). These GWA studies support a role for CYP1A2 in the pharmacogenetics of caffeine. In addition, variants of *ADORA2A* (gene encoding adenosine A_{2A} receptors) also influence caffeine preference (Cornelis et al. 2011) and sleep disruption by caffeine (Rétey et al. 2007; Byrne et al. 2012).

Because melatonin is also degraded by CYP1A2, caffeine and melatonin compete for the same metabolizing enzyme. The half-life of caffeine during melatonin release may be prolonged and, in turn, caffeine consumption may delay the nocturnal peak of melatonin (Härtter et al. 2006; Braam et al. 2013). Habitual caffeine intake should, thus, be taken into account to understand individual efficacy of melatonin and novel melatonin receptor agonists.

3.4.4 γ-Hydroxybutyrate (GHB)

So far, no studies have investigated the relevance of functional genetic variants of $GABA_B$ receptors or polymorphisms related to GHB metabolizing pathways for individual drug responses in narcolepsy patients treated for EDS and cataplexy. By contrast, GHB intolerance was proposed to possibly arise from reduced activity of the GABA metabolizing enzyme, succinic semialdehyde dehydrogenase (Berner 2013).

3.5 Pharmacotherapy of Sleep-Related Movement Disorders

3.5.1 Dopamine $D_{2/3}$ Receptor Agonists

Polymorphisms of *DRD2* and *DRD3* genes (encoding dopamine D_2 and D_3 receptors) impact the effects of the dopamine receptor agonists used to treat RLS (Agundez et al. 2013). Pharmacogenetic evidence also linked a *Ser9Gly* polymorphism of *DRD3* (SNP-Id: rs6280) with response to pramipexole in Parkinson's disease (PD) patients, but not with the *Taq1A* polymorphism of *DRD2* (SNP-Id: rs1800497) (Liu et al. 2009). In another study, however, both rs1800497 and a *MspI* polymorphisms of *DRD3* (SNP-Id: rs4646996) were related to discontinued use of pramipexole and ropinirole in PD (Arbouw et al. 2009).

The D_2 receptor agonist ropinirole is degraded by CYP1A2 and to a lesser extent by CYP3A4 (Kaye and Nicholls 2000), whereas the metabolic pathways for pramipexole are not yet clear (Agundez et al. 2013). A polymorphism of the gene encoding the solute carrier protein SLC22A1 (SNP-Id: rs622342) was linked to higher doses of pramipexole and reduced survival time after initiation of L-dopa therapy (Becker et al. 2011). These effects may be caused by reduced drug uptake from the small intestine.

3.5.2 Ligands of $\alpha_2\delta$ Subunit of Voltage-Gated Calcium Channels

Mutations and deletions of the *CACNA2D1* gene coding for the $\alpha_2\delta$ subunit family, which has five isoforms targeted by gabapentin and pregabalin, were linked to several medical disorders and increased sensitivity to opioid treatment (Rhodin et al. 2013). Moreover, a *CACNA1A* mutation in mice leading to impaired G protein-coupled inhibition was associated with reduced sleep induction by adenosine and wake promotion by caffeine (Deboer et al. 2013). Interestingly, recent evidence indicates that genetic variants of *CACNA1C* are associated with sleep latency and sleep quality (Byrne et al. 2013; Parsons et al. 2013). It may, hence, be expected that such polymorphisms modulate the efficacy of drugs or environmental influences that affect sleep.

Gabapentin-like compounds undergo negligible metabolism and are primarily eliminated as unchanged drug by renal excretion. The c.1507C>T polymorphism (SNP-Id: rs1050152) of the gene encoding the solute carrier protein SLC22A4 affects gabapentin transport in vitro, yet homozygous T-allele carriers showed no enhanced renal clearance of gabapentin when compared to carriers of the "wild-type" C-allele (Urban et al. 2007).

4 Pharmacogenetic Dissection of Sleep-Wake Circuitries in Humans

As mentioned above, converging lines of research employing optogenetic and chemogenetic methods in animal models have pointed to new structures and pathways regulating sleep and wakefulness. For example, dopaminergic signaling appeared for a long time to have no crucial role in sleep-wake regulation.

Accumulating evidence now suggests that antagonistic adenosinergic-dopaminergic interactions particularly in the striatum play important roles in sleep-wake regulation (for reviews, see Lazarus et al. 2017; Wisor 2018). Based on this evidence, A_{2A}-D_2 receptor-positive striatopallidal cells may serve as new target for therapeutic intervention in sleep-wake disorders. Preclinical human pharmacology provides a bridge between basic (sleep-wake) research and clinical (sleep-wake) medicine and is an essential step in the development of novel (sleep-wake) therapeutics.

Rational treatments of sleep-wake disorders should strengthen the physiological processes underlying sleep-wake regulation. According to this rationale, an "ideal" novel hypnotic can be expected to promote the same physiological processes that underlie elevated sleep need and increased sleep intensity, as reflected by the prevalence of theta/alpha (~6–10 Hz) and delta (<4 Hz) frequency activity in the electroencephalogram (EEG) in wakefulness and sleep. Sleep deprivation, the strongest challenge of the sleep-wake-dependent, homeostatic facet of sleep-wake regulation, not only alters in highly predictable fashion electrical brain activity in these frequencies but also impairs a wide range of measures derived from neurobehavioral performance tasks. In the laboratory, neurobehavioral deficits of sleep deprivation are accurately indexed by increased lapses and slowed reaction times on the psychomotor vigilance task (PVT). This task has been considered a gold-standard measurement of sustained vigilant attention in sleep-wake research (Lim and Dinges 2010). Performance on the PVT is reliably impaired by elevated sleep need and reflects distinct changes in neuronal activity (Nir et al. 2017; Maire et al. 2018). Importantly, these consequences of sleep deprivation vary widely among individuals and are in part genetically determined (Van Dongen et al. 2004; Lim et al. 2012). Intriguingly, however, the neurophysiological and neurobehavioral variables do not typically show a clear association with each other but rather develop seemingly independently. Thus, it has been suggested that the neurophysiological and neurobehavioral consequences of sleep loss are likely governed by separate mechanisms. In the following section, an experimental pharmacogenetic approach to systematically dissect these sleep-wake regulatory processes in healthy humans will be outlined. Collectively, the results demonstrate distinctly different roles for adenosinergic and dopaminergic pathways in the regulation of sleep-wake-dependent neurophysiological and behavioral functions of the brain.

4.1 ADOAR2A Gene Variants Contribute to Caffeine Sensitivity and Neurophysiological and Behavioral Effects of Sleep Deprivation

Through competitive blockade of adenosine A_1 and A_{2A} receptors in the central nervous system, caffeine attenuates the buildup of sleep pressure during wakefulness and delays the endogenous circadian clock (Landolt et al. 2004; Burke et al. 2015). The interference with these physiological sleep-wake regulatory mechanisms can disturb the quality of sleep (Clark and Landolt 2017). The heritability of coffee-attributed sleep disturbances equals roughly 40% (Luciano et al. 2007).

The major psychostimulant ingredient of coffee is caffeine (Fredholm et al. 1999), which blocks A_{2A} receptors to promote wakefulness (Huang et al. 2005). The human *ADORA2A* gene is located on chromosome 22q11.2, and functional genetic variation of *ADORA2A* is an important determinant of sleep disruption by caffeine. More specifically, a combined sleep deprivation and caffeine study provided evidence that the c.1976T>C polymorphism of *ADORA2A* (SNP-ID: rs5751876) modulates individual sensitivity to subjective and objective effects of caffeine on sleep (Rétey et al. 2007). Remarkably, this notion was confirmed in a large GWA study in 2,402 twins and their families (Byrne et al. 2012). The polymorphism rs5751876 forms linkage disequilibria with several other variants of *ADORA2A* that modulate actions of caffeine on sleep and attentional domains that are commonly impaired by sleep deprivation (Bodenmann et al. 2012; Renda et al. 2015). In addition, caffeine-sensitive and caffeine-insensitive individuals appear to be differently affected by sleep loss (Rétey et al. 2006). This observation supports the notion that the functional state of A_{2A} receptors determines the accumulation of sleep need during prolonged wakefulness, both in mice and humans (Hayaishi et al. 2004; Bodenmann et al. 2012; Landolt et al. 2012; Rupp et al. 2013).

4.2 Adenosine A_{2A} Receptors, Dopamine Transporters, and Dopamine D_2 Receptors Are Co-localized in the Striatum

The distribution of adenosine receptors in the central nervous system is highly heterogeneous. The A_{2A} receptor subtype is primarily expressed in the striate nuclei (caudate nucleus, putamen, nucleus accumbens [NAc], globus pallidus) and olfactory tubercle (Fig. 2). Accumulating evidence suggests that the striatum, the NAc in particular, plays critical roles in wake promotion by caffeine and in sleep-wake regulation (Lazarus et al. 2011; Zhang et al. 2013; Holst and Landolt 2015). Apart from widespread glutamatergic inputs from several cortical and subcortical areas, the striatum receives dopaminergic inputs via axons originating in the *substantia nigra pars compacta* and the ventral tegmental area (VTA) of the brainstem. Extracellular dopamine levels in NAc and medial PFC fluctuate across sleep-wake states in rats (Lena et al. 2005). Combined behavioral, chemogenetic, and optogenetic manipulations in freely moving mice suggest a fundamental role for the VTA dopaminergic circuitry in maintaining wakefulness and linking motivated behaviors with sleep-wake regulation (Eban-Rothschild et al. 2016; Oishi et al. 2017a).

Similar to adenosine A_{2A} receptors, also the dopamine reuptake transporter (DAT) is highly expressed in the striatum (Fig. 2). The DAT transports extracellular dopamine from the synaptic cleft into the presynaptic terminals for repackaging into vesicles. The deletion of the *dat* gene in mice reduces NREM sleep time and increases wakefulness (Wisor et al. 2001). Intriguingly, the $Dat^{-/-}$ knockout mice are hypersensitive to the wake-promoting effects of caffeine. More specifically, relative to wild-type littermates, the knockout animals exhibit a threefold and fivefold greater increase in wake time after 2.5 and 10 mg/kg caffeine administration

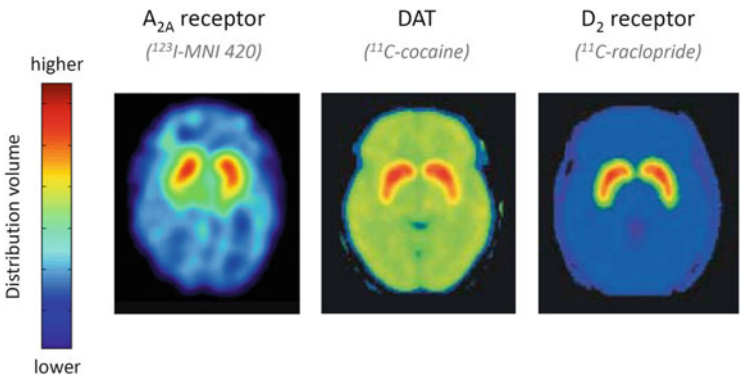

Fig. 2 A_{2A} receptors, dopamine transporters (DAT), and D_2 receptors are co-localized on dorsal and ventral striatal neurons. The distribution volumes (color-coded) of selective radioligands that bind to these receptors and transporters on the level of the basal ganglia in humans were reconstructed with single photon emission computer tomography (^{123}I-MNI 420) and positron emission tomography (^{11}C-cocaine and ^{11}C-raclopride) imaging. The figure has been composed of published data by Seibyl et al. (2012), Volkow et al. (2012)

when compared to vehicle (Wisor et al. 2001). Based on these data in animals, it was hypothesized that functional variance not only in *CYP1A2* and *ADORA2A* (see above) but also in the *DAT1* gene may modulate the response to caffeine in humans.

4.3 DAT1 Genotype Modulates Caffeine Sensitivity and Neurophysiological and Behavioral Effects of Sleep Deprivation

The human *DAT1* gene contains a common VNTR polymorphism in its 3′-untranslated region. Linkage and associations of this polymorphism (SNP-ID: rs28363170) with various psychiatric disorders (e.g., attention-deficit hyperactivity disorder, PD, alcoholism, schizophrenia) have been reported, suggesting that it is functionally relevant (Pinsonneault et al. 2011). In addition, in vitro studies revealed an effect of this polymorphism on gene expression (Pinsonneault et al. 2011). The VNTR can exist in forms of 3–13 repeats, but two alleles are most common: 9 repeats (9R) or 10 repeats (10R) of a 40-bp sequence in exon 15. Several studies in humans quantified striatal DAT availability with SPECT (single photon emission computer tomography) and PET (positron emission tomography): these studies consistently reported lower striatal DAT binding in 10R homozygotes when compared to 9R allele carriers. On average, 10R homozygotes exhibit 10–15% lower striatal DAT availability than 9R allele carriers (Costa et al. 2011; Faraone et al. 2014). Are 10R homozygotes, thus, more sensitive to caffeine than 9R carriers, analogous to $Dat^{-/-}$ mice?

Indeed, this seems to be the case. In a survey among 485 individuals, a significantly larger proportion of 10R homozygotes (39%) rated themselves as being

sensitive to psychostimulant action of caffeine when compared to 9R allele carriers (Holst et al. 2014). In the case of 9R homozygotes, only 2 out of 23 individuals (9%) were caffeine sensitive. The respective number was 33% in 9R/10R heterozygotes. A subsequent controlled study in the laboratory confirmed that the *DAT1* VNTR predicts the effect of caffeine on EEG markers of sleep need. The stimulant attenuated the rebound in EEG slow waves following prolonged wakefulness in *DAT1* 10R homozygotes only (Holst et al. 2014). From the perspective of sleep-wake regulatory mechanisms, it is important to note that this polymorphism associated with the repercussions of sleep deprivation on sleep EEG markers of increased sleep drive (Holst et al. 2014). In addition, it modulated time-on-task effects and performance instability on the PVT, as well as on the effects of the DAT inhibitor, modafinil, on markers of sleep need (Lim et al. 2012; Holst et al. 2014; Satterfield et al. 2017). Taken together, convergent findings in animals and humans suggest that A_{2A} receptors and DAT are involved in the wake-promoting effects of caffeine and modafinil. They further indicate that adenosine-dopamine interactions in the striatum are important for sleep-wake regulation and the behavioral consequences of sleep deprivation.

4.4 DRD2 Genotype Modulates Neurophysiological and Neurobehavioral Consequences of Sleep Deprivation

Increasing evidence demonstrates the presence of co-expressed A_{2A}-D_2 receptor complexes on distinct subpopulations of medium spiny neurons, the principle neuronal cell type of the dorsal and ventral striatum (Azdad et al. 2009; Ferre et al. 2010; Casado-Anguera et al. 2016). Brain imaging also demonstrates that D_2 receptors are mainly expressed in the striatum (Fig. 2). Striatal outputs are primarily composed of two types of projection neurons: indirect medium spiny neurons (iMSNs) that express D_2-type receptors and direct medium spiny neurons expressing D_1-type receptors (Ferre et al. 2010; Yager et al. 2015). Activation of iMSNs causes a net inhibition of the thalamus, which is part of the fronto-striatal circuit (Yager et al. 2015). Brain imaging of human subjects while performing a working memory task revealed that gene variants encoding DAT and D_2 receptor proteins interact to regulate fronto-striatal circuit activity (Bertolino et al. 2009). Moreover, striatal D_2 receptor availability after sleep deprivation is reduced when compared to baseline (Volkow et al. 2012) and regulates brain activation during performance of a visual attention task (Tomasi et al. 2016).

The *DRD2* gene includes a functional c.957C>T polymorphism (SNP-ID: rs6277) that affects levels and stability of mRNA such that C-allele carriers exhibit 15–20% enhanced striatal D_2 receptor availability when compared to T-allele carriers (Hirvonen et al. 2009). This polymorphism alone and in interaction with the presence of either 9R and 10R alleles of *DAT1* was found to modulate the evolution of waking EEG markers of accumulating sleep pressure, subjective sleepiness, as well as performance on the PVT across prolonged waking (Holst et al. 2017). More specifically, individuals homozygous for the C allele of *DRD2* and the

10R allele of *DAT1* were more severely affected with attentional lapses after 40 h of wakefulness than other genotypic variants. A recent study confirmed that homozygous carriers of the C allele of the *DRD2* c.957C>T polymorphism are impaired by sleep deprivation in vigilant attention (Whitney et al. 2017). Remarkably, however, the same individuals were resilient to the effects of prolonged waking on reversal learning, a measure of cognitive flexibility (Whitney et al. 2017). Consistent with the notion that the consequences of sleep deprivation are trait-like but *task-specific*, the data collectively suggest that striato-thalamo-cortical dopaminergic pathways contribute to the regulation of neurophysiological and neurobehavioral consequences of sleep loss in humans.

4.5 COMT Genotype Modulates Neurobehavioral Consequences of Sleep Deprivation Without Affecting Neurophysiological Markers of Elevated Sleep Need

The *COMT* gene encodes the main enzyme degrading catecholamines in the PFC, COMT, which regulates dopaminergic neurotransmission by converting DA to 3-methoxytyramine (Matsumoto et al. 2003). The genotype of the Val158Met polymorphism of *COMT* (SNP-Id: rs4680) has a consistent impact on PFC functioning (Egan et al. 2001; Tunbridge et al. 2006; Bodenmann et al. 2009b). Nevertheless, some effects of this genetic variant only become apparent when the DA system is challenged, such as after sleep deprivation (Bodenmann et al. 2009a; Satterfield et al. 2018; Valomon et al. 2018). More specifically, Val/Val, Val/Met, and Met/Met allele carriers perform similarly in baseline. Yet, after a night without sleep, Val/Met heterozygotes produced twice as many attentional lapses than Met/Met homozygotes (Valomon et al. 2018). Furthermore, the wake-promoting agent modafinil was virtually ineffective in mitigating impaired PVT performance after sleep loss in Met/Met homozygotes. By contrast, in homozygous Val/Val allele carriers, the same dose of modafinil maintained virtually optimal performance throughout 40 h of wakefulness (Bodenmann et al. 2009a).

To test the hypothesis that PFC dopaminergic signaling regulated by COMT causally contributes to neurobehavioral and neurophysiological markers of sleep loss, the interaction of *COMT* genotype, tolcapone, and sleep deprivation on lapses of attention and neurophysiological markers of sleep-wake regulation was investigated in a controlled laboratory study. Tolcapone is a brain penetrant inhibitor of COMT that leads to specific increases in PFC dopaminergic tone without affecting striatal dopaminergic transmission (Farrell et al. 2012). It was expected that tolcapone would mitigate the effects of sleep deprivation primarily in Val/Val homozygotes because it counteracts the overactive COMT and associated deficit in PFC dopaminergic function. Unexpectedly, tolcapone had no beneficial effect in any COMT genotype. By contrast, it increased attentional lapses even further, particularly in Val/Met and Met/Met allele carriers (Valomon et al. 2018). The data suggest an inverted U-shape relationship between PFC dopaminergic signaling

and sustained attention after sleep loss. They further demonstrate that beyond and above COMT genotype, a preexisting sleep debt strongly impacts on the effects of a pharmacological increase of dopaminergic neurotransmission on brain functions.

In contrast to these behavioral effects, genetically determined and pharmacologically induced differences in COMT activity do not affect established EEG markers of elevated sleep pressure in wakefulness and sleep challenged by acute total sleep deprivation (Bodenmann and Landolt 2010; Valomon et al. 2018).

4.6 Converging Evidence for Distinct Adenosinergic/Dopaminergic Pathways Mediating Neurophysiological and Behavioral Consequences of Sleep Loss

Among the four major dopaminergic pathways in the brain, three pathways originating in the midbrain are especially important for interpreting the findings discussed above. They consist of the *nigrostriatal pathway* projecting from the substantia nigra to the dorsal striatum (caudate nucleus and putamen), which is involved in motor control; the *mesolimbic pathway* originating in the VTA and innervating several structures of the limbic system (including the NAc), which is associated with reward, cognition, reinforcement, and motivational salience; and the *mesocortical pathway*, also originating in the VTA, yet having widespread projections to the cerebral cortex, being essential for normal cognitive functions of the dorsolateral PFC, attentional control, motivation, and emotional processes. The regulation of DA metabolism and uptake and, thus, dopaminergic neurotransmission depends on the different pathways, such that DAT and D_2 receptors regulate nigrostriatal/mesolimbic circuit activity, while COMT controls dopaminergic transmission in the mesocortical pathway.

The systematic human pharmacogenetic dissection of adenosine-associated loci and dopamine-related loci discussed above demonstrates that the distinct adenosinergic-dopaminergic signaling pathways differently modulate the response to sleep loss and wake-promoting agents on neurophysiological markers of sleep need and neurobehavioral deficits (Fig. 3). The *ADORA2A* c.1976T>C polymorphism (Rétey et al. 2007; Rupp et al. 2013), an *ADORA2A* haplotype (Bodenmann et al. 2012), the *DAT1* VNTR polymorphism (Holst et al. 2014; Satterfield et al. 2017), and the *DRD2* c.957C>T polymorphism (Holst et al. 2017; Whitney et al. 2017) modulate both EEG and distinct behavioral markers of elevated sleep need and the effects of caffeine and modafinil on these markers. The findings suggest that striatal iMSNs, which co-express high densities of adenosine A_{2A} and dopamine D_2 receptors, mediate these consequences of sleep loss. It is tempting to speculate that the NAc is essential in these processes. Thus, elegant studies in animal models have demonstrated that caffeine and modafinil promote wakefulness by blocking A_{2A} receptors on GABAergic neurons of the NAc that co-express A_{2A} and D_2 receptors (Lazarus et al. 2011; Qiu et al. 2016; Oishi et al. 2017b). These neurons have major projections to the ventral pallidum and may induce sleep by inhibiting the ascending arousal pathways and the thalamus.

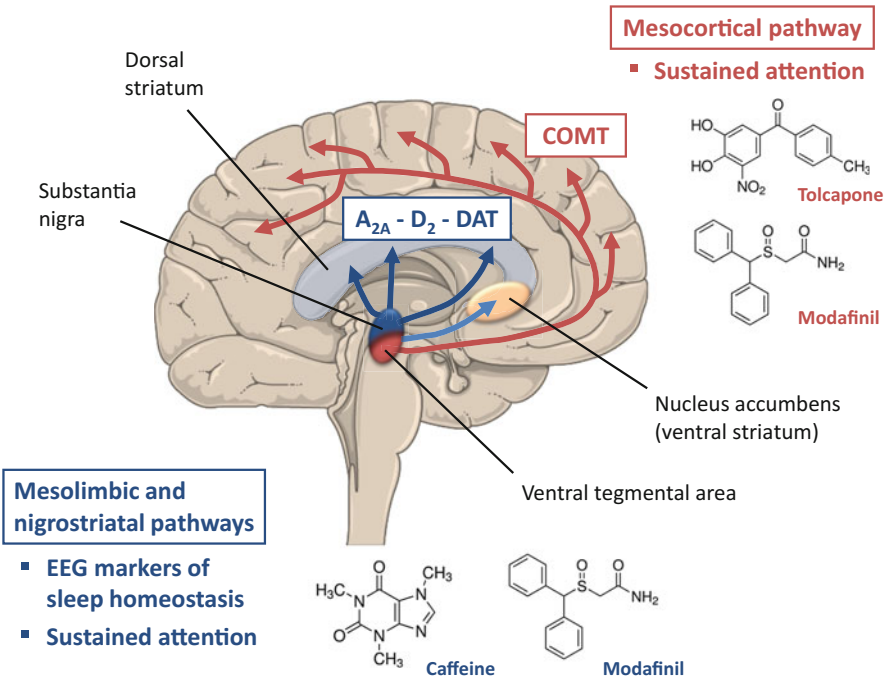

Fig. 3 Pharmacogenetic dissection of adenosinergic-dopaminergic pathways with caffeine (A_{2A} receptor antagonist), modafinil (DAT inhibitor), and tolcapone (COMT inhibitor) reveals different contributions of dopaminergic pathways to sleep-wake regulation and behavioral consequences of sleep loss. The mesolimbic/nigrostriatal dopaminergic pathway (blue) regulates neurophysiological markers of sleep homeostasis in wakefulness and sleep and contributes to impaired sustained attention. Together with animal studies, findings support the notion that the nucleus accumbens, which expresses high densities of dopamine D_2 and adenosine A_{2A} receptors, may be an essential player in these functions. The mesocortical dopaminergic pathway (red) does not affect neurophysiological markers of sleep deprivation. *COMT* catechol-O-methyltransferase, *DAT* dopamine transporter

In contrast to *ADORA2A*, *DAT1*, and *DRD2*, genetic variation of *COMT* did not impact on neurophysiological markers of sleep need (Bodenmann et al. 2009a; Valomon et al. 2018). Nevertheless, *COMT* genotype and pharmacologic interference with PFC dopaminergic tone with modafinil and tolcapone consistently affect the consequences of sleep loss on PVT lapses (Bodenmann et al. 2009a; Valomon et al. 2018). Given that tolcapone leaves striatal dopaminergic neurotransmission unaltered, this finding strongly indicates that the mesocortical pathway contributes to the regulation of attentional lapses during prolonged wakefulness without affecting sleep-wake regulatory mechanisms.

5 Concluding Remarks

5.1 Prospects of Pharmacogenetics for Personalized Sleep-Wake Medicine and New Drug Discovery

Not only gene polymorphisms but a host of environmental factors, including epigenetics, as well as comorbidity and co-medications, cause variability in expression and function of drug targets and pharmacokinetic processes in each individual. The genotyping of single genes is, thus, of limited use for guiding individual pharmacotherapy. Rather, the implementation of deep phenotyping strategies to test individual target and enzyme functions likely provides a more promising approach to achieve personalized dosing of sleep-wake drugs (e.g., Hohmann et al. 2016; Urry et al. 2016).

Powerful new tools to interrogate sleep-wake regulating circuits have revealed new molecular and cellular markers, as well as novel network mechanisms and pathways that regulate wakefulness and sleep. While the ascending arousal system was first described as a reticular network of neurons projecting widely to the cortex and the spinal cord (Moruzzi and Magoun 1949), it has become clear in the last few years that wakefulness and sleep are governed by distinct populations and subtypes of neurons (Varin and Bonnavion 2018). Although specific, these neuronal systems are often redundant and reinforce each other. Future research should clarify their causal associations with sleep homeostasis and the circadian clock, as well as sleep and circadian rhythm disturbances in humans. For example, it should be clarified whether A_{2A}-D_2 receptor-positive striatopallidal neurons regulate sleep homeostasis or rather promote sleep in the absence of motivational stimuli (cf. Oishi et al. 2017b). Such studies may identify critical targets within newly identified sleep and circadian pathways for pharmacological interventions.

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References

Abad VC, Guilleminault C (2017) New developments in the management of narcolepsy. Nat Sci Sleep 9:39–57

Adamantidis A, Luthi A (2018) Optogenetic dissection of sleep-wake states in vitro and in vivo. Handb Exp Pharmacol. https://doi.org/10.1007/164_2018_94

Agundez JAG, Garcia-Martin E, Alonso-Navarro H, Jimenez-Jimenez FJ (2013) Anti-Parkinson's disease drugs and pharmacogenetic considerations. Expert Opin Drug Metab Toxicol 9:859–874

Akutsu T, Kobayashi K, Sakurada K, Ikegaya H, Furihata T, Chiba K (2007) Identification of human cytochrome P450 isozymes involved in diphenhydramine N-demethylation. Drug Metab Dispos 35:72–78

Alston M, Cain SW, Rajaratnam SMW (2018) Advances of melatonin-based therapies in the treatment of disturbed sleep and mood. Handb Exp Pharmacol (in press)

American Academy of Sleep Medicine (2014) International classification of sleep disorders, 3rd edn. American Academy of Sleep Medicine, Darien

Arbouw MEL, Movig KLL, Egberts TCG, Poels PJE, van Vugt JPP, Wessels JAM, van der Straaten RJHM, Neef C, Guchelaar HJ (2009) Clinical and pharmacogenetic determinants for the discontinuation of non-ergoline dopamine agonists in Parkinson's disease. Eur J Clin Pharmacol 65:1245–1251

Azdad K, Chavez M, Bischop PD, Wetzelaer P, Marescau B, De Deyn PP, Gall D, Schiffmann SN (2009) Homeostatic plasticity of striatal neurons intrinsic excitability following dopamine depletion. PLoS One 4:e6908

Banerjee D, Vitiello MV, Grunstein RR (2004) Pharmacotherapy for excessive daytime sleepiness. Sleep Med Rev 8:339–354

Batllori M, Molero-Luis M, Arrabal L, Heras JD, Fernandez-Ramos JA, Gutierrez-Solana LG, Ibanez-Mico S, Domingo R, Campistol J, Ormazabal A, Sedel F, Opladen T, Zouvelou B, Pons R, Garcia-Cazorla A, Lopez-Laso E, Artuch R (2017) Urinary sulphatoxymelatonin as a biomarker of serotonin status in biogenic amine-deficient patients. Sci Rep 7:14675

Baumann CR (2018) Clinical sleep-wake disorders I: focus on hypersomnias and movement disorders during sleep. Handb Exp Pharmacol. https://doi.org/10.1007/164_2018_126

Becker ML, Visser LE, van Schaik RHN, Hofman A, Uitterlinden AG, Stricker BHC (2011) OCT1 polymorphism is associated with response and survival time in anti-Parkinsonian drug users. Neurogenetics 12:79–82

Berner J (2013) Sodium oxybate intolerance associated with familial serum acylcarnitine elevation. J Clin Sleep Med 9:71–72

Bertolino A, Fazio L, Di Giorgio A, Blasi G, Romano R, Taurisano P, Caforio G, Sinibaldi L, Ursini G, Popolizio T, Tirotta E, Papp A, Dallapiccola B, Borrelli E, Sadee W (2009) Genetically determined interaction between the dopamine transporter and the D-2 receptor on prefronto-striatal activity and volume in humans. J Neurosci 29:1224–1234

Black SW, Morairty SR, Chen TM, Leung AK, Wisor JP, Yamanaka A, Kilduff TS (2014) GABA (B) agonism promotes sleep and reduces cataplexy in murine narcolepsy. J Neurosci 34:6485–6494

Bodenmann S, Landolt HP (2010) Effects of modafinil on the sleep EEG depend on Val158Met genotype of COMT. Sleep 33:1027–1035

Bodenmann S, Xu S, Luhmann UFO, Arand M, Berger W, Jung HH, Landolt HP (2009a) Pharmacogenetics of modafinil after sleep loss: catechol-O-methyltransferase genotype modulates waking functions but not recovery sleep. Clin Pharmacol Ther 85:296–304

Bodenmann S, Rusterholz T, Durr R, Stoll C, Bachmann V, Geissler E, Jaggi-Schwarz K, Landolt HP (2009b) The functional Val158Met polymorphism of COMT predicts interindividual differences in brain alpha oscillations in young men. J Neurosci 29:10855–10862

Bodenmann S, Hohoff C, Freitag C, Deckert J, Rétey JV, Bachmann V, Landolt H-P (2012) Polymorphisms of *ADORA2A* modulate psychomotor vigilance and the effects of caffeine on neurobehavioral performance and sleep EEG after sleep deprivation. Br J Pharmacol 165:1904–1913

Braam W, Keijzer H, Boudier HS, Didden R, Smits M, Curfs L (2013) CYP1A2 polymorphisms in slow melatonin metabolisers: a possible relationship with autism spectrum disorder? J Intellect Disabil Res 57:993–1000

Brockmöller J, Meineke I, Kirchheiner J (2007) Pharmacokinetics of mirtazapine: enantioselective effects of the CYP2D6 ultra rapid metabolizer genotype and correlation with adverse effects. Clin Pharmacol Ther 81:699–707

Brüss M, Bonisch H, Buhlen M, Nothen MM, Propping P, Gothert M (1999) Modified ligand binding to the naturally occurring Cys-124 variant of the human serotonin 5-HT1B receptor. Pharmacogenetics 9:95–102

Burgess HJ, Emens JS (2018) Drugs used in circadian sleep-wake rhythm disturbances. Sleep Med Clin 13:231–241

Burke TM, Markwald RR, McHill AW, Chinoy ED, Snider JA, Bessman SC, Jung CM, O'Neill JS, Wright KP (2015) Effects of caffeine on the human circadian clock in vivo and in vitro. Sci Transl Med 7:305ra146

Byrne EM, Johnson J, McRae AF, Nyholt DR, Medland SE, Gehrman PR, Heath AC, Madden PAF, Montgomery GW, Chenevix-Trench G, Martin NG (2012) A genome-wide association study of caffeine-related sleep disturbance: confirmation of a role for a common variant in the adenosine receptor. Sleep 35:967–975

Byrne EM, Gehrman PR, Medland SE, Nyholt DR, Heath AC, Madden PAF, Hickie IB, Van Duijn CM, Henders AK, Montgomery GW, Martin NG, Wray NR, Consortium C (2013) A genome-wide association study of sleep habits and insomnia. Am J Med Genet B Neuropsychiatr Genet 162B:439–451

Cai K, Nanga RPR, Lamprou L, Schinstine C, Elliott M, Hariharan H, Reddy R, Epperson CN (2012) The impact of gabapentin administration on brain GABA and glutamate concentrations: a 7T H-1-MRS study. Neuropsychopharmacology 37:2764–2771

Casado-Anguera V, Bonaventura J, Moreno E, Navarro G, Cortes A, Ferre S, Casado V (2016) Evidence for the heterotetrameric structure of the adenosine A(2A)-dopamine D-2 receptor complex. Biochem Soc Trans 44:595–600

Castelnovo A, Lopez R, Proserpio P, Nobili L, Dauvilliers Y (2018) NREM sleep parasomnias as disorders of sleep-state dissociation. Nat Rev Neurol 14:470–481

Chen JS, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, Kolachana BS, Hyde TM, Herman MM, Apud J, Egan MF, Kleinman JE, Weinberger DR (2004) Functional analysis of genetic variation in catechol-o-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet 75:807–821

Clark I, Landolt HP (2017) Coffee, caffeine, and sleep: a systematic review of epidemiological studies and randomized controlled trials. Sleep Med Rev 31:70–78

Claustrat B, Brun J, Chazot G (2005) The basic physiology and pathophysiology of melatonin. Sleep Med Rev 9:11–24

Cope DW, Wulff P, Oberto A, Aller MI, Capogna M, Ferraguti F, Halbsguth C, Hoeger H, Jolin HE, Jones A, Mckenzie ANJ, Ogris W, Poeltl A, Sinkkonen ST, Vekovischeva OY, Korpi ER, Sieghart W, Sigel E, Somogyi P, Wisden W (2004) Abolition of zolpidem sensitivity in mice with a point mutation in the GABA(A) receptor gamma 2 subunit. Neuropharmacology 47:17–34

Cornelis MC, Monda KL, Yu K, Paynter N, Azzato EM, Bennett SN, Berndt SI, Boerwinkle E, Chanock S, Chatterjee N, Couper D, Curhan G, Heiss G, Hu FB, Hunter DJ, Jacobs K, Jensen MK, Kraft P, Landi MT, Nettleton JA, Purdue MP, Rajaraman P, Rimm EB, Rose LM, Rothman N, Silverman D, Stolzenberg-Solomon R, Subar A, Yeager M, Chasman DI, van Dam RM, Caporaso NE (2011) Genome-wide meta-analysis identifies regions on 7p21 (AHR) and 15q24 (CYP1A2) as determinants of habitual caffeine consumption. PLoS Genet 7: e1002033

Costa A, Riedel M, Muller U, Moller HJ, Ettinger U (2011) Relationship between SLC6A3 genotype and striatal dopamine transporter availability: a meta-analysis of human single photon emission computed tomography studies. Synapse 65:998–1005

Cui DH, Cabalu T, Yee KL, Small J, Li XD, Liu B, Maciolek C, Smith S, Liu W, McCrea JB, Prueksaritanont T (2016) In vitro and in vivo characterisation of the metabolism and disposition of suvorexant in humans. Xenobiotica 46:882–895

Dauvilliers Y, Neidhart E, Billiard M, Tafti M (2002) Sexual dimorphism of the catechol-O-methyltransferase gene in narcolepsy is associated with response to modafinil. Pharmacogenomics J 2:65–68

De Berardis D, Fornaro M, Serroni N, Campanella D, Rapini G, Olivieri L, Srinivasan V, Iasevoli F, Tomasetti C, De Bartolomeis A, Valchera A, Perna G, Mazza M, Di Nicola M, Martinotti G, Di Giannantonio M (2015) Agomelatine beyond borders: current evidences of its efficacy in disorders other than major depression. Int J Mol Sci 16:1111–1130

de Leon J, Nikoloff DM (2008) Paradoxical excitation on diphenhydramine may be associated with being a CYP2D6 ultrarapid metabolizer: three case reports. CNS Spectr 13:133–135

Deboer T, van Diepen HC, Ferrari MD, Van den Maagdenberg AMJM, Meijer JH (2013) Reduced sleep and low adenosinergic sensitivity in Cacna1a R192Q mutant mice. Sleep 36:127–136

Dresler M, Spoormaker VI, Beitinger P, Czisch M, Kimura M, Steiger A, Holsboer F (2014) Neuroscience-driven discovery and development of sleep therapeutics. Pharmacol Ther 141:300–334

Eban-Rothschild A, Rothschild G, Giardino WJ, Jones JR, de Lecea L (2016) VTA dopaminergic neurons regulate ethologically relevant sleep-wake behaviors. Nat Neurosci 19:1356–1366

Eban-Rothschild A, Appelbaum L, de Lecea L (2018) Neuronal mechanisms for sleep/wake regulation and modulatory drive. Neuropsychopharmacology 43:937–952

Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Goldman D, Weinberger DR (2001) Effect of COMT Val(108/158) met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci U S A 98:6917–6922

Ekmekcioglu C (2006) Melatonin receptors in humans: biological role and clinical relevance. Biomed Pharmacother 60:97–108

Evans WE, Relling MV (1999) Pharmacogenomics: translating functional genomics into rational therapeutics. Science 286:487–491

Faraone SV, Spencer TJ, Madras BK, Zhang-James Y, Biederman J (2014) Functional effects of dopamine transporter gene genotypes on in vivo dopamine transporter functioning: a meta-analysis. Mol Psychiatry 19:880–889

Farrell SM, Tunbridge EM, Braeutigam S, Harrison PJ (2012) COMT Val(158)Met genotype determines the direction of cognitive effects produced by catechol-O-methyltransferase inhibition. Biol Psychiatry 71:538–544

Ferre S, Lluis C, Justinova Z, Quiroz C, Orru M, Navarro G, Canela EI, Franco R, Goldberg SR (2010) Adenosine-cannabinoid receptor interactions. Implications for striatal function. Br J Pharmacol 160:443–453

Finelli LA, Landolt HP, Buck A, Roth C, Berthold T, Borbély AA, Achermann P (2000) Functional neuroanatomy of human sleep states after zolpidem and placebo: a $H_2{}^{15}O$-PET study. J Sleep Res 9:161–173

Fredholm BB, Bättig K, Holmen J, Nehlig A, Zvartau EE (1999) Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev 51:83–133

Froehlich TE, Epstein JN, Nick TG, Castro MSM, Stein MA, Brinkman WB, Graham AJ, Langberg JM, Kahn RS (2011) Pharmacogenetic predictors of methylphenidate dose-response in attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 50:1129–1139

Garcia-Borreguero D, Silber MH, Winkelman JW, Hogl B, Bainbridge J, Buchfuhrer M, Hadjigeorgiou G, Inoue Y, Manconi M, Oertel W, Ondo W, Winkelmann J, Allen RP (2016) Guidelines for the first-line treatment of restless legs syndrome/Willis-Ekbom disease, prevention and treatment of dopaminergic augmentation: a combined task force of the IRLSSG, EURLSSG, and the RLS-foundation. Sleep Med 21:1–11

Garcia-Borreguero D, Guitart X, Garcia Malo C, Cano-Pumarega I, Granizo JJ, Ferre S (2018) Treatment of restless legs syndrome/Willis-Ekbom disease with the non-selective ENT1/ENT2 inhibitor dipyridamole: testing the adenosine hypothesis. Sleep Med 45:94–97

Goforth HW (2009) Low-dose doxepin for the treatment of insomnia: emerging data. Expert Opin Pharmacother 10:1649–1655

Gottselig JM, Adam M, Rétey JV, Khatami R, Achermann P, Landolt HP (2006) Random number generation during sleep deprivation: effects of caffeine on response maintenance and stereotypy. J Sleep Res 15:31–40

Gunes A, Dahl ML (2008) Variation in CYP1A2 activity and its clinical implications: influence of environmental factors and genetic polymorphisms. Pharmacogenomics 9:625–637

Härtter S, Korhonen T, Lundgren S, Rane A, Tolonen A, Turpeinen M, Laine K (2006) Effect of caffeine intake 12 or 24 h prior to melatonin intake and CYP1A2*1F polymorphism on CYP1A2 phenotyping by melatonin. Basic Clin Pharmacol Toxicol 99:300–304

Haufroid V, Hantson P (2015) CYP2D6 genetic polymorphisms and their relevance for poisoning due to amfetamines, opioid analgesics and antidepressants. Clin Toxicol 53:501–510

Hayaishi O, Urade Y, Eguchi N, Huang Z-L (2004) Genes for prostaglandin D synthase and receptor as well as adenosine A_{2A} receptor are involved in the homeostatic regulation of NREM sleep. Arch Ital Biol 142:533–539

Hedner J, Zou D (2018) Drug therapy in obstructive sleep apnea. Sleep Med Clin 13:203

Hirvonen MM, Laakso A, Nagren K, Rinne JO, Pohjalainen T, Hietala J (2009) C957T polymorphism of dopamine D2 receptor gene affects striatal DRD2 in vivo availability by changing the receptor affinity. Synapse 63:907–912

Hohmann N, Haefeli WE, Mikus G (2016) CYP3A activity: towards dose adaptation to the individual. Expert Opin Drug Metab Toxicol 12:479–497

Holmes C, McCulley M, Nicoll JAR, Alder JT, Chen CPLH, Francis PT (2007) Genetic variation in the 5-HT2A receptor and altered neocortical [H-3] ketanserin binding in Alzheimer's disease. Neurosci Lett 420:58–60

Holst SC, Landolt HP (2015) Sleep homeostasis, metabolism, and adenosine. Curr Sleep Med Rep 1:27–37

Holst SC, Landolt HP (2018) Sleep-wake neurochemistry. Sleep Med Clin 13:137–146

Holst SC, Bersagliere A, Bachmann V, Berger W, Achermann P, Landolt HP (2014) Dopaminergic role in regulating neurophysiological markers of sleep homeostasis in humans. J Neurosci 34:566–573

Holst SC, Valomon A, Landolt HP (2016) Sleep pharmacogenetics: personalized sleep-wake therapy. Annu Rev Pharmacol Toxicol 56:577–603

Holst SC, Muller T, Valomon A, Seebauer B, Berger W, Landolt HP (2017) Functional polymorphisms in dopaminergic genes modulate neurobehavioral and neurophysiological consequences of sleep deprivation. Sci Rep 7:45982

Houghton WC, Scammell TE, Thorpy M (2004) Pharmacotherapy for cataplexy. Sleep Med Rev 8:355–366

Hoyer D, Jacobson LH (2013) Orexin in sleep, addiction and more: is the perfect insomnia drug at hand? Neuropeptides 47:477–488

Huang YS, Guilleminault C (2009) Narcolepsy: action of two gamma-aminobutyric acid type B agonists, baclofen and sodium oxybate. Pediatr Neurol 41:9–16

Huang ZL, Qu WM, Eguchi N, Chen JF, Schwarzschild MA, Fredholm BB, Urade Y, Hayaishi O (2005) Adenosine A_{2A}, but not A_1, receptors mediate the arousal effect of caffeine. Nat Neurosci 8:858–859

Hung CC, Chen PL, Huang WM, Tai JJ, Hsieh TJ, Ding ST, Hsieh YW, Liou HH (2013) Gene-wide tagging study of the effects of common genetic polymorphisms in the a subunits of the GABA(A) receptor on epilepsy treatment response. Pharmacogenomics 14:1849–1856

Idzikowski C (2014) The pharmacology of human sleep, a work in progress? Curr Opin Pharmacol 14:90–96

Ingelman-Sundberg M (2004) Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future. Trends Pharmacol Sci 25:193–200

Iwata N, Cowley DS, Radel M, Roy-Byrne PP, Goldman D (1999) Relationship between a GABA (A)alpha 6 Pro385Ser substitution and benzodiazepine sensitivity. Am J Psychiatry 156:1447–1449

Kang RH, Choi MJ, Paik JW, Hahn SW, Lee MS (2007) Effect of serotonin receptor 2A gene polymorphism on mirtazapine response in major depression. Int J Psychiatry Med 37:315–329

Karamitri A, Renault N, Clement N, Guillaume JL, Jockers R (2013) Minireview: toward the establishment of a link between melatonin and glucose homeostasis: association of melatonin MT2 receptor variants with type 2 diabetes. Mol Endocrinol 27:1217–1233

Kato M, Serretti A (2010) Review and meta-analysis of antidepressant pharmacogenetic findings in major depressive disorder. Mol Psychiatry 15:473–500

Kaye CM, Nicholls B (2000) Clinical pharmacokinetics of ropinirole. Clin Pharmacokinet 39:243–254

Kerb R (2006) Implications of genetic polymorphisms in drug transporters for pharmacotherapy. Cancer Lett 234:4–33

Kereszturi E, Tarnok Z, Bognar E, Lakatos K, Farkas L, Gadoros J, Sasvari-Szekely M, Nemoda Z (2008) Catechol-O-methyltransferase Val158Met polymorphism is associated with methylphenidate response in ADHD children. Am J Med Genet B Neuropsychiatr Genet 147B:1431–1435

Kirchheiner J, Meineke I, Muller G, Roots L, Brockmoller J (2002) Contributions of CYP2D6, CYP2C9 and CYP2C19 to the biotransformation of E- and Z-doxepin in healthy volunteers. Pharmacogenetics 12:571–580

Kopp C, Rudolph U, Low K, Tobler I (2004) Modulation of rhythmic brain activity by diazepam: GABA(A) receptor subtype and state specificity. Proc Natl Acad Sci U S A 101:3674–3679

Krystal AD, Richelson E, Roth T (2013) Review of the histamine system and the clinical effects of H-1 antagonists: basis for a new model for understanding the effects of insomnia medications. Sleep Med Rev 17:263–272

Landolt HP, Rétey JV, Tönz K, Gottselig JM, Khatami R, Buckelmüller I, Achermann P (2004) Caffeine attenuates waking and sleep electroencephalographic markers of sleep homeostasis in humans. Neuropsychopharmacology 29:1933–1939

Landolt H-P, Rétey JV, Adam M (2012) Reduced neurobehavioral impairment from sleep deprivation in older adults: contribution of adenosinergic mechanisms. Front Neurol 3:62

Lankford DA (2011) Tasimelteon for insomnia. Expert Opin Investig Drugs 20:987–993

Lazarus M, Shen HY, Cherasse Y, Qu WM, Huang ZL, Bass CE, Winsky-Sommerer R, Semba K, Fredholm BB, Boison D, Hayaishi O, Urade Y, Chen JF (2011) Arousal effect of caffeine depends on adenosine A(2A) receptors in the shell of the nucleus accumbens. J Neurosci 31:10067–10075

Lazarus M, Chen JF, Huang ZL, Urade Y, Fredholm BB (2017) Adenosine and sleep. Handb Exp Pharmacol. https://doi.org/10.1007/164_2017_36

Lena I, Parrot S, Deschaux O, Muffat-Joly S, Sauvinet V, Renaud B, Suaud-Chagny MF, Gottesmann C (2005) Variations in extracellular levels of dopamine, noradrenaline, glutamate, and aspartate across the sleep-wake cycle in the medial prefrontal cortex and nucleus accumbens of freely moving rats. J Neurosci Res 81:891–899

Li T, Chen CK, Hu X, Ball D, Lin SK, Chen W, Sham PC, Loh EW, Murray RM, Collier DA (2004) Association analysis of the DRD4 and COMT genes in methamphetamine abuse. Am J Med Genet B Neuropsychiatr Genet 129B:120–124

Lim J, Dinges DF (2010) A meta-analysis of the impact of short-term sleep deprivation on cognitive variables. Psychol Bull 136:375–389

Lim J, Ebstein R, Tse CY, Monakhov M, Lai PS, Dinges DF, Kwok K (2012) Dopaminergic polymorphisms associated with time-on-task declines and fatigue in the Psychomotor Vigilance Test. PLoS One 7:e33767

Lin JS, Sergeeva OA, Haas HL (2011) Histamine H-3 receptors and sleep-wake regulation. J Pharmacol Exp Ther 336:17–23

Liu YZ, Tang BS, Yan XX, Liu J, Ouyang DS, Nie LN, Fan L, Li Z, Ji W, Hu DL, Wang D, Zhou HH (2009) Association of the DRD2 and DRD3 polymorphisms with response to pramipexole in Parkinson's disease patients. Eur J Clin Pharmacol 65:679–683

Loftfield E, Cornelis MC, Caporaso N, Yu K, Sinha R, Freedman N (2018) Association of coffee drinking with mortality by genetic variation in caffeine metabolism: findings from the UK Biobank. JAMA Intern Med 178:1086–1097

Lozupone M, Panza F, Stella E, La Montagna M, Bisceglia P, Miscio G, Galizia I, Daniele A, di Mauro L, Bellomo A, Logroscino G, Greco A, Seripa D (2017) Pharmacogenetics of neurological and psychiatric diseases at older age: has the time come? Expert Opin Drug Metab Toxicol 13:259–277

Luciano M, Zhu G, Kirk KM, Gordon SD, Heath AC, Montgomery GW, Martin NG (2007) "No thanks, it keeps me awake": the genetics of coffee-attributed sleep disturbance. Sleep 30:1378–1386

Luppi PH, Fort P (2018) Neuroanatomical and neurochemical bases of vigilance states. Handb Exp Pharmacol. https://doi.org/10.1007/164_2017_84

Maire M, Reichert CF, Gabel V, Viola AU, Phillips C, Berthomier C, Borgwardt S, Cajochen C, Schmidt C (2018) Human brain patterns underlying vigilant attention: impact of sleep debt, circadian phase and attentional engagement. Sci Rep 8:970

Mamelak M (2009) Narcolepsy and depression and the neurobiology of gammahydroxybutyrate. Prog Neurobiol 89:193–219

Matsumoto M, Weickert CS, Akil M, Lipska BK, Hyde TM, Herman MM, Kleinman JE, Weinberger DR (2003) Catechol O-methyltransferase mRNA expression in human and rat brain: evidence for a role in cortical neuronal function. Neuroscience 116:127–137

Mazzotti DR, Guindalini C, Pellegrino R, Barrueco KF, Santos-Silva R, Bittencourt LRA, Tufik S (2011) Effects of the adenosine deaminase polymorphism and caffeine intake on sleep parameters in a large population sample. Sleep 34:399–402

McGrane IR, Leung JG, St Louis EK, Boeve BF (2015) Melatonin therapy for REM sleep behavior disorder: a critical review of evidence. Sleep Med 16:19–26

Mignot EJM (2012) A practical guide to the therapy of narcolepsy and hypersomnia syndromes. Neurotherapeutics 9:739–752

Mitler MM, Walsleben J, Sangal RB, Hirshkowitz M (1998) Sleep latency on the maintenance of wakefulness test (MWT) for 530 patients with narcolepsy while free of psychoactive drugs. Electroencephalogr Clin Neurophysiol 107:33–38

Moresco M, Riccardi LN, Pizza F, Zenesini C, Caporali L, Plazzi G, Pelotti S (2016) Pharmacogenetics and treatment response in narcolepsy type 1: relevance of the polymorphisms of the drug transporter gene ABCB1. Clin Neuropharmacol 39:18–23

Moruzzi G, Magoun HW (1949) Brain stem and EEG activation. Electroencephalogr Clin Neurophysiol 1:455–473

Neukamm MA, Vogt S, Hermanns-Clausen M, Naue J, Thierauf A, Auwarter V (2013) Fatal doxepin intoxication – suicide or slow gradual intoxication? Forensic Sci Int 227:82–84

Nir Y, Andrillon T, Marmelshtein A, Suthana N, Cirelli C, Tononi G, Fried I (2017) Selective neuronal lapses precede human cognitive lapses following sleep deprivation. Nat Med 23:1474–1480

Nittur N, Konofal E, Dauvilliers Y, Franco P, Leu-Semenescu S, De Cock VC, Inocente CO, Bayard S, Scholtz S, Lecendreux M, Arnulf I (2013) Mazindol in narcolepsy and idiopathic and symptomatic hypersomnia refractory to stimulants: a long-term chart review. Sleep Med 14:30–36

Oishi Y, Suzuki Y, Takahashi K, Yonezawa T, Kanda T, Takata Y, Cherasse Y, Lazarus M (2017a) Activation of ventral tegmental area dopamine neurons produces wakefulness through dopamine D-2-like receptors in mice. Brain Struct Funct 222:2907–2915

Oishi Y, Xu Q, Wang L, Zhang BJ, Takahashi K, Takata Y, Luo YJ, Cherasse Y, Schiffmann SN, d'Exaerde AD, Urade Y, Qu WM, Huang ZL, Lazarus M (2017b) Slow-wave sleep is controlled by a subset of nucleus accumbens core neurons in mice. Nat Commun 8:734

Park JY, Kim KA, Park PL, Lee OJ, Kang DK, Shon JH, Liu KH, Shin JG (2006) Effect of CYP3A5*3 genotype on the pharmacokinetics and pharmacodynamics of alprazolam in healthy subjects. Clin Pharmacol Ther 79:590–599

Park HJ, Park JK, Kim SK, Cho AR, Kim JW, Yim SV, Chung JH (2011) Association of polymorphism in the promoter of the melatonin receptor 1A gene with schizophrenia and with insomnia symptoms in schizophrenia patients. J Mol Neurosci 45:304–308

Parsons MJ, Lester KJ, Barclay NL, Nolan PM, Eley TC, Gregory AM (2013) Replication of genome-wide association studies (GWAS) loci for sleep in the British G1219 cohort. Am J Med Genet B Neuropsychiatr Genet 162B:431–438

Patel KV, Aspesi AV, Evoy KE (2015) Suvorexant: a dual orexin receptor antagonist for the treatment of sleep onset and sleep maintenance insomnia. Ann Pharmacother 49:477–483

Pinsonneault JK, Han DD, Burdick KE, Kataki M, Bertolino A, Malhotra AK, Gu HH, Sadee W (2011) Dopamine transporter gene variant affecting expression in human brain is associated with bipolar disorder. Neuropsychopharmacology 36:1644–1655

Proctor A, Bianchi MT (2012) Clinical pharmacology in sleep medicine. ISRN Pharmacol 2012:914168

Qiu MH, Yao QL, Vetrivelan R, Chen MC, Lu J (2016) Nigrostriatal dopamine acting on globus pallidus regulates sleep. Cereb Cortex 26:1430–1439

Ramaekers JG, Conen S, de Kam PJ, Braat S, Peeters P, Theunissen EL, Ivgy-may N (2011) Residual effects of esmirtazapine on actual driving performance: overall findings and an exploratory analysis into the role of CYP2D6 phenotype. Psychopharmacology 215:321–332

Rasmussen BB, Brix TH, Kyvik KO, Brosen K (2002) The interindividual differences in the 3-demthylation of caffeine alias CYP1A2 is determined by both genetic and environmental factors. Pharmacogenetics 12:473–478

Renda G, Committeri G, Zimarino M, Di Nicola M, Tatasciore A, Ruggieri B, Ambrosini E, Viola V, Antonucci I, Stuppia L, De Caterina R (2015) Genetic determinants of cognitive responses to caffeine drinking identified from a double-blind, randomized, controlled trial. Eur Neuropsychopharmacol 25:798–807

Rétey JV, Adam M, Gottselig JM, Khatami R, Dürr R, Achermann P, Landolt H-P (2006) Adenosinergic mechanisms contribute to individual differences in sleep-deprivation induced changes in neurobehavioral function and brain rhythmic activity. J Neurosci 26:10472–10479

Rétey JV, Adam M, Khatami R, Luhmann UFO, Jung HH, Berger W, Landolt H-P (2007) A genetic variation in the adenosine A_{2A} receptor gene (*ADORA2A*) contributes to individual sensitivity to caffeine effects on sleep. Clin Pharmacol Ther 81:692–698

Rhodin A, Gronbladh A, Ginya H, Nilsson KW, Rosenblad A, Zhou Q, Enlund M, Hallberg M, Gordh T, Nyberg F (2013) Combined analysis of circulating beta-endorphin with gene polymorphisms in OPRM1, CACNAD2 and ABCB1 reveals correlation with pain, opioid sensitivity and opioid-related side effects. Mol Brain 6:8

Riemann D, Perlis ML (2009) The treatments of chronic insomnia: a review of benzodiazepine receptor agonists and psychological and behavioral therapies. Sleep Med Rev 13:205–214

Rivara S, Pala D, Bedini A, Spadoni G (2015) Therapeutic uses of melatonin and melatonin derivatives: a patent review (2012–2014). Expert Opin Ther Pat 25:425–441

Roden DM, Wilke RA, Kroemer HK, Stein CM (2011) Pharmacogenomics the genetics of variable drug responses. Circulation 123:1661–1670

Rupp TL, Wesensten NJ, Newman R, Balkin TJ (2013) PER3 and ADORA2A polymorphisms impact neurobehavioral performance during sleep restriction. J Sleep Res 22:160–165

Sachse C, Brochmoller J, Bauer S, Roots I (1999) Functional significance of a C → A polymorphism in intron I of the cytochrome P450 CYP1A2 gene tested with caffeine. Br J Clin Pharmacol 47:445–449

Sadee W, Dai Z (2005) Pharmacogenetics/genomics and personalized medicine. Hum Mol Genet 14:R207–R214

Saper CB, Fuller PM (2017) Wake-sleep circuitry: an overview. Curr Opin Neurobiol 44:186–192

Saruwatari J, Matsunaga M, Ikeda K, Nakao M, Oniki K, Seo T, Mihara S, Marubayashi T, Kamataki T, Nakagawa K (2006) Impact of CYP2D6*10 on H1-antihistamine-induced hypersomnia. Eur J Clin Pharmacol 62:995–1001

Satterfield BC, Wisor JP, Schmidt MA, Van Dongen HPA (2017) Time-on-task effect during sleep deprivation in healthy young adults is modulated by dopamine transporter genotype. Sleep 40: zsx167

Satterfield BC, Hinson JM, Whitney P, Schmidt MA, Wisor JP, Van Dongen HPA (2018) Catechol-O-methyltransferase (COMT) genotype affects cognitive control during total sleep deprivation. Cortex 99:179–186

Saxvig IW, Wilhelmsen-Langeland A, Pallesen S, Vedaa O, Nordhus IH, Bjorvatn B (2014) A randomized controlled trial with bright light and melatonin for delayed sleep phase disorder: effects on subjective and objective sleep. Chronobiol Int 31:72–86

Seibyl J, Russell D, Jennings D, Marek K (2012) Neuroimaging over the course of Parkinson's disease: from early detection of the at-risk patient to improving pharmacotherapy of later-stage disease. Semin Nucl Med 42:406–414

Shen M, Shi Y, Xiang P (2013) CYP3A4 and CYP2C19 genetic polymorphisms and zolpidem metabolism in the Chinese Han population: a pilot study. Forensic Sci Int 227:77–81

Siller N, Egerer G, Weiss J, Mikus G (2014) Prolonged sedation of lorazepam due to absent UGT2B4/2B7 glucuronidation. Arch Toxicol 88:179–180

Song L, Du Q, Jiang X, Wang L (2014) Effect of CYP1A2 polymorphism on the pharmacokinetics of agomelatine in Chinese healthy male volunteers. J Clin Pharm Ther 39:204–209

Spiegelhalder K, Nissen C, Riemann D (2017) Clinical sleep-wake disorders II: focus on insomnia and circadian rhythm sleep disorders. Handb Exp Pharmacol. https://doi.org/10.1007/164_2017_40

Stocking EM, Letavic MA (2008) Histamine H(3) antagonists as wake-promoting and pro-cognitive agents. Curr Top Med Chem 8:988–1002

Sulem P, Gudbjartsson DF, Geller F, Prokopenko I, Feenstra B, Aben KKH, Franke B, den Heijer M, Kovacs P, Stumvoll M, Magi R, Yanek LR, Becker LC, Boyd HA, Stacey SN, Walters GB, Jonasdottir A, Thorleifsson G, Holm H, Gudjonsson SA, Rafnar T, Bjornsdottir G, Becker DM, Melbye M, Kong A, Tonjes A, Thorgeirsson T, Thorsteinsdottir U, Kiemeney LA, Stefansson K (2011) Sequence variants at CYP1A1-CYP1A2 and AHR associate with coffee consumption. Hum Mol Genet 20:2071–2077

Thompson MD, Xhaard H, Sakurai T, Rainero I, Kukkonen JP (2014) OX1 and OX2 orexin/hypocretin receptor pharmacogenetics. Front Neurosci 8:57

Thorpy MJ, Dauvilliers Y (2015) Clinical and practical considerations in the pharmacologic management of narcolepsy. Sleep Med 16:9–18

Tobler I, Kopp C, Deboer T, Rudolph U (2001) Diazepam-induced changes in sleep: role of the alpha 1 GABA(A) receptor subtype. Proc Natl Acad Sci U S A 98:6464–6469

Tomasi D, Wang GJ, Volkow ND (2016) Association between striatal dopamine D-2/D-3 receptors and brain activation during visual attention: effects of sleep deprivation. Transl Psychiatry 6: e828

Toth K, Csukly G, Sirok D, Belic A, Kiss A, Hafra E, Deri M, Menus A, Bitter I, Monostory K (2016) Optimization of clonazepam therapy adjusted to patient's CYP3A status and NAT2 genotype. Int J Neuropsychopharmacol 19

Trenkwalder C, Paulus W (2010) Restless legs syndrome: pathophysiology, clinical presentation and management. Nat Rev Neurol 6:337–346

Trenkwalder C, Benes H, Grote L, Garcia-Borreguero D, Hogl B, Hopp M, Bosse B, Oksche A, Reimer K, Winkelmann J, Allen RP, Kohnen R, Grp RS (2013) Prolonged release oxycodone-naloxone for treatment of severe restless legs syndrome after failure of previous treatment: a double-blind, randomised, placebo-controlled trial with an open-label extension. Lancet Neurol 12:1141–1150

Treur JL, Gibson M, Taylor AE, Rogers PJ, Munafò MR (2018) Investigating genetic correlations and causal effects between caffeine consumption and sleep behaviours. J Sleep Res 27(5): e12695

Tunbridge EM, Harrison PJ, Weinberger DR (2006) Catechol-o-methyltransferase, cognition, and psychosis: Val(158)Met and beyond. Biol Psychiatry 60:141–151

Tyree SM, de Lecea L (2017) Optogenetic investigation of arousal circuits. Int J Mol Sci 18:1773

Ujike H, Harano M, Inada T, Yamada M, Komiyama T, Sekine Y, Sora I, Iyo M, Katsu T, Nomura A, Nakata K, Ozaki N (2003) Nine- or fewer repeat alleles in VNTR polymorphism

of the dopamine transporter gene is a strong risk factor for prolonged methamphetamine psychosis. Pharmacogenomics J 3:242–247
Urban TJ, Yang C, Castro RA, Taylor TR, Huang CC, Stryke D, Johns SJ, Kawamoto M, Carlson EJ, Ferrin TE, Burchard EG, Giacomini KM (2007) Functional effects of protein sequence polymorphisms in the organic cation/ergothioneine transporter OCTN1 (SLC22A4). Pharmacogenet Genomics 17:773–782
Urry E, Jetter A, Landolt HP (2016) Assessment of CYP1A2 enzyme activity in relation to type-2 diabetes and habitual caffeine intake. Nutr Metab 13:66
Urry E, Jetter A, Holst SC, Berger W, Spinas GA, Langhans W, Landolt HP (2017) A case-control field study on the relationships among type 2 diabetes, sleepiness and habitual caffeine intake. J Psychopharmacol 31:233–242
Valomon A, Holst SC, Borrello A, Weigend S, Muller T, Berger W, Sommerauer M, Baumann CR, Landolt HP (2018) Effects of COMT genotype and tolcapone on lapses of sustained attention after sleep deprivation in healthy young men. Neuropsychopharmacology 43:1599–1607
Van Dongen HPA, Baynard MD, Maislin G, Dinges DF (2004) Systematic interindividual differences in neurobehavioral impairment from sleep loss: evidence of trait-like differential vulnerability. Sleep 27:423–433
Varin C, Bonnavion P (2018) Pharmacosynthetic deconstruction of sleep-wake circuits in the brain. Handb Exp Pharmacol (in press)
Volkow ND, Fowler JS, Logan J, Alexoff D, Zhu W, Telang F, Wang GJ, Jayne M, Hooker JM, Wong C, Hubbard B, Carter P, Warner D, King P, Shea C, Xu YW, Muench L, Apelskog-Torres K (2009) Effects of modafinil on dopamine and dopamine transporters in the male human brain clinical implications. JAMA 301:1148–1154
Volkow ND, Tomasi D, Wang GJ, Telang F, Fowler JS, Logan J, Benveniste H, Kim R, Thanos PK, Ferre S (2012) Evidence that sleep deprivation downregulates dopamine D2R in ventral striatum in the human brain. J Neurosci 32:6711–6717
Weinhold SL, Seeck-Hirschner M, Nowak A, Hallschmid M, Goder R, Baier PC (2014) The effect of intranasal orexin-A (hypocretin-1) on sleep, wakefulness and attention in narcolepsy with cataplexy. Behav Brain Res 262:8–13
Whitney P, Hinson JM, Satterfield BC, Grant DA, Honn KA, Van Dongen HPA (2017) Sleep deprivation diminishes attentional control effectiveness and impairs flexible adaptation to changing conditions. Sci Rep 7:16020
Wilt TJ, MacDonald R, Brasure M, Olson CM, Carlyle M, Fuchs E, Khawaja IS, Diem S, Koffel E, Ouellette J, Butler M, Kane RL (2016) Pharmacologic treatment of insomnia disorder: an evidence report for a clinical practice guideline by the American College of Physicians. Ann Int Med 165:103–112
Winkelmann J, Allen RP, Hogl B, Inoue Y, Oertel W, Salminen AV, Winkelman JW, Trenkwalder C, Sampaio C (2018) Treatment of restless legs syndrome: evidence-based review and implications for clinical practice (Revised 2017). Mov Disord 33(7):1077–1091
Wisor JP (2018) Dopamine and wakefulness: pharmacology, genetics and circuitry. Handb Exp Pharmacol. https://doi.org/10.1007/164_2018_95
Wisor JP, Nishino S, Sora I, Uhl GH, Mignot E, Edgar DM (2001) Dopaminergic role in stimulant-induced wakefulness. J Neurosci 21:1787–1794
Yager LM, Garcia AF, Wunsch AM, Ferguson SM (2015) The ins and outs of the striatum: role in drug addiction. Neuroscience 301:529–541
Zhang JP, Xu Q, Yuan XS, Cherasse Y, Schiffmann SN, d'Exaerde AD, Qu WM, Urade Y, Lazarus M, Huang ZL, Li RX (2013) Projections of nucleus accumbens adenosine A (2A) receptor neurons in the mouse brain and their implications in mediating sleep-wake regulation. Front Neuroanat 7:43

Clinical Sleep-Wake Disorders I: Focus on Hypersomnias and Movement Disorders During Sleep

Christian R. Baumann

Contents

Abstract

Central disorders of hypersomnolence are characterized by daily periods of irrepressible need to sleep or daytime lapses into sleep, as defined in the current version of the International Criteria of Sleep Disorders. Thus, the unifying symptom is excessive daytime sleepiness which is not caused by any other sleep-wake disorder. Relevant disorders including narcolepsy type 1 and 2, idiopathic hypersomnia, Kleine-Levin syndrome, and insufficient sleep syndrome will be discussed. Other central disorders of hypersomnolence include hypersomnias due to medical or psychiatric disorders or because of medication or substance use.

In sleep-related movement disorders, the cardinal symptom consists of simple, often stereotyped movements occurring during sleep. The most frequent disorder

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in this category of sleep-wake disorders is restless legs syndrome, which is often associated with period limb movements during sleep.

Keywords
Excessive daytime sleepiness · Hypersomnia · Multiple sleep latency test · Narcolepsy · Polysomnography · Restless legs

1 Central Disorders of Hypersomnolence

Central disorders of hypersomnolence (formerly known as hypersomnias) (Table 1) are characterized by daily periods of irrepressible need to sleep or daytime lapses into sleep, as defined in the current version of the International Criteria of Sleep Disorders (AASM 2014).

1.1 Narcolepsy Type 1

Narcolepsy type 1, formerly known as narcolepsy with cataplexy, is a chronic hypersomnia disorder which is characterized by excessive daytime sleepiness and symptoms of disturbed rapid eye movement (REM) regulation.

Table 1 Central disorders of hypersomnolence and sleep-related movement disorders, classified along the International Criteria for Sleep Disorders, third edition (ICSD-3) (AASM 2014)

Central disorders of hypersomnolence
Narcolepsy type 1
Narcolepsy type 2
Idiopathic hypersomnia
Kleine-Levin syndrome
Hypersomnia due to a medical disorder
Hypersomnia due to a medication or substance
Hypersomnia associated with a psychiatric disorder
Insufficient sleep syndrome
Sleep-related movement disorders
Restless legs syndrome
Periodic limb movement disorder
Sleep-related leg cramps
Sleep-related bruxism
Sleep-related rhythmic movement disorder
Benign sleep myoclonus of infancy
Propriospinal myoclonus at sleep onset
Sleep-related movement disorder due to a medical disorder
Sleep-related movement disorder due to a medication or substance
Sleep-related movement disorder, unspecified

Epidemiology Narcolepsy affects about 1–2 in 4,000 people and usually begins in childhood or adolescence (Hublin et al. 1994; Scammell 2015), yet there are significant differences in prevalence between different populations. Most epidemiological studies in narcolepsy are burdened with limitations. For instance, most are only questionnaire-based. There are few epidemiological studies based on multiple sleep latency tests (MSLT). There is, however, increasing evidence that MSLT criteria for narcolepsy are neither intraindividually stable nor specific for narcolepsy (Trotti et al. 2013; Marti et al. 2009). Misinterpretation of MSLT results therefore might have caused an overestimation of narcolepsy in some studies. In addition, there is a marked diagnostic delay due to unawareness of the disorder and its symptoms both in patients and in treating physicians (Taddei et al. 2016). This delay not only causes impairment of quality of life of affected patients but also most likely leads to an underestimation of the frequency of narcolepsy. Altogether, there are no unambiguous epidemiological studies in the field.

Symptomatology Chronic excessive daytime sleepiness is mandatory for the diagnosis of narcolepsy type 1 (AASM 2014). It is defined as increased sleep propensity during daytime, which may lead to sleep attacks even in non-adequate situations. Such sleepiness is present every day, also when patients allow themselves adequate sleep times. Nighttime sleep and short naps usually improve sleepiness for a brief period of time which typically does not exceed 1–2 h.

Narcolepsy type 1 patients typically suffer from cataplexy, i.e., loss of voluntary muscle tone upon emotions that is not accompanied by a decrease or loss of consciousness. This loss of muscle tone is often only partial, e.g., affecting head or neck muscles, but may also manifest itself with generalized muscle tone loss and consecutively with falls. The duration of cataplexy attacks is usually only seconds to few minutes. Longer attacks up to status cataplecticus may occur upon discontinuation of anti-cataplectic treatment. Although typical cataplexy is considered pathognomonic for narcolepsy type 1, its presence is no longer sufficient to make the diagnosis in chronically sleepy patients (AASM 2014). Like hallucinations and sleep paralysis, cataplexy is interpreted as intrusion of REM sleep-like behavioral states into wakefulness (Scammell 2015).

Hallucinations that occur when patients fall asleep (hypnagogic) or wake up (hypnopompic) are reported by many narcolepsy patients and may affect different sensory systems. Most often, however, they are visual and somehow threatening. Sleep paralysis also occurs during transitions between wakefulness and sleep and lasts seconds to few minutes. During such episodes, patients cannot move their voluntary muscles, but eye movements and breathing are unimpaired. Neither hallucinations nor sleep paralysis are specific for narcolepsy.

Most narcolepsy patients complain about disturbed, i.e., fragmented nocturnal sleep. They often wake up during the night but then quickly fall back into sleep. Other symptoms that are more prevalent than in the general population are increased body weight, depression, obstructive sleep apnea, periodic limb movements during sleep, and parasomnias.

Pathophysiology Narcolepsy type 1 is considered a disorder of hypocretin (orexin) signaling deficiency, as it has been shown to be associated with a selective and almost complete loss of wakefulness-promoting hypocretin neurons in the posterolateral hypothalamus (Peyron et al. 2000; Thannickal et al. 2000). Hypocretin deficiency is conceptualized to underlie not only excessive daytime sleepiness and enhanced REM sleep pressure but also state behavioral instability, i.e., enhanced likelihood to switch from wakefulness to sleep and vice versa (Saper et al. 2005) (Fig. 1).

The cause of hypocretin neuronal loss is still unclear, but several observations indicate an autoimmune cause: the selectivity of neuronal destruction, the tight association with human leukocyte antigen (HLA) alleles, particularly HLA-DQB1*06:02, and the assumption that streptococcal and H1N1 influenza A virus infection and H1N1 vaccination might act as triggers (Cvetkovic-Lopes et al. 2010; Hallmayer et al. 2009; Mignot et al. 2001; Kornum et al. 2017). Nevertheless, the final proof for an autoimmune cause is still lacking.

The finding of a marked increase of wake-maintaining histamine neurons in the tuberomammillary nucleus in narcolepsy type 1 patients suggests that other neurotransmitter systems are likely to contribute to the clinical picture in those patients (Valko et al. 2013; John et al. 2013).

Diagnosis The current diagnostic criteria request chronic excessive daytime sleepiness and one of the following findings: either pathologically decreased cerebrospinal fluid hypocretin levels or a combination of typical cataplexy and typical findings on the MSLT, i.e., a low mean sleep latency below 8 min and multiple sleep-onset rapid eye movement (REM) sleep periods in 4–5 nap opportunities (AASM 2014). In the presence of low hypocretin levels, the diagnosis is usually straightforward and can

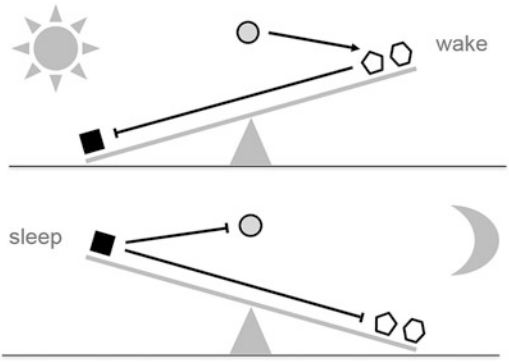

Fig. 1 Hypocretin (orexin) neurons (gray circle) in the posterolateral hypothalamus excite wake-promoting monoaminergic (white pentagon) and cholinergic (white hexagon) in the brainstem and posterior hypothalamus. In addition, it is assumed that these neurons stabilize the flip-flop switch between sleep and wakefulness. GABAergic and galaninergic neurons (black square) in the ventrolateral preoptic area inhibit hypocretin and other wake-promoting neuronal systems and ultimately promote sleep (Adapted and simplified from Saper et al. 2005)

be considered solid, yet the alternative criteria of cataplexy in combination with low mean sleep latencies can be misleading. Cataplexy is usually not observed in the doctor's practice and therefore relies on patient's history. The diagnosis of cataplexy can be difficult, even for experienced specialists (Pizza et al. 2018). In addition, low MSLT mean sleep latencies are common in society, mostly due to widespread insufficient sleep. In narcolepsy, mean sleep latencies are typically very low, i.e., below 3–4 min. In case of any diagnostic doubts – given the therapeutic consequences – a lumbar tap should be performed to measure hypocretin levels.

HLA-DQB1*06:02 can be assessed, but due to the fact that this allele is also common in the non-narcoleptic population, only negative HLA findings are helpful in the diagnosis, as they make the diagnosis of narcolepsy type 1 most unlikely.

Treatment Most patients are treated with a combined non-pharmacological (e.g., sleep hygiene with scheduled daytime naps) and pharmacological approach. In many countries worldwide, stimulants such as modafinil (100–400 mg daily), armodafinil (100–250 mg per day), or methylphenidate (10–60 mg per day) are approved for the treatment of excessive daytime sleepiness (Scammell 2015; Kornum et al. 2017). A novel inverse histamine H_3 receptor agonist, pitolisant (9–36 mg daily), has been approved in several countries to improve sleepiness, and recent evidence suggests that the compound might also exert some beneficial effect on cataplexy (Szakacs et al. 2017). Sodium oxybate, an agonist on $GABA_B$ and possibly specific gamma-hydroxybutyric acid (GHB) receptors, is administered twice at night (at bedtime and 3–4 h later, up to 9 g per day) and consolidates nocturnal sleep and improves sleepiness but also cataplexy, hallucinations, and sleep paralysis. Alternative – and mostly off-label – strategies to reduce cataplexy consist of antidepressants that increase serotonin and/or noradrenaline neurotransmission, e.g., the serotonin-noradrenaline reuptake inhibitor venlafaxine (37.5–300 mg per day).

1.2 Narcolepsy Type 2

Narcolepsy type 2 is characterized by excessive daytime sleepiness in combination with increased REM sleep pressure. In contrast to narcolepsy type 1 with its specific biomarker (deficient hypocretin in cerebrospinal fluid) and clear-cut clinical and electrophysiological findings, the diagnosis of narcolepsy type 2 is burdened with unspecific findings and therefore challenging (Baumann et al. 2014). The current diagnostic criteria require chronic sleepiness and a positive MSLT (as in narcolepsy type 1: mean sleep latency $\leq$8 min and two or more sleep-onset REM periods), but MSLT results appear particularly unreliable in narcolepsy type 2 (Ruoff et al. 2018). In addition, there is no history of cataplexy, and hypocretin levels in cerebrospinal fluid – if measured – are within the normal range (AASM 2014). Most importantly, any other cause for these symptoms should be excluded. As insufficient sleep can produce sleepiness and positive MSLT findings, this differential diagnosis should be ruled out before making the diagnosis of narcolepsy type 2 (see below).

Because of the high level of diagnostic vagueness, the real epidemiology of narcolepsy type 2 remains obscure. On the one hand, given the long diagnostic delay between narcolepsy symptoms and diagnosis, it is conceivable that narcolepsy type 2 is underdiagnosed (Taddei et al. 2016). On the other hand, however, the clinical and electrophysiological overlap with insufficient sleep syndrome makes it even more likely that narcolepsy type 2 is heavily overdiagnosed (Marti et al. 2009). Even more, it remains open whether or not narcolepsy type 2 is a distinct entity or merely a prodromal phase of narcolepsy type 1 which often begins with excessive sleepiness, whereas cataplexy can emerge years later. In the absence of biomarkers for the disease, therefore, there is a need of large multicenter studies applying strict clinical and electrophysiological assessments to obtain a better view on narcolepsy type 2.

To diagnose narcolepsy type 2, actigraphy over at least 7 days should be recorded prior to polysomnography and MSLT, to rule out insufficient sleep or shift work. In case that actigraphy is not reimbursed by insurance or not available, sleep logs should be administered, although poor compliance can produce invalid results. Likewise, medical or environmental conditions that might cause sleepiness should be searched for. Positive HLA-DQB1*06:02 can be indicative of narcolepsy, yet is unspecific.

The therapy of excessive daytime sleepiness is the same as in narcolepsy type 1 (see above).

1.3 Idiopathic Hypersomnia

Idiopathic hypersomnia is a chronic neurological disorder, which presents with excessive daytime sleepiness, non-restorative sleep, and typically increased sleep duration per 24 h. As indicated by nomenclature, the underlying pathophysiology is not known.

Epidemiology The prevalence of idiopathic hypersomnia is unknown, yet its frequency is considered much lower than narcolepsy (Trotti 2017). Using population-based data of 19,136 noninstitutionalized individuals, some authors found a combination of excessive daytime sleepiness with non-restorative sleep of at least 9 h in 0.5% of the population, but as much as these symptoms may be typical for idiopathic hypersomnia, they are not specific, as there might be a significant overlap with depression and other neuropsychiatric disorders (Ohayon et al. 2013).

Symptomatology As in narcolepsy, the core symptom of idiopathic hypersomnia is chronic excessive daytime sleepiness, i.e., irresistible need to fall asleep during daytime, including unwanted episodes of daytime sleep. As always, sleepiness must not be caused by any other sleep-wake, medical, neurological, or psychiatric disorder. A very typical expression of idiopathic hypersomnia is prolonged nocturnal sleep, usually above 10 h per day. In addition, patients with idiopathic hypersomnia typically report that sleep is not refreshing, neither nocturnal sleep nor daytime naps.

A prolonged and marked difficulty to wake up after sleep that is referred to as sleep inertia or sleep drunkenness is a helpful clinical sign to distinguish idiopathic hypersomnia from other central disorders with hypersomnolence (Kretzschmar et al. 2016). This clinical feature impairs quality of life of patients with idiopathic hypersomnia considerably, as affected subjects never feel fully refreshed (Ozaki et al. 2012). In addition, many patients report subjective cognitive deficits, e.g., memory or attention problems.

Diagnosis Apart from excessive daytime sleepiness, increased sleep need per 24 h is the most important diagnostic feature. The current diagnostic criteria demand chronic subjective sleepiness, absence of cataplexy, absence of enhanced REM sleep pressure upon polysomnography or MSLT tests, and either short MSLT mean latencies (below 8 min) or a total sleep time of at least 11 h per 24 h on a 24-h polysomnography or wrist actigraphy over at least 7 days with unrestricted sleep opportunities (AASM 2014). Notably, insufficient sleep must be ruled out. While these criteria are clearly improved compared to the previous edition of the diagnostic manual – in the absence of specific biomarkers – some uncertainty remains. Because the test-retest reliability of the MSLT must be considered poor (Trotti et al. 2013), sleepiness and the absence of cataplexy or enhanced REM sleep pressure are probably not specific enough for a definite diagnosis of idiopathic hypersomnia. Therefore, actigraphy should be performed whenever possible, first to exclude insufficient sleep syndrome and second to prove increased sleep need, i.e., hypersomnia sensu strictu. Polysomnography should reveal very high sleep efficiency (usually above 95%), and 24-h polysomnography, if performed, can be helpful to show long and highly consolidated nocturnal sleep. In addition, in the absence of sleep inertia, the diagnosis of idiopathic hypersomnia should be doubted.

Treatment Non-pharmacological treatment strategies such as good sleep hygiene with regular sleep periods and scheduled daytime naps are largely ineffective, mostly due to sleep inertia. On the other hand, in a recent survey among patients suffering from hypersomnia disorders, caffeine intake was reported by more than 80% of idiopathic hypersomnia patients to be a beneficial "non-pharmacological" strategy to help manage the symptoms of the disease (Neikrug et al. 2017). Only recently, few randomized and controlled trials in idiopathic hypersomnia patients have been published, yet all pharmacological medication of those patients at present remains off-label. Given their outcomes, it must be concluded that modafinil partially reduces subjective excessive daytime sleepiness and increases vigilance, but patients remain significantly affected even on treatment (Mayer et al. 2015; Philip et al. 2014). It has been reported that a significant portion of idiopathic hypersomnia patients achieved complete symptom control with stimulants (Ali et al. 2009). Unfortunately, we cannot confirm this finding in our population of idiopathic hypersomnia patients. In the contrary, we find pharmacological treatment of sleepiness and sleep inertia in those patients largely unsatisfying. Whether or not additional or alternative treatment strategies such as other stimulants, antagonistic modulation of $GABA_A$ receptors (e.g., clarithromycin), gamma-hydroxybutyrate, or histamine H_3 receptor inverse

agonists are helpful for therapy-refractory idiopathic hypersomnia, patients need to be examined in future studies. Only clarithromycin has been studied for the treatment of non-narcoleptic hypersomnia disorders and exerted some beneficial effects on sleepiness but not on vigilance (Trotti et al. 2015).

1.4 Kleine-Levin Syndrome

Kleine-Levin syndrome is a rare central disorder of hypersomnolence of unknown origin, with recurring episodes of hypersomnia in combination with neuropsychiatric symptoms.

Epidemiology There are no large epidemiological studies of Kleine-Levin syndrome, yet it is considered a very rare disease which affects mostly adolescents (Lavault et al. 2015).

Symptomatology The key symptom is recurrent hypersomnia, i.e., repeated episodes of sleepiness, tiredness, and increased sleep times. The average sleep time per 24 h during those episodes is 18 h, and patients are not refreshed after sleep yet remain exhausted, tired, and irritable (Arnulf et al. 2008). When awake, patients often exhibit marked neuropsychiatric disturbances during those episodes, including cognitive impairment, apathy, confusion, altered perception up to derealization, abnormal behaviors such as hyperphagia or hypersexuality, and psychiatric symptoms including decreased mood, anxiety, aggressiveness, or regressive behavior (Arnulf 2015). In addition, migraine-like symptoms such as headache and photo- and phonophobia are not uncommon. Episodes last usually a few days up to months, but the mean duration is 13 days (Lavault et al. 2015). Episodes occur regularly, with a mean interval between episodes of 6 months (Arnulf 2015). Beyond the age of 30 years, the disease often disappears.

Diagnosis The current diagnostic criteria demand recurrent episodes of severe sleepiness lasting 2 days up to several weeks, usually more often than once per year and at least every 18 months, with at least one neuropsychiatric feature during the episode and normal alertness, cognition, mood, and behavior between episodes (AASM 2014). Sleep recordings are not mandatory, but a 24-h polysomnography during episodes confirms increased sleep duration. Brain imaging is usually normal, and EEG recordings during episodes reveal unspecific slowing of background activity (Papacostas and Hadjivasilis 2000).

Treatment Symptomatic therapy of Kleine-Levin syndrome is challenging. Stimulants can be probed during episodes, and neuropsychiatric symptoms can be treated with classical compounds, e.g., benzodiazepines against anxiety or neuroleptics against psychotic symptoms. Relatives or caregivers should be informed about aberrant behaviors during episodes and about monitoring regular food intake during episodes to avoid unwanted weight gain.

1.5 Insufficient Sleep Syndrome

Insufficient sleep syndrome is not a pathology-driven disease but a collection of symptoms, particularly excessive daytime sleepiness, caused by chronic sleep restriction and/or irregular sleep-wake behavior.

Epidemiology Given the laborious confirmation of clear-cut insufficient sleep syndrome (see below), epidemiological studies are uncommon and mostly unreliable. A Japanese cross-sectional study, for instance, performed a web-based questionnaire in young adults and diagnosed insufficient sleep syndrome in 11% (Morita et al. 2015). If we interpret shift work as an important contributor to insufficient sleep, then prevalence number may rise up to 29% (Kecklund and Axelsson 2016).

Symptomatology Leading symptom is chronic excessive daytime sleepiness, but sleepiness is usually improved or even absent during holidays with prolonged nocturnal sleep hours or without shift work. Subjects with insufficient sleep syndrome are prone to reduced performance, error-proneness, poor academic performance, impaired decision-making, increased risk-seeking, and neuropsychiatric symptoms including depression, irritability, and impulsiveness (Van Dongen et al. 2003; Lee et al. 2015; Maric et al. 2017).

Diagnosis The current diagnostic criteria require chronic excessive daytime sleepiness (AASM 2014). In addition, the patients' sleep time must be shorter than expected for age, sleep duration is longer on weekends or during vacation, the patient curtails sleep time by an alarm clock or similar strategies, and extension of total sleep time results in resolution of symptoms.

Although the listing of insufficient sleep syndrome in the ICSD-3 criteria is most important, the diagnostic criteria as such pose some significant challenges:

First, it is difficult to establish whether a patient's sleep time is usually shorter than expected for age, as there is a large variability of individual sleep need in society, including long- and short-sleepers. In addition, the criteria offer history-taking, sleep logs, or actigraphy for assessing usual sleep times, but particularly history-taking and sleep logs largely depend on self-perception, forthrightness, and compliance, and a deficit in any of those traits produces false results.

Second, how much longer should an individual sleep during weekends or vacations to fulfill the criteria? Is a 1-h difference enough, or should it be at least 2 h, or should it be decided on an individual basis?

Third, the criterion of extending total sleep time and repeating sleepiness tests is important, but most sleep laboratories will not be able (i.e., refunded) for repeated objective measurements of sleepiness before and after sleep extension, and actigraphy to monitor sleep extension is not reimbursed in many countries. Thus, feasibility of this criterion must be questioned, given the actual reimbursement policies.

Fourth, the circadian aspect of insufficient sleep is completely neglected. For instance, insufficient sleep because of shift work or because of irregular sleep times because of social distractions is not taken into account in the current criteria.

Treatment Causal therapy would be to prolong sleep duration, along with a regular sleep-wake pattern, but experience tells that this is difficult for many affected subjects. Shift workers cannot simply change their sleep habits without endangering their job, and young adults are often not ready to abstain from two or three party nights with very late bedtimes. Altogether, the issue of insufficient sleep syndrome remains exigent, both from a diagnostic and from a therapeutic perspective.

2 Sleep-Related Movement Disorders

In sleep-related movement disorders, the cardinal symptom consists of simple, often stereotyped movements occurring during sleep (AASM 2014) (Table 1).

2.1 Restless Legs Syndrome

Restless legs syndrome is one of the most prevalent neurological disorders and is characterized by an urge to move the limbs, mostly the legs, with a circadian pattern and a predominance during periods of rest and with improvement upon moving.

Epidemiology In Europe, the prevalence of restless legs syndrome may be higher than 5%, whereas it is below 1% in African countries (Koo 2015). Women are more often affected than men. Several conditions are associated to restless legs syndrome, particularly iron deficiency, end-stage renal disease, and pregnancy.

Symptomatology and Diagnosis The diagnosis of restless legs syndrome depends primarily on the description of symptoms by the affected patient. The current diagnostic guidelines essentially rely on four criteria (AASM 2014; Allen et al. 2014):

1. Urge to move the legs, typically accompanied by unpleasant sensations in the legs.
2. Symptoms get worse during rest.
3. Symptoms get worse during evening and night hours.
4. Moving the legs improves urge and unpleasant symptoms.

In addition, it is required to exclude potential mimics of restless legs syndrome, such as rheumatoid disorders, akathisia, painful legs and moving toes, positional discomfort, sleep-related cramps, or myalgia (Hening et al. 2009).

In addition, supportive criteria can help making a correct diagnosis, particularly in patients with doubtful symptom presentation (AASM 2014; Allen et al. 2014):

1. Positive family history with affected first-degree relatives.
2. Symptoms respond clearly to dopaminergic treatment.
3. Presence of periodic limb movements during sleep.

Periodic limb movements during sleep can be assessed with polysomnography or with leg actigraphy and consist of repetitive Babinski-like leg movements occurring every 5–90 s (Iber et al. 2007; Cippà et al. 2013). Importantly, periodic limb movements during sleep are not at all specific for restless legs syndrome, as they can be observed in more than 25% of recorded healthy subjects (Haba-Rubio et al. 2016).

Pathophysiology Three factors appear significantly to contribute to the expression of restless legs syndrome: (1) dopamine-related pathology, (2) iron deficiency, and (3) genetics. Dopaminergic drugs are most effective to treat restless legs syndrome, yet novel evidence points toward a hyperdopaminergic state involved in the disease (Earley et al. 2014). Similarly, iron replacement often improves restless legs syndrome considerably in patients with low or even normal iron and ferritin levels, and altered brain iron acquisition has been observed in restless legs syndrome (Connor et al. 2011). Finally, several genome-wide association studies revealed restless legs syndrome susceptibility loci (Stefansson et al. 2007; Winkelmann et al. 2007, 2011).

Treatment Before considering pharmacological treatment, current medication should be carefully checked. Dopamine antagonists such as neuroleptics or metoclopramide should be discontinued, if possible. If depression is present, bupropion is considered a first-line treatment, as there is evidence that it does not – opposed to other antidepressants – worsen restless legs syndrome (Bayard et al. 2011).

At the same time, iron panels should be assessed early in all patients, as restless legs syndrome is tightly linked to iron deficiency. The current expert-driven guidelines note that ferric carboxymaltose (1,000 mg) is effective for treating moderate to severe restless legs syndrome in those with serum ferritin <300 μg/l and could be used as first-line treatment in adults. Furthermore, the expert panel states that oral iron (65 mg elemental iron) is possibly effective for treating restless legs syndrome in those with serum ferritin ≤ 75 μg/l (Allen et al. 2018).

A combined task force published guidelines for the first-line treatment of restless legs syndrome (Garcia-Borreguero et al. 2016). The task force recommends α2β receptor ligands for the first-line treatment of restless legs, with gabapentin (600–1,200 mg per day) being approved in several countries including the USA and Japan. This recommendation is based on the observation that dopaminergic agents pose the highest risk for augmentation (see below). Alternative first-line treatment options remain dopaminergic agents, preferably in low doses (pramipexole, up to 0.5–0.75 mg; ropinirole, up to 4 mg; rotigotine, up to 3 mg – all of them approved for restless legs syndrome treatment in many countries), to keep the risk of augmentation as low as possible. Levodopa is more likely to cause augmentation than dopamine receptor agonists (Bassetti et al. 2011). Opioids can be considered for

patients with treatment-refractory restless legs syndrome, by individually weighing opioid risks and benefits.

Augmentation The main features of augmentation include (1) earlier begin of symptoms in the day compared to before medication was started, (2) need of higher doses or earlier medication intake to control symptoms compared to when medication was started, (3) increased intensity of symptoms since medication was started, and/or (4) spreading of symptoms to formerly unaffected parts of the body (typically the arms and hands) (Garcia-Borreguero et al. 2016). The incidence rate of augmentation appears being highest with levodopa, high with shorter-acting dopamine receptor agonists, and lower with longer-acting dopamine receptor agonists (Garcia-Borreguero et al. 2016).

If augmentation is diagnosed, exacerbating factors should be eliminated, i.e., low serum ferritin or exacerbating drugs. In case of mild augmentation, two options are recommended (Garcia-Borreguero et al. 2016): to keep the same dopamine receptor agonist but to split the same dose, to advance the dose toward earlier intake, or to increase dose but keep it below approved daily dose or, alternatively, to switch to an α2β receptor ligand or a low-dose longer-acting dopamine receptor agonist such as transdermal rotigotine. In case of failure of these strategies or of severe augmentation, it is recommended to reduce or better to eliminate shorter-acting dopamine receptor agonists and to start with rotigotine or another longer-acting dopamine receptor agonist or an α2β receptor ligand or a long-acting opiate.

2.2 Other Sleep-Related Movement Disorders in Adults

Periodic limb movement disorder is diagnosed in the presence of periodic limb movements during sleep in combination with either insomnia or excessive daytime sleepiness but without symptoms of restless legs syndrome. The entity, however, remains to some extent controversial, and diagnosis and treatment should be made and initiated with reluctance.

Sleep-related leg cramps are characterized by painful nocturnal cramps or muscle contractions during wakefulness or sleep. Cramps beyond the distal limbs or during daytime should prompt a search for an underlying neuromuscular disorder. There are no thorough studies nor approved medication for sleep-related leg cramps.

Sleep-related bruxism presents with clenching or grinding of teeth during sleep and may lead to dental erosion, temporal headache, jaw muscle pain, and arousal of bedpartners. To protect teeth, dental appliances are usually recommended, whereas pharmacological interventions (i.e., with benzodiazepines or botulinum toxin) are clearly second line.

Sleep-related rhythmic movement disorder, including body rocking or head banging, is diagnosed in the presence of rhythmic and markedly stereotyped body movements at the transition from wakefulness to sleep, which are not of epileptic origin and which ultimately lead to sleep disruption with daytime consequences or to injuries. These behaviors are frequent in infants and become rarer later on, yet they

may sometimes persist into adulthood. Clonazepam can be probed as off-label treatment.

Propriospinal myoclonus is a rare form of spinal myoclonus at sleep onset, which typically starts in midthoracic muscular segments and then propagates down and up in the spinal cord (Antelmi and Provini 2015). This propagation can produce typical irregular and jerky movements such as sitting up or jackknife-like movements. Diagnosis and exclusion of a functional movement disorder warrants a combined polysomnography with multi-segmental electromyography electrodes.

3 Summary

Apart from narcolepsy type 1, the pathophysiology of neurological sleep-wake disorders remains largely unknown. Similarly, narcolepsy type 1 is the only hypersomnia or sleep-related movement disorder which can be diagnosed with specific biomarkers. The treatment of hypersomnia disorders is still primarily based on stimulants and restless legs syndrome on α2β receptor ligands or dopaminergic drugs. Whether or not hypocretin-based treatments or immunotherapies will be successfully introduced in narcolepsy remains elusive. Also, novel noninvasive non-pharmacological technologies for hypersomnia disorders are not available as of yet.

Conflicts of Interest Dr. Baumann reports no conflicts of interests in regard to this work.

References

Ali M, Auger RR, Slocumb NL et al (2009) Idiopathic hypersomnia: clinical features and response to treatment. J Clin Sleep Med 5(6):562–568

Allen RP, Picchietti DL, Garcia-Borreguero D et al (2014) Restless legs syndrome/Willis-Ekbom disease diagnostic criteria: updated International Restless Legs Syndrome Study Group (IRLSSG) consensus criteria – history, rationale, description, and significance. Sleep Med 15 (8):860–873

Allen RP, Picchietti DL, Auerbach M et al (2018) Evidence-based and consensus clinical practice guidelines for the iron treatment of restless legs syndrome/Willis-Ekbom disease in adults and children: an IRLSSG task force report. Sleep Med 41:27–44

American Academy of Sleep Medicine (AASM) (2014) The International Classification of Sleep Disorders – third edition (ICSD-3). American Academy of Sleep Medicine, Darien

Antelmi E, Provini F (2015) Propriospinal myoclonus: the spectrum of clinical and neurophysiological phenotypes. Sleep Med Rev 22:54–63

Arnulf I (2015) Kleine-Levin syndrome. Sleep Med Clin 10:151–161

Arnulf I, Lin L, Gadoth N et al (2008) Kleine-Levin syndrome: a systematic study of 108 patients. Ann Neurol 63:482–493

Bassetti CL, Bornatico F, Fuhr P, Schwander J, Kallweit U, Mathis J, Swiss RLS study group (2011) Pramipexole versus dual release levodopa in restless legs syndrome: a double blind, randomised, cross-over trial. Swiss Med Wkly w13274:141

Baumann CR, Mignot E, Lammers GJ et al (2014) Challenges in diagnosing narcolepsy without cataplexy: a consensus statement. Sleep 37(6):1035–1042

Bayard M, Bailey B, Acharya D et al (2011) F. Bupropion and restless legs syndrome: a randomized controlled trial. J Am Board Fam Med 24(4):422–428
Cippà MA, Baumann CR, Siccoli MM, Bassetti CL, Poryazova R, Werth E (2013) Actigraphic assessment of periodic leg movements in patients with restless legs syndrome. J Sleep Res 22 (5):589–592
Connor JR, Ponnuru P, Wang XS, Patton SM, Allen RP, Earley CJ (2011) Profile of altered brain iron acquisition in restless legs syndrome. Brain 134(Pt 4):959–968
Cvetkovic-Lopes V, Bayer L, Dorsaz S et al (2010) Elevated tribbles homolog 2-specific antibody levels in narcolepsy patients. J Clin Invest 120(3):713–719
Earley CJ, Connor J, Garcia-Borreguero D et al (2014) Altered brain iron homeostasis and dopaminergic function in restless legs syndrome (Willis-Ekbom disease). Sleep Med 15 (11):1288–1301
Garcia-Borreguero D, Silber MH, Winkelman JW et al (2016) Guidelines for the first-line treatment of restless legs syndrome/Willis-Ekbom disease, prevention and treatment of dopaminergic augmentation: a combined task force of the IRLSSG, EURLSSG, and the RLS-foundation. Sleep Med 21:1–11
Haba-Rubio J, Marti-Soler H, Marques-Vidal P et al (2016) Prevalence and determinants of periodic limb movements in the general population. Ann Neurol 79(3):464–474
Hallmayer J, Faraco J, Lin L et al (2009) Narcolepsy is strongly associated with the T-cell receptor alpha locus. Nat Genet 41(6):708–711
Hening WA, Allen RP, Washburn M, Lesage SR, Earley CJ (2009) The four diagnostic criteria for Restless Legs Syndrome are unable to exclude confounding conditions ("mimics"). Sleep Med 10(9):976–981
Hublin C, Kaprio J, Partinen M, Koskenvuo M, Heikkila K, Koskimies S, Guilleminault C (1994) The prevalence of narcolepsy: an epidemiological study of the Finnish Twin Cohort. Ann Neurol 35(6):709–716
Iber C, Ancoli-Israel S, Chesson A et al (2007) The AASM manual for the scoring of sleep and associated events. American Academy of Sleep Medicine, Westchester
John J, Thannickal TC, McGregor R et al (2013) Greatly increased numbers of histamine cells in human narcolepsy with cataplexy. Ann Neurol 74(6):786–793
Kecklund G, Axelsson J (2016) Health consequences of shift work and insufficient sleep. BMJ 355: i5210
Koo BB (2015) Restless leg syndrome across the globe: epidemiology of the restless legs syndrome/Willis-Ekbom disease. Sleep Med Clin 10(3):189–205
Kornum BR, Knudsen S, Ollila HM, Pizza F, Jennum PJ, Dauvilliers Y, Overeem S (2017) Narcolepsy. Nat Rev Dis Primers 3:16100
Kretzschmar U, Werth E, Sturzenegger C et al (2016) Which diagnostic findings in disorders with excessive daytime sleepiness are really helpful? A retrospective study. J Sleep Res 25 (3):307–313
Lavault S, Golmard J, Groos E et al (2015) Kleine-Levin syndrome in 120 patients: differential diagnosis and long episodes. Ann Neurol 77:529–540
Lee YJ, Park J, Kim S, Cho SJ, Kim SJ (2015) Academic performance among adolescents with behaviorally induced insufficient sleep syndrome. J Clin Sleep Med 11(1):61–68
Maric A, Montvai E, Werth E et al (2017) Insufficient sleep: enhanced risk-seeking relates to low local sleep intensity. Ann Neurol 82(3):409–418
Marti I, Valko PO, Khatami R, Bassetti CL, Baumann CR (2009) Multiple sleep latency measures in narcolepsy and behaviourally induced insufficient sleep syndrome. Sleep Med 10:1146–1150
Mayer G, Benes H, Young P et al (2015) Modafinil in the treatment of idiopathic hypersomnia without long sleep time-a randomized, double-blind, placebo-controlled study. J Sleep Res 24 (1):74–81
Mignot E, Lin L, Rogers W et al (2001) Complex HLA-DR and -DQ interactions confer risk of narcolepsy-cataplexy in three ethnic groups. Am J Hum Genet 68(3):686–699

Morita Y, Sasai-Sakuma T, Asaoka S, Inoue Y (2015) Prevalence and correlates of insufficient sleep syndrome in Japanese young adults: a web-based cross-sectional study. J Clin Sleep Med 11:1163–1169

Neikrug AB, Crawford MR, Ong JC (2017) Behavioral sleep medicine services for hypersomnia disorders: a survey study. Behav Sleep Med 15(2):158–171

Ohayon MM, Reynolds CF 3rd, Dauvilliers Y (2013) Excessive sleep duration and quality of life. Ann Neurol 73(6):785–794

Ozaki A, Inoue Y, Hayashida K et al (2012) Quality of life in patients with narcolepsy with cataplexy, narcolepsy without cataplexy, and idiopathic hypersomnia without long sleep time: comparison between patients on psychostimulants, drug-naive patients and the general Japanese population. Sleep Med 13(2):200–206

Papacostas SS, Hadjivasilis V (2000) The Kleine-Levin syndrome. Report of a case and review of the literature. Eur Psychiatry 15:231–235

Peyron C, Faraco J, Rogers W et al (2000) A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 6(9):991–997

Philip P, Chaufton C, Taillard J et al (2014) Modafinil improves real driving performance in patients with hypersomnia: a randomized double-blind placebo-controlled crossover clinical trial. Sleep 37(3):483–487

Pizza F, Antelmi E, Vandi S et al (2018) The distinguishing motor features of cataplexy: a study from video recorded attacks. Sleep [Epub ahead of print]

Ruoff C, Pizza F, Trotti LM et al (2018) The MSLT is repeatable in narcolepsy type 1 but not narcolepsy type 2: a retrospective patient study. J Clin Sleep Med 14(1):65–74

Saper CB, Scammell TE, Lu J (2005) Hypothalamic regulation of sleep and circadian rhythms. Nature 437:1257–1263

Scammell TE (2015) Narcolepsy. N Engl J Med 373(27):2654–2662

Stefansson H, Rye DB, Hicks A et al (2007) A genetic risk factor for periodic limb movements in sleep. N Engl J Med 357(7):639–647

Szakacs Z, Dauvilliers Y, Mikhaylov V et al (2017) Safety and efficacy of pitolisant on cataplexy in patients with narcolepsy: a randomised, double-blind, placebo-controlled trial. Lancet Neurol 16 (3):200–207

Taddei RN, Werth E, Poryazova R, Baumann CR, Valko PO (2016) Diagnostic delay in narcolepsy type 1: combining the patients' and the doctors' perspectives. J Sleep Res 25(6):709–715

Thannickal TC, Moore RY, Nienhuis R et al (2000) Reduced number of hypocretin neurons in human narcolepsy. Neuron 27(3):469–474

Trotti LM (2017) Idiopathic hypersomnia. Sleep Med Clin 12(3):331–344

Trotti LM, Staab BA, Rye DB (2013) Test-retest reliability of the multiple sleep latency test in narcolepsy without cataplexy and idiopathic hypersomnia. J Clin Sleep Med 9(8):789–795

Trotti LM, Saini P, Bliwise DL et al (2015) Clarithromycin in gamma-aminobutyric acid-related hypersomnolence: a randomized, crossover trial. Ann Neurol 78(3):454–465

Valko PO, Gavrilov YV, Yamamoto M et al (2013) Increase of histaminergic tuberomammillary neurons in narcolepsy. Ann Neurol 74(6):794–804

Van Dongen HP, Maislin G, Mullington JM, Dinges DF (2003) The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. Sleep 26(2):117–126

Winkelmann J, Schormair B, Lichtner P et al (2007) Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions. Nat Genet 39(8):1000–1006

Winkelmann J, Czamara D, Schormair B et al (2011) Genome-wide association study identifies novel restless legs syndrome susceptibility loci on 2p14 and 16q12.1. PLoS Genet 7(7): e1002171

Clinical Sleep–Wake Disorders II: Focus on Insomnia and Circadian Rhythm Sleep Disorders

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Contents

Abstract

Insomnia and circadian rhythm sleep disorders affect large proportions of the population and have pronounced effects on quality of life and daytime performance. While the neurobiology of insomnia is not yet fully understood, circadian rhythm sleep disorders are assumed to be caused by a mismatch between the individual circadian phase position and the desired sleep–wake schedule. Benzodiazepines and non-benzodiazepine positive allosteric $GABA_A$ receptor modulators improve sleep onset and maintenance in the short-term treatment of

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insomnia. However, tolerance and dependence are important side effects. Sedating antidepressants are frequently prescribed for insomnia, however, only few randomised controlled trials have been published so far. Melatonin and melatonin receptor agonists are considered to be an option for the treatment of insomnia especially because of their minimal abuse potential and safety. First data on orexin (aka hypocretin) receptor antagonists are promising, however, the risk-benefit ratio needs to be further evaluated. With respect to circadian rhythm sleep disorders, there is solid evidence from meta-analyses supporting the use of melatonin in jet lag disorder to accelerate entrainment to the new time zone, and in delayed sleep phase disorder to advance sleep–wake rhythms. In addition to that, there is evidence supporting the use of melatonin in patients with shift work disorder in order to promote daytime sleep after night shifts.

Keywords
Aetiology • Circadian rhythm sleep disorders • Insomnia • Pharmacological treatment

1 Definition and Epidemiology

Insomnia is a common mental disorder characterised by difficulties initiating or maintaining sleep associated with impairments of daytime functioning (American Psychiatric Association 2013; Morin et al. 2015). Approximately 6–10% of the population suffers from insomnia meeting diagnostic criteria (Ohayon 2002). Women are more frequently affected than men (Zhang and Wing 2006) and there is an increasing prevalence with older age (Ohayon 2002). In most patients with insomnia, the disorder is a chronic condition. More than 70% of those experiencing insomnia today will still suffer from it next year (Morin et al. 2009a). While often viewed as a nuisance symptom in clinical practice, chronic insomnia is associated with a severely reduced quality of life (Kyle et al. 2010) and cognitive impairments (Fortier-Brochu et al. 2012). Furthermore, longitudinal studies suggest that insomnia is a risk factor for other mental disorders, especially depression (Baglioni et al. 2011), as well as for cardiovascular morbidity and mortality (Laugsand et al. 2011, 2014; Sofi et al. 2014). In line with this, insomnia leads to a huge increase in health care consumption, work disability and absenteeism, and, consequently, to very high costs for our societies (Léger and Bayon 2010). For the US, the costs of insomnia due to low work performance and absenteeism have been estimated to exceed 60 billion $ per year (Kessler et al. 2011).

It is assumed that circadian rhythm sleep disorders are also very common although the exact prevalence rate is unknown (Sack et al. 2007a). They are characterised by a misalignment between the endogenous circadian rhythm and the natural external day–night rhythm (Bjorvatn and Pallesen 2009). These disorders include conditions that are related to exogenously determined alterations in sleep timing (e.g. shift work disorder and jet lag disorder), as well as disorders that are related to endogenous causes (e.g. delayed sleep phase disorder and

advanced sleep phase disorder). Older age appears to be a risk factor for shift work intolerance (Sack et al. 2007a) and advanced sleep phase disorder (Sack et al. 2007b). On the contrary, younger age appears to be a risk factor for delayed sleep phase disorder (Sack et al. 2007b), which may also be more prevalent in men than in women (Adan and Natale 2002). Moreover, clinical experience suggests that circadian rhythm sleep disorders are typically associated with complaints of insomnia or daytime sleepiness associated with daytime impairments and reduced quality of life.

2 Aetiology

Despite the above-mentioned huge socio-economic impact of chronic insomnia, the neurobiological mechanisms are not yet fully understood. Some of the most frequently cited models of the disorder suggest that cognitive, emotional and physiological hyperarousal are important factors for the development and maintenance of insomnia (Riemann et al. 2010, 2015; Espie et al. 2006; Harvey 2002; Perlis et al. 1997). While it is difficult to find a formal definition of this construct in the scientific literature, it is usually assumed that hyperarousal is the opposite of the relaxation state required for restorative sleep. Cognitive hyperarousal involves excessive worry and rumination which have been frequently shown to be associated with insomnia (e.g. Spiegelhalder et al. 2012; Fernández-Mendoza et al. 2010). In contrast, there are surprisingly few empirical studies investigating the role of emotional processes for insomnia (Baglioni et al. 2010). These data suggest that dysfunctional emotional reactivity may mediate the interaction between cognitive and physiological hyperarousal and may thus contribute to the maintenance of insomnia. Physiological hyperarousal is assumed to be reflected in increased electroencephalographic (EEG) beta power (Perlis et al. 2001), an increased number of arousals during REM sleep (Feige et al. 2008; Riemann et al. 2012), increased cortisol levels (Vgontzas et al. 2001), increased body temperature (Lack et al. 2008), as well as increased heart rate and altered heart rate variability during sleep (Stein and Pu 2012). Despite this literature, the central nervous system correlates, underpinnings and consequences of hyperarousal in insomnia are largely unknown thereby deterring the development of neurobiologically informed treatment approaches. For example, the spatial localisation of brain activity related to increased EEG beta power during sleep has not been investigated in patients with insomnia up to now. Furthermore, it is unclear how increased beta power interferes with sleep-promoting brain circuits and leads to the subjective experience of sleep initiation and maintenance problems.

To investigate brain function in insomnia patients, some researchers used positron emission tomography or single photon emission computed tomography (Hasler et al. 2012; Nofzinger et al. 2004; Smith et al. 2002). A frequently cited study by Nofzinger et al. (2004) reported an increased brain metabolism in a distributed network of cortical and subcortical brain regions including the ascending reticular activating system, hypothalamus, thalamus, amygdala, hippocampus, insula, and

anterior cingulate and prefrontal cortices. These findings suggested an association of insomnia with alterations in the general arousal system, the emotion-regulating system, and the cognitive system leading to the assumption that insomnia is a disorder of corticolimbic overactivity that interferes with sleep-promoting brain structures (see also Saper et al. 2005, p. 1262). In addition to this literature, functional magnetic resonance imaging (fMRI) studies suggest that insomnia patients' daytime performance in neuropsychological tasks is associated with a hypoactivation of task-related brain areas, especially in fronto-striatal networks (Stoffers et al. 2014; Drummond et al. 2013; Altena et al. 2008). A further fMRI study suggests that insomnia patients show an increased amygdala reactivity to the presentation of sleep-related stimuli (Baglioni et al. 2014), which is in line with the literature on preferential attention allocation to sleep-related stimuli in those with insomnia (Harris et al. 2015). Moreover, it is a plausible hypothesis that insomnia is characterised by functional alterations of the default-mode network as this network is involved in self-referential processing, introspection, worry and rumination (Hamilton et al. 2011). However, the evidence concerning this hypothesis is currently inconclusive (Regen et al. 2016; Drummond et al. 2013). Most importantly with respect to pharmacological treatments, there are several studies using magnetic resonance spectroscopy to investigate γ-aminobutyric acid (GABA) levels in patients with insomnia. The majority of these studies suggest decreased GABA levels in those with insomnia (Meyerhoff et al. 2014; Plante et al. 2012; Winkelman et al. 2008), however, it should be noted that there are also conflicting findings (Morgan et al. 2012; Spiegelhalder et al. 2016).

With respect to the aetiology of circadian rhythm sleep disorders, it is crucial to understand the function of the so-called master circadian clock in the suprachiasmatic nucleus. This well-described group of neurons generates a 24-h rhythm via an autoregulatory feedback loop which can be influenced by light or melatonin (Lu and Zee 2006). While the delayed sleep phase disorder is assumed to be caused by an abnormally delayed circadian clock (Weitzman et al. 1981), the opposite is assumed for the advanced sleep phase disorder. Both disorders involve a continuing mismatch between the schedule desired by the individual or the social environment and the individual circadian rhythm leading to daytime impairment. In shift work disorder and jet lag disorder, the requirements of the environment are altered which also leads to a continuing or temporary mismatch with the individual circadian rhythm.

3 Treatment

There are a variety of treatments for insomnia (Morin et al. 2015; Bootzin and Epstein 2011; Riemann and Perlis 2009). The most commonly used are benzodiazepines (BZs) and non-benzodiazepine positive allosteric $GABA_A$ receptor modulators (GARMs; Huedo-Medina et al. 2012; Glass et al. 2005; Duendar et al. 2004; Nowell et al. 1997), off-label treatment with sedating antidepressants (Buscemi et al. 2007), melatonin and melatonin receptor agonists (Kuriyama et al.

2014), herbal substances (Leach and Page 2015) and cognitive behavioural therapy for insomnia (Trauer et al. 2015; Wu et al. 2015). Moreover, orexin (aka hypocretin) receptor agonists have been and currently are developed for the treatment of insomnia (e.g. Michelson et al. 2014). Both BZs/GARMs and melatonin/melatonin receptor agonists have also been investigated in the context of circadian rhythm sleep disorders and are frequently used in these conditions.

It should be noted that cognitive behavioural therapy for insomnia is considered by many, including us, as the first-line treatment for insomnia and both timed light exposure and behavioural interventions targeting sleep–wake schedules are recommended treatments for circadian rhythm sleep disorders. However, the current chapter focuses exclusively on pharmacological approaches to treating insomnia and circadian rhythm sleep disorders.

3.1 Pharmacological Treatment with BZs and GARMs

BZs and GARMs are probably the most widely prescribed pharmacological substances for the treatment of insomnia (see Table 1). These substances vary considerably in half-life ranging from 2–4 h (zolpidem) to 48–120 h (flurazepam). In addition, many of the BZs have pharmacologically active metabolites with substantially longer half-life. The intake of substances with long half-life is associated with a substantial risk of daytime impairment on the following day, the intake of substances with short half-life is usually limited with respect to the effects on sleep maintenance problems. The onset of action of BZs/GARMs is rapid, and most of these drugs are recommended to be taken just shortly prior to or even after going to bed.

Table 1 Benzodiazepines and non-benzodiazepine agonistic $GABA_A$ receptor modulators frequently used for the treatment of insomnia

Drug	Usual dose (mg)	t½ (h)
Benzodiazepines		
Flunitrazepam	0.5–2	16–35
Flurazepam	15–30	48–120
Lormetazepam	0.5–1	8–15
Nitrazepam	5–10	25–35
Temazepam	10–30	8–20
Triazolam	0.125–0.250	1.4–4.6
Agonistic $GABA_A$ receptor modulators (non-benzodiazepine structure)		
Zolpidem	5–10	2–4
Zopiclon	3.75–7.5	5–6
Nonhypnotics sometimes used to aid sleep		
Clonazepam	0.5–3	30–40
Diazepam	5–10	30–100

The mechanism of BZs and GARMs is an allosteric modulation of the GABA-A receptor complex (Downing et al. 2005). BZs and GARMs bind to this receptor complex which increases the inhibition caused by GABA. GARMs bind more specifically to a certain type of GABA-A receptors than BZs, however, it is a matter of debate whether this leads to a different pharmacological profile of these substances. In general, the inhibitory mechanism of BZs and GARMs is comparably powerful as GABA-A receptors are ubiquitous in the central nervous system, comprising up to 30% of all synapses (Mendelson 2005). The GABA-A receptor is also involved in the anxiolytic, muscle relaxant, and anticonvulsant effects of BZs and GARMs which are associated with some of the most frequently observed side effects of these substances (e.g. risk of nocturnal falls associated with muscle relaxation).

Several meta-analyses on the efficacy of BZs and GARMs in the short-term treatment of insomnia have been published. The first of these included studies on several BZs as well as zolpidem (half-life of approximately 2.5 h) as the most widely prescribed GARMs (Nowell et al. 1997). Twenty-two randomised, double-blind, placebo-controlled clinical trials with 1,894 patients were included in this analysis. The median duration of treatment with BZs or GARMs was 7 days. The combined effect sizes for all substances in comparison to placebo were 0.71 for total sleep time, 0.56 for sleep onset latency, 0.65 for the number of nocturnal awakenings, and 0.62 for sleep quality (all statistically significant), thus indicating medium-sized effects.

Duendar et al. (2004) analysed potential differences between BZs and GARMs. In this meta-analysis, 24 randomised, double-blind, placebo-controlled clinical trials with 3,909 patients were included with a treatment duration between 1 night and 6 weeks. Only few statistically significant differences between specific substances were revealed, e.g. there was some evidence suggesting that zaleplon has a more pronounced effect on sleep onset latency and a less pronounced effect on total sleep time than zolpidem. In summary, the authors concluded that BZs and GARMs do not differ substantially from each other with respect to their efficacy and safety profiles. Moreover, the authors stated that the quality of the data was limited by non-standardised outcomes and a variety in the level of information provided across studies which made study comparisons difficult to make.

Another meta-analysis focused on the efficacy and risks of using BZs and GARMs in elderly patients with insomnia (age $>$60 years; Glass et al. 2005). In this meta-analysis, 24 randomised, double-blind, placebo-controlled clinical trials with 2,417 patients were included. In comparison with placebo, BZs and GARMs had small but significant effects on total sleep time, the number of awakenings and sleep quality, however, cognitive adverse events were 4.8 times more common, psychomotor adverse events were 2.6 times more common, and reports of daytime fatigue were 3.8 times more common in the intervention groups. Moreover, an increased incidence of falls and motor vehicle accidents was reported with active drugs compared to placebo. Accordingly, the main conclusion of the authors of this meta-analysis was that in elderly patients the benefits of BZs and GARMs may not justify the risks.

A further meta-analysis on the efficacy of GARMs was provided by Huedo-Medina et al. (2012) who focused on the analysis of datasets from the US Food and Drug Administration. These data contain all published and unpublished trials up to the date of drug approval and are, thus, less prone to publication bias than the data used in previous meta-analyses. Overall, 13 studies with 4,378 patients were analysed. The combined effect size for the GARMs compared to placebo was 0.36 for polysomnographically determined sleep onset latency which was the primary outcome of the meta-analysis. However, there were no statistically significant effects of GARMs on those variables that were investigated in a smaller number of studies, namely polysomnographically determined total sleep time, wake after sleep onset or number of awakenings.

Given the chronic nature of insomnia, long-term pharmacological treatment is an important issue that needs to be addressed, especially with respect to the abuse potential of BZs and GARMs (see Hajak et al. 2003). This has led to prescription guidelines restricting the intake of BZs and GARMs to 3–4 weeks. This creates, however, a paradoxical situation, keeping in mind that insomnia is a chronic disorder. Unfortunately, there are only few published investigations on the long-term treatment with BZs and GARMs. Ancoli-Israel et al. (2005) investigated an open-label phase in which patients self-administered zaleplon for up to 12 months following two randomised, double-blind placebo-controlled clinical trials. The safety profile in this sample was similar to the one observed in the short-term trials, and, most notably, discontinuation of zaleplon intake was not associated with rebound insomnia. In another study, Krystal et al. (2003) investigated the effects of eszopiclone in a randomised, double-blind, placebo-controlled clinical trial including 788 insomnia patients. The initial gains in sleep continuity were remarkably stable across the 6-month treatment period and there was no evidence of tolerance. Krystal et al. (2008) provided another randomised, double-blind, placebo-controlled clinical trial in which zolpidem was administered for up to 6 months. Again, an absence of tolerance to drug effects was reported and there was no rebound effect during the first three nights of discontinuation. However, Morin et al. (2009b) reported that co-administration of zolpidem in addition to cognitive behavioural therapy for insomnia reduces the long-term therapeutic effect of the psychotherapy. In summary, there is still too little data on the development of tolerance, abuse and dependence in the context of BZ and GARM administration. From our point of view, these issues are, however, of utmost importance for the evaluation of BZs and GARMs for the treatment of chronic insomnia.

In the context of circadian rhythm sleep disorders, clinical guidelines recommend BZs or GARMs as an option for jet lag-induced insomnia in short-term treatment and as an option to promote daytime sleep in night shift workers (Morgenthaler et al. 2007). However, the balance of risks and benefits is not clear and the abuse potential of these substances and potential carryover effects after daytime use need to be considered.

3.2 Pharmacological Treatment with Sedating Antidepressants

Sedating antidepressants like amitriptyline, doxepin, mirtazapine, trazodone, trimipramine or agomelatine are frequently used off-label for the treatment of insomnia (Bertisch et al. 2014; Walsh and Schweitzer 1999). These substances are approved for the treatment of depression and are usually used at lower doses in the context of insomnia treatment. As half-lives range from 9 h (trazodone) to 30 h (amitriptyline), all of these substances are associated with a significant risk of next-day impairments. Sedating antidepressants are a heterogeneous class of substances acting on several neurotransmitter systems in the CNS. The sedating properties are assumed to be mainly driven by the antagonism at the histamine H1 receptor. Anticholinergic effects are responsible for many of the adverse effects, e.g., decreased salivation, decreased gastrointestinal motility or increased heart rate. Polysomnographic investigations in depressed individuals indicate that sedating antidepressants improve sleep continuity, trazodone increases the amount of slow wave sleep, and amitriptyline and doxepin suppress rapid eye movement sleep (Buysse et al. 2005). Moreover, doxepin and mirtazapine are assumed to increase the number of periodic limb movements during sleep and the incidence of the restless legs syndrome.

Only few randomised, double-blind, placebo-controlled clinical trials on the efficacy and safety of sedating antidepressants in the context of clinically diagnosed insomnia have been published. The largest number of clinical trials supporting the efficacy of sedating antidepressants for insomnia has been published for doxepin. The recommended dose for insomnia treatment in adults is 3–6 mg 1 h prior to bedtime (Krystal et al. 2010, 2011; Roth et al. 2007; for studies using larger doses, see also Rodenbeck et al. 2003; Hajak et al. 2001). Doxepin has potent histamine H_1 receptor-blocking activity, a bioavailability of about 25%, and a plasma half-life of around 17 h (51 h for one of its main metabolites desmethyldoxepin). The most commonly observed side effects in clinical trials were fatigue, dizziness, dry mouth, constipation and hypotension. However, at dosages $\leq$6 mg, the safety profile of doxepin was indistinguishable from placebo in a recent clinical trial (Krystal et al. 2011). In summary, the effects on sleep in insomnia patients appear to be significant but smaller than the ones of BZs and GARMs (Winkler et al. 2014; Buscemi et al. 2007).

Walsh et al. (1998) investigated the efficacy of trazodone (50 mg) in comparison with zolpidem (10 mg), and placebo in a randomised, double-blind, clinical trial that included 306 insomnia patients. During the first week of treatment, both trazodone and zolpidem produced significant gains in comparison with placebo in terms of sleep onset latency and total sleep time. However, during the second week of treatment, only the zolpidem group maintained a significantly shorter sleep onset latency in comparison with placebo.

In the only randomised, double-blind clinical trial on trimipramine in insomnia patients, Riemann et al. (2002) investigated the efficacy of this substance in comparison with lormetazepam (1 mg) and placebo in 60 insomnia patients. The study design allowed flexible dosing of trimipramine in the range of 50–200 mg.

Active treatment was administered for 4 weeks followed by a 2-week discontinuation phase. Trimipramine significantly increased polysomnographically determined sleep efficiency in comparison to placebo while lormetazepam did not. The impact on subjective variables was also more pronounced in the trimipramine group than in the lormetazepam group. In addition, there was no evidence for rebound insomnia or withdrawal symptoms in the trimipramine group during 2 weeks of drug discontinuation. However, adverse events were reported in 79% of patients in the trimipramine group, 78% of patients in the placebo group, but only 33% of patients in the lormetazepam group.

In summary, more data is needed to evaluate the efficacy and safety of sedating antidepressants for the treatment of insomnia. Moreover, there is no evidence concerning the use of these substances in the treatment of circadian rhythm sleep disorders (Morgenthaler et al. 2007). The clinical trials summarised above suggest that sedating antidepressants are effective in alleviating insomnia symptoms. Thus, given the minimal abuse potential, these substances are a therapeutic alternative to BZs and GARMs. However, the adverse events profile and the need of cardiovascular monitoring during active treatment should be borne in mind when using sedating antidepressants in the clinical management of insomnia.

3.3 Pharmacological Treatment with Melatonin and Melatonin Receptor Agonists

Melatonin is an endogenous hormone produced by the pineal gland. The rhythm of secretion of melatonin is generated by the suprachiasmatic nucleus: the secretion is suppressed during daytime, increases in the evening, and peaks during nighttime (Claustrat et al. 2005). Light is able to suppress melatonin secretion (Lewy et al. 1980). As a synthetic pharmacological substance, melatonin is available as an over-the-counter medication in the US. Moreover, both melatonin and melatonin receptor agonists are also available as synthetic drugs on a physician prescription basis (ramelteon in the U.S.; Circadin in Europe). Ramelteon is a melatonin receptor agonist acting at both the MT1 and MT2 receptor subtypes and has been approved by the US Food and Drug Administration for the treatment of sleep onset problems. Circadin is a prolonged-release formulation of melatonin acting also at both melatonin receptor subtypes as an agonist and has been approved by the European Medicines Agency for the short-term treatment (3 months) of insomnia patients who are aged 55 or over.

The rationale for using melatonin in insomnia is based on findings that the endogenous hormone is critically involved in the regulation of circadian rhythms and sleep–wake patterns. Melatonin is also supposed to be involved in retinal light sensitivity, systemic immunity, antioxidative defences and glucose regulation (Claustrat et al. 2005). Over-the-counter melatonin has a half-life of 40–60 min, ramelteon of approximately 1.4 h, and Circadin of approximately 2.5 h. The recommended doses are 3–5 mg for over-the-counter melatonin, 8–16 mg for ramelteon, and 2 mg for Circadin.

In a recent meta-analysis, clinical data from randomised, double-blind placebo-controlled trials of ramelteon for insomnia have been summarised by Kuriyama et al. (2014). This meta-analysis included 13 clinical studies with 5,812 insomnia patients. The mean treatment duration was 38 days. The intake of ramelteon led to significant improvements in subjective sleep onset latency and sleep quality as well as polysomnographically determined total sleep time and sleep efficiency. However, the effects were comparably small and, thus, of questionable clinical relevance. Overall, melatonin and melatonin receptor agonists are considered to be an option for the treatment of insomnia especially because of their minimal abuse potential and safety. Overall, the published literature on over-the-counter melatonin, ramelteon, and Circadin does not suggest any serious adverse effects of these substances including a lack of impact on next-day performance.

Herxheimer and Petrie (2002) performed a meta-analysis on studies investigating the efficacy of melatonin for jet lag disorder and came to a very positive evaluation of its effectiveness for this condition. The authors also concluded that the evidence suggests that the positive effects of melatonin are probably greater when more time zones are crossed, and probably smaller for westward travels (see also Beaumont et al. 2004). A further meta-analysis focused on the effects of melatonin on delayed sleep phase disorder (van Geijlswijk et al. 2010). The analysis of nine randomised, double-blind, placebo-controlled clinical trials including 317 adults and children suggested that melatonin has pronounced effects on the clock hour of sleep onset and reduces sleep onset latency significantly by approximately 23 min. Thus, the authors concluded that melatonin is effective in advancing sleep–wake rhythms in patients with delayed sleep phase disorder. Accordingly, clinical guidelines recommend the use of melatonin in jet lag disorder to accelerate entrainment to the new time zone, and in delayed sleep phase disorder to advance sleep–wake rhythms, and, in addition to that, in patients with shift work disorder (prior to daytime sleep). Moreover, the intake of melatonin is recommended to be considered as an option in advanced sleep phase disorder with the aim of delaying sleep–wake rhythms (Morgenthaler et al. 2007).

3.4 Pharmacological Treatment with Orexin Receptor Antagonists

In 2014, the first orexin receptor antagonist, suvorexant, has been approved by the US Food and Drug Administration for the treatment of sleep-onset and sleep-maintenance insomnia. In the lateral hypothalamus, about 70,000 orexin neurons are involved in generating and maintaining wakefulness. Preclinical work suggests that blocking these neurons leads to a reduction of arousal and improved sleep onset and maintenance. Suvorexant is an antagonist at both the orexin A receptor and the orexin B receptor (Yin et al. 2015) and the first hypnotic substance that suppresses wakefulness by acting on the orexinergic system. This is in contrast to the more common pharmacological approach to strengthen the sleep drive (e.g. by the intake of BZs and GARMs). Some substances that specifically block either the orexin A

receptor or the orexin B receptor are currently in development, however, as there are no published clinical trials on these substances, the effects of receptor selectivity remain unknown.

Clinical trials on the effects of suvorexant are promising and show that it is effective for up to a year without significant rebound insomnia (Herring et al. 2012, 2016; Michelson et al. 2014). Most notably, Michelson et al. (2014) conducted a phase 3 study comparing suvorexant ($n = 522$) with placebo ($n = 259$) in a 1-year treatment phase and a 2-month discontinuation phase. In this trial, suvorexant had sustained effects on subjective total sleep time (60 min increase in the suvorexant group vs. 33 min in the placebo group), the number of adverse events during the 1-year treatment phase was similar in both groups, and the data from the discontinuation phase did not suggest significant rebound or withdrawal effects. However, we have previously commented that somnolence was reported by 13% of the patients in the suvorexant group compared with only 3% of the patients in the placebo group (Riemann and Spiegelhalder 2014). While somnolence was reported to be mostly mild or moderate in severity in this clinical trial and a further study does not suggest pronounced effects on next-morning driving performance (Vermeeren et al. 2015), it should be noted that the development of another orexin receptor antagonist, almorexant, was stopped in 2011 after concerns about side effects were raised. Of further note, the published data on the efficacy of suvorexant is more compelling for daily doses of 30–40 mg in comparison of daily doses of 10–20 mg (see Michelson et al. 2014). However, based on safety considerations, the US Food and Drug Administration clearly stated that the total daily dose of suvorexant should not exceed 20 mg (see also Kripke 2015 for a critical evaluation of the risk-benefit ratio of suvorexant).

Up to now, there is no evidence concerning the use of orexin receptor antagonists in the clinical management of circadian rhythm sleep disorders.

3.5 Pharmacological Treatment with Herbal Substances

Herbal substances have been used as over-the-counter sleep medications for centuries. The substances that have been most rigorously studied are valerian, chamomile, kava and wuling. A recently published meta-analysis identified ten randomised, controlled trials investigating the efficacy of these substances in insomnia, however, there were not statistically significant differences between any herbal substance and placebo for any of the measures of clinical efficacy (Leach and Page 2015). Moreover, the majority of studies did not describe the methodology in sufficient detail, to assess the scientific quality of this research. To the best of our knowledge, there are no randomised, double-blind, placebo-controlled clinical trials on the efficacy of herbal substances for circadian rhythm sleep disorders. Thus, in summary, there is currently no evidence supporting the use of herbal substances for insomnia or circadian rhythm sleep disorders.

4 Summary

BZs and GARMs like zolpidem (the most commonly prescribed drug for insomnia) improve sleep onset and maintenance in the short-term treatment of insomnia. However, tolerance and dependence are important side effects, which have led to prescription guidelines restricting the intake of BZs and GARMs to 3–4 weeks. This creates a paradoxical situation, keeping in mind that insomnia is a chronic disorder. Sedating antidepressants are frequently prescribed for insomnia, however, only few randomised controlled trials have been published so far. Melatonin and melatonin receptor agonists are considered to be an option for the treatment of insomnia especially because of their minimal abuse potential and safety, however, the effects on sleep onset and maintenance appear to be smaller than the ones of BZs, GARMs and sedating antidepressants. First data on orexin receptor antagonists for insomnia are promising, however, the risk–benefit ratio needs to be further evaluated. With respect to herbal substances, there is currently no evidence supporting their use in patients with insomnia.

Cognitive-behavioural therapy for insomnia is a safe and effective alternative to pharmacological treatment, and, most importantly, it does exert stable long-term effects (e.g. Morin et al. 2009b). However, there is a substantial proportion of subjects who do not respond or remit during treatment. In particular, adherence to the behavioural strategies is known to be extremely difficult due to increased daytime sleepiness during the initial phase of implementation (Kyle et al. 2014). Furthermore, CBT-I is not largely available and mainly offered in specialised research settings. Insofar the present situation reflects a very dreary outlook for patients with chronic insomnia and new treatment strategies are urgently needed that are safe and effective.

With respect to circadian rhythm sleep disorders, there is solid evidence from meta-analyses supporting the use of melatonin in jet lag disorder to accelerate entrainment to the new time zone, and in delayed sleep phase disorder to advance sleep–wake rhythms. In addition to that, there is evidence supporting the use of melatonin in patients with shift work disorder in order to promote daytime sleep after night shifts. In addition, timed light exposure and behavioural interventions targeting sleep–wake schedules are recommended treatments for circadian rhythm disorders.

Conflicts of Interest

Kai Spiegelhalder declares no competing interests. Christoph Nissen has received speaker honoraria from Servier and serves on the advisory board of Vanda Pharmaceuticals. Dieter Riemann received an honorarium from Abbvie in 2014 for consultation on the development of new drugs for neurodegenerative disorders.

References

Adan A, Natale V (2002) Gender differences in morningness-eveningness preference. Chronobiol Int 19:709–720

Altena E, Van Der Werf YD, Sanz-Arigita EJ et al (2008) Prefrontal hypoactivation and recovery in insomnia. Sleep 31:1271–1276

American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders (DSM-5), 5th edn. American Psychiatric Publishing, Arlington

Ancoli-Israel S, Richardson GS, Mangano RM et al (2005) Long-term use of sedative hypnotics in older patients with insomnia. Sleep Med 6:107–113

Baglioni C, Spiegelhalder K, Lombardo C et al (2010) Sleep and emotions: a focus on insomnia. Sleep Med Rev 14:227–238

Baglioni C, Battagliese G, Feige B et al (2011) Insomnia as a predictor of depression: a meta-analytic evaluation of longitudinal epidemiological studies. J Affect Disord 135:10–19

Baglioni C, Spiegelhalder K, Regen W et al (2014) Insomnia disorder is associated with increased amygdala reactivity to insomnia-related stimuli. Sleep 37:1907–1917

Beaumont M, Batéjat D, Piérard C et al (2004) Caffeine or melatonin effects on sleep and sleepiness after rapid eastward transmeridian travel. J Appl Physiol 96:50–58

Bertisch SM, Herzig SJ, Winkelman JW et al (2014) National use of prescription medications for insomnia: NHANES 1999–2010. Sleep 37:343–349

Bjorvatn B, Pallesen S (2009) A practical approach to circadian rhythm sleep disorders. Sleep Med Rev 13:47–60

Bootzin RR, Epstein DR (2011) Understanding and treating insomnia. Annu Rev Clin Psychol 7:435–458

Buscemi N, Vandermeer B, Friesen C et al (2007) The efficacy and safety of drug treatments for chronic insomnia in adults: a meta-analysis of RCTs. J Gen Intern Med 22:1335–1350

Buysse DJ, Schweitzer PK, Moul DE (2005) Clinical pharmacology of other drugs used as hypnotics. In: Kryger MH, Roth T, Dement WC (eds) Principles and practice of sleep medicine, 4th edn. Saunders, Philadelphia, pp 452–467

Claustrat B, Brun J, Chazot G (2005) The basic physiology and pathophysiology of melatonin. Sleep Med Rev 9:11–24

Downing SS, Lee YT, Farb DH et al (2005) Benzodiazepine modulation of partial agonist efficacy and spontaneously active GABA A receptors supports an allosteric model of modulation. Br J Pharmacol 145:894–906

Drummond SP, Walker M, Almklov E et al (2013) Neural correlates of working memory performance in primary insomnia. Sleep 36:1307–1316

Duendar Y, Dodd S, Strobl J et al (2004) Comparative efficacy of newer hypnotic drugs for the short-term management of insomnia: a systematic review and meta-analysis. Hum Psychopharmacol 19:305–322

Espie CA, Broomfield NM, Macmahon KM et al (2006) The attention-intention-effort pathway in the development of psychophysiologic insomnia: a theoretical review. Sleep Med Rev 10:215–245

Feige B, Al-Shajlawi A, Nissen C et al (2008) Does REM sleep contribute to subjective wake time in primary insomnia? A comparison of polysomnographic and subjective sleep in 100 patients. J Sleep Res 17:180–190

Fernández-Mendoza J, Vela-Bueno A, Vgontzas AN et al (2010) Cognitive-emotional hyperarousal as a premorbid characteristic of individuals vulnerable to insomnia. Psychosom Med 72:397–403

Fortier-Brochu E, Beaulieu-Bonneau S, Ivers H et al (2012) Insomnia and daytime cognitive performance: a meta-analysis. Sleep Med Rev 16:83–94

Glass J, Lanctôt KL, Herrmann N et al (2005) Sedative hypnotics in older people with insomnia: meta-analysis of risks and benefits. BMJ 331:1169

Hajak G, Rodenbeck A, Voderholzer U et al (2001) Doxepin in the treatment of primary insomnia: a placebo-controlled, double-blind, polysomnographic study. J Clin Psychiatry 62:453–463
Hajak G, Müller WE, Wittchen HU et al (2003) Abuse and dependence potential for the non-benzodiazepine hypnotics zolpidem and zopiclone: a review of case reports and epidemiological data. Addiction 98:1371–1378
Hamilton JP, Furman DJ, Chang C et al (2011) Default-mode and task-positive network activity in major depressive disorder: implications for adaptive and maladaptive rumination. Biol Psychiatry 70:327–333
Harris K, Spiegelhalder K, Espie CA et al (2015) Sleep-related attentional bias in insomnia: a state-of-the-science review. Clin Psychol Rev 42:16–27
Harvey AG (2002) A cognitive model of insomnia. Behav Res Ther 40:869–893
Hasler BP, Germain A, Nofzinger EA et al (2012) Chronotype and diurnal patterns of positive affect and affective neural circuitry in primary insomnia. J Sleep Res 21:515–526
Herring WJ, Snyder E, Budd K et al (2012) Orexin receptor antagonism for treatment of insomnia: a randomized clinical trial of suvorexant. Neurology 79:2265–2274
Herring WJ, Connor KM, Ivgy-May N et al (2016) Suvorexant in patients with insomnia: results from two 3-month randomized controlled clinical trials. Biol Psychiatry 79:136–148
Herxheimer A, Petrie KJ (2002) Melatonin for the prevention and treatment of jet lag. Cochrane Database Syst Rev 2:CD001520
Huedo-Medina TB, Kirsch I, Middlemass J et al (2012) Effectiveness of non-benzodiazepine hypnotics in treatment of adult insomnia: meta-analysis of data submitted to the Food and Drug Administration. BMJ 345:e8343
Kessler RC, Berglund PA, Coulouvrat C et al (2011) Insomnia and the performance of US workers: results from the America insomnia survey. Sleep 34:1161–1171
Kripke DF (2015) Is suvorexant a better choice than alternative hypnotics? F1000Res 4:456
Krystal AD, Walsh JK, Laska E et al (2003) Sustained efficacy of eszopiclone over 6 months of nightly treatment: results of a randomized, double-blind, placebo-controlled study in adults with chronic insomnia. Sleep 26:793–799
Krystal AD, Erman M, Zammit GK et al (2008) Long-term efficacy and safety of zolpidem extended-release 12.5 mg, administered 3 to 7 nights per week for 24 weeks, in patients with chronic primary insomnia: a 6-month, randomized, double-blind, placebo-controlled, parallel-group, multicenter study. Sleep 31:79–90
Krystal AD, Durrence HH, Scharf M et al (2010) Efficacy and safety of doxepin 1 mg and 3 mg in a 12-week sleep laboratory and outpatient trial of elderly subjects with chronic primary insomnia. Sleep 33:1553–1561
Krystal AD, Lankford A, Durrence HH et al (2011) Efficacy and safety of doxepin 3 and 6 mg in a 35-day sleep laboratory trial in adults with chronic primary insomnia. Sleep 34:1433–1442
Kuriyama A, Honda M, Hayashino Y (2014) Ramelteon for the treatment of insomnia in adults: a systematic review and meta-analysis. Sleep Med 15:385–392
Kyle SD, Morgan K, Espie CA (2010) Insomnia and health-related quality of life. Sleep Med Rev 14:69–82
Kyle SD, Miller CB, Rogers Z et al (2014) Sleep restriction therapy for insomnia is associated with reduced objective total sleep time, increased daytime somnolence, and objectively impaired vigilance: implications for the clinical management of insomnia disorder. Sleep 37:229–237
Lack LC, Gradisar M, Van Someren EJ et al (2008) The relationship between insomnia and body temperatures. Sleep Med Rev 12:307–317
Laugsand LE, Vatten LJ, Platou C et al (2011) Insomnia and the risk of acute myocardial infarction: a population study. Circulation 124:2073–2081
Laugsand LE, Strand LB, Platou C et al (2014) Insomnia and the risk of incident heart failure: a population study. Eur Heart J 35:1382–1393
Leach MJ, Page AT (2015) Herbal medicine for insomnia: a systematic review and meta-analysis. Sleep Med Rev 24:1–12
Léger D, Bayon V (2010) Societal costs of insomnia. Sleep Med Rev 14:379–389

Lewy AJ, Wehr TA, Goodwin FK et al (1980) Light suppresses melatonin secretion in humans. Science 210:1267–1269
Lu BS, Zee PC (2006) Circadian rhythm sleep disorders. Chest 130:1915–1923
Mendelson WB (2005) Hypnotic medications: mechanisms of action and pharmacological effects. In: Kryger MH, Roth T, Dement WC (eds) Principles and practice of sleep medicine, 4th edn. Saunders, Philadelphia, pp 444–451
Meyerhoff DJ, Mon A, Metzler T et al (2014) Cortical gamma-aminobutyric acid and glutamate in posttraumatic stress disorder and their relationships to self-reported sleep quality. Sleep 37:893–900
Michelson D, Snyder E, Paradis E et al (2014) Safety and efficacy of suvorexant during 1-year treatment of insomnia with subsequent abrupt treatment discontinuation: a phase 3 randomised, double-blind, placebo-controlled trial. Lancet Neurol 13:461–471
Morgan PT, Pace-Schott EF, Mason GF et al (2012) Cortical GABA levels in primary insomnia. Sleep 35:807–814
Morgenthaler TI, Lee-Chiong T, Alessi C et al (2007) Practice parameters for the clinical evaluation and treatment of circadian rhythm sleep disorders. An American Academy of Sleep Medicine report. Sleep 30:1445–1459
Morin CM, Bélanger L, LeBlanc M et al (2009a) The natural history of insomnia: a population-based 3-year longitudinal study. Arch Intern Med 169:447–453
Morin CM, Vallières A, Guay B et al (2009b) Cognitive behavioral therapy, singly and combined with medication, for persistent insomnia: a randomized controlled trial. JAMA 301:2005–2015
Morin CM, Drake C, Harvey AG et al (2015) Insomnia disorder. Nat Rev Dis Primers 1:15026
Nofzinger EA, Buysse DJ, Germain A et al (2004) Functional neuroimaging evidence for hyperarousal in insomnia. Am J Psychiatry 161:2126–2128
Nowell PD, Mazumdar S, Buysse DJ et al (1997) Benzodiazepines and zolpidem for chronic insomnia: a meta-analysis of treatment efficacy. JAMA 278:2170–2177
Ohayon MM (2002) Epidemiology of insomnia: what we know and what we still need to learn. Sleep Med Rev 6:97–111
Perlis ML, Giles DE, Mendelson WB et al (1997) Psychophysiological insomnia: the behavioural model and a neurocognitive perspective. J Sleep Res 6:179–188
Perlis ML, Smith MT, Andrews PJ et al (2001) Beta/gamma EEG activity in patients with primary and secondary insomnia and good sleeper controls. Sleep 24:110–117
Plante DT, Jensen JE, Schoerning L et al (2012) Reduced γ-aminobutyric acid in occipital and anterior cingulate cortices in primary insomnia: a link to major depressive disorder? Neuropsychopharmacology 37:1548–1557
Regen W, Kyle SD, Nissen C et al (2016) Objective sleep disturbances are associated with greater waking resting-state connectivity between the retrosplenial cortex/hippocampus and various nodes of the default mode network. J Psychiatry Neurosci 41:295–303
Riemann D, Perlis ML (2009) The treatments of chronic insomnia: a review of benzodiazepine receptor agonists and psychological and behavioral therapies. Sleep Med Rev 13:205–214
Riemann D, Spiegelhalder K (2014) Orexin receptor antagonists: a new treatment for insomnia? Lancet Neurol 13:441–443
Riemann D, Voderholzer U, Cohrs S et al (2002) Trimipramine in primary insomnia: results of a polysomnographic double-blind controlled study. Pharmacopsychiatry 35:165–174
Riemann D, Spiegelhalder K, Feige B et al (2010) The hyperarousal model of insomnia: a review of the concept and its evidence. Sleep Med Rev 14:19–31
Riemann D, Spiegelhalder K, Nissen C et al (2012) REM sleep instability – a new pathway for insomnia? Pharmacopsychiatry 45:167–176
Riemann D, Nissen C, Palagini L et al (2015) The neurobiology, investigation, and treatment of chronic insomnia. Lancet Neurol 14:547–558
Rodenbeck A, Cohrs S, Jordan W et al (2003) The sleep-improving effects of doxepin are paralleled by a normalized plasma cortisol secretion in primary insomnia. A placebo-

controlled, double-blind, randomized, cross-over study followed by an open treatment over 3 weeks. Psychopharmacology 170:423–428
Roth T, Rogowski R, Hull S et al (2007) Efficacy and safety of doxepin 1 mg, 3 mg, and 6 mg in adults with primary insomnia. Sleep 30:1555–1561
Sack RL, Auckley D, Auger RR et al (2007a) Circadian rhythm sleep disorders: part I, basic principles, shift work and jet lag disorders. An American Academy of Sleep Medicine review. Sleep 30:1460–1483
Sack RL, Auckley D, Auger RR et al (2007b) Circadian rhythm sleep disorders: part II, advanced sleep phase disorder, delayed sleep phase disorder, free-running disorder, and irregular sleep-wake rhythm. An American Academy of Sleep Medicine review. Sleep 30:1484–1501
Saper CB, Scammell TE, Lu J (2005) Hypothalamic regulation of sleep and circadian rhythms. Nature 437:1257–1263
Smith MT, Perlis ML, Chengazi VU et al (2002) Neuroimaging of NREM sleep in primary insomnia: a Tc-99-HMPAO single photon emission computed tomography study. Sleep 25:325–335
Sofi F, Cesari F, Casini A et al (2014) Insomnia and risk of cardiovascular disease: a meta-analysis. Eur J Prev Cardiol 21:57–64
Spiegelhalder K, Regen W, Feige B et al (2012) Sleep-related arousal versus general cognitive arousal in primary insomnia. J Clin Sleep Med 8:431–437
Spiegelhalder K, Regen W, Nissen C et al (2016) Magnetic resonance spectroscopy in patients with insomnia: a repeated measurement study. PLoS One 11:e0156771
Stein PK, Pu Y (2012) Heart rate variability, sleep and sleep disorders. Sleep Med Rev 16:47–66
Stoffers D, Altena E, van der Werf YD et al (2014) The caudate: a key node in the neuronal network imbalance of insomnia? Brain 137:610–620
Trauer JM, Qian MY, Doyle JS et al (2015) Cognitive behavioral therapy for chronic insomnia: a systematic review and meta-analysis. Ann Intern Med 163:191–204
van Geijlswijk IM, Korzilius HP, Smits MG (2010) The use of exogenous melatonin in delayed sleep phase disorder: a meta-analysis. Sleep 33:1605–1614
Vermeeren A, Sun H, Vuurman EF et al (2015) On-the-road driving performance the morning after bedtime use of suvorexant 20 and 40 mg: a study in non-elderly healthy volunteers. Sleep 38:1803–1813
Vgontzas AN, Bixler EO, Lin HM et al (2001) Chronic insomnia is associated with nyctohemeral activation of the hypothalamic-pituitary-adrenal axis: clinical implications. J Clin Endocrinol Metab 86:3787–3794
Walsh JK, Schweitzer PK (1999) Ten-year trends in the pharmacological treatment of insomnia. Sleep 22:371–375
Walsh JK, Erma M, Erwin CW et al (1998) Subjective hypnotic efficacy of trazodone and zolpidem in DSMIII-R primary insomnia. Hum Psychopharmacol 13:191–198
Weitzman ED, Czeisler CA, Coleman RM et al (1981) Delayed sleep phase syndrome. A chronobiological disorder with sleep-onset insomnia. Arch Gen Psychiatry 38:737–746
Winkelman JW, Buxton OM, Jensen JE et al (2008) Reduced brain GABA in primary insomnia: preliminary data from 4T proton magnetic resonance spectroscopy (1H-MRS). Sleep 31:1499–1506
Winkler A, Auer C, Doering BK et al (2014) Drug treatment of primary insomnia: a meta-analysis of polysomnographic randomized controlled trials. CNS Drugs 28:799–816
Wu JQ, Appleman ER, Salazar RD et al (2015) Cognitive behavioral therapy for insomnia comorbid with psychiatric and medical conditions: a meta-analysis. JAMA Intern Med 175:1461–1472
Yin J, Mobarec JC, Kolb P et al (2015) Crystal structure of the human OX2 orexin receptor bound to the insomnia drug suvorexant. Nature 519:247–250
Zhang B, Wing YK (2006) Sex differences in insomnia: a meta-analysis. Sleep 29:85–93

GABA Receptors and the Pharmacology of Sleep

W. Wisden, X. Yu, and N. P. Franks

Contents

Abstract

Current GABAergic sleep-promoting medications were developed pragmatically, without making use of the immense diversity of $GABA_A$ receptors. Pharmacogenetic experiments are leading to an understanding of the circuit mechanisms in the hypothalamus by which zolpidem and similar compounds induce sleep at α2βγ2-type $GABA_A$ receptors. Drugs acting at more selective receptor types, for example, at receptors containing the α2 and/or α3 subunits

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expressed in hypothalamic and brain stem areas, could in principle be useful as hypnotics/anxiolytics. A highly promising sleep-promoting drug, gaboxadol, which activates αβδ-type receptors failed in clinical trials. Thus, for the time being, drugs such as zolpidem, which work as positive allosteric modulators at $GABA_A$ receptors, continue to be some of the most effective compounds to treat primary insomnia.

Keywords
Benzodiazepine • GABA-A receptor • GABA-B receptor • Gaboxadol • Histamine • Sedation • Steroid • THIP • Z-drug • Zolpidem

1 Introduction

For primary insomniacs, those otherwise healthy people who cannot get to sleep or maintain sleep, cognitive behavioural therapy is recommended as the first route to better sleep (Schroeck et al. 2016; Wafford and Ebert 2008). If this fails, sleeping medication is then prescribed. These medications, known as sedatives or hypnotics, are generally controlled substances, requiring a medical doctor's authorization. In this chapter, we look at the sedatives/hypnotics used to treat primary insomnia that work by enhancing inhibitory GABAergic transmission. There have been various reviews on sleep medication and $GABA_A$ receptors (Greenblatt and Roth 2012; Lancel and Steiger 1999; Nutt and Stahl 2010; Wafford and Ebert 2008; Winsky-Sommerer 2009). In spite of the passage of time, these reviews remain excellent resources on drug structures, the history of how these drugs were developed and their clinical effects. The $GABA_A$ receptor drug zolpidem (Ambien) remains the first pharmacological line of attack for treating insomnia (Bertisch et al. 2014; Lancel and Steiger 1999; Mignot 2013; Nutt and Stahl 2010). One survey of about 32,300 adults (healthy and not in hospital) found that 3% of them (about 970 people) had been prescribed zolpidem for insomnia in the preceding month (Bertisch et al. 2014). In fact, in the USA, there were 53.4 million prescriptions for zolpidem (Ambien) in 2010 (Greenblatt and Roth 2012). This amounted in 2006 to $1.5 billion of sales for the company that then principally marketed zolpidem, Sanofi (Morris 2013).

2 Natural Sleep and Hypnosis/Sedation

Sedatives are substances that depress the central nervous system, producing calmness and relaxation, less anxiety and more sleepiness; and hypnotics are defined as substances which induce sleep, so the words "sedative" and "hypnotic" tend to be used interchangeably (Wafford and Ebert 2008). In the GABA field, there is much work done on selective anxiolytics that do not cause sedation, but an anxiolytic drug is also likely to be sleep promoting if sleep latency was measured in a calm environment – in practice, for humans, it would be unlikely that there would be

an anxiolytic that does not influence sleep propensity, since relaxation is part of the preparation for getting ready to sleep.

Some hypnotic/sedative drugs induce vigilance states partially resembling natural NREM sleep, with a delta to theta ratio in the EEG that could be classified as NREM-like. The power of the EEG at different frequencies reflects the degree of synchronization of neural currents at those frequencies in the neocortex and hippocampus. A sleep-like EEG produced by a sedative/hypnotic often has a high delta to theta frequency power ratio but sometimes with an overall lower power than occurring in NREM sleep, which may represent a less deep sleep. For certain compounds (e.g. propofol, etomidate, barbiturates, inhalational compounds), as drug dosage is increased, sedation deepens into general anaesthesia (Franks 2008; Franks and Lieb 1994; Garcia et al. 2010), and the EEG becomes flat, with periodic bursts of large amplitude spikes, a phenomenon termed "burst suppression" (see for example Fig. 6 in Reynolds et al. 2003).

In this chapter, we take drug-induced sedation/hypnosis to mean a NREM-like activity measured in the EEG, reduced or zero movement with the subject being harder to arouse and with a lower body temperature. Even within this drug-induced sedative/hypnotic state, there are differences between drugs and receptor classes. Sedation is easier to define on human subjects, because verbal responses and visual observation reveal if the person is conscious or in an intermediate state and this can be combined with EEG, heart rate and respiratory recordings (polysomnography).

Sedation as a concept has been used rather loosely in experiments on animals. Researchers studying sedation, especially with regard to benzodiazepine and zolpidem action, have tended to separate drug-induced sedation and drug-induced sleep into separate concepts: e.g. benzodiazepine-induced sedation and benzodiazepine-induced sleep. This has added some confusion to the field (Tobler et al. 2001; Winsky-Sommerer 2009). This distinction came about because many experimental studies have defined an animal as sedated by just one parameter, which is if the animal moves less in the open-field assay following drug treatment or if it becomes ataxic. But often the EEG was not measured in these studies on sedation (Crestani et al. 2000; McKernan et al. 2000; Rudolph et al. 1999). Animals could be quietly awake or have a changed motor function independent of sleep through, e.g. catalepsy or cerebellar interference. When, for example, Purkinje cells in the mouse cerebellum were made selectively sensitive to zolpidem, zolpidem given systemically to these mice caused ataxia and reduced the time they could stay on a rotating rod, but the mice were not sedated (Wulff et al. 2007). An EEG analysis on sedation is more incisive, because reduced or changed movement alone is an insufficient criterion to define sedation, and as part of the EEG scoring, movement is implicitly measured, as the EMG is used to help score the vigilance states.

Although we think that reduced movement alone is not an adequate criterion to define sedation, it has also been argued that there is too much reliance on the EEG to characterize vigilance states, without paying enough attention to the obvious feature of how the animal appears visually (Coenen and van Luijtelaar 1991). There is sometimes a dissociation between the EEG and the behaviour of the

animal, so that "a quantitative analysis of the EEG alone might give misleading information about the effects of a compound on the state of vigilance" (Coenen and van Luijtelaar 1991). The anaesthetic urethane, whose molecular targets are still unknown, produces strong and sustained theta in the EEG (Coenen and van Luijtelaar 1991). A strong delta power component in the EEG does not always mean NREM sleep or sedation either. The EEG can contain a strong delta profile in fear-induced freezing (frontal cortex) (Karalis et al. 2016) or following atropine administration (Qiu et al. 2015) or following THIP and muscimol administration, where mice are awake, but have NREM-like oscillations in their prefrontal cortex (Vyazovskiy et al. 2005, 2007; Winsky-Sommerer et al. 2007), and also awake $GABA_A$ receptor δ subunit knockout mice have strong δ oscillations. In the case of muscimol, the state produced is catalepsy, where the animals do not move because the muscles have no tone, similar to REM sleep, but the mice are not asleep (Vyazovskiy et al. 2007).

3 The Perfect Sleeping Pill?

A good "sleeping pill" should reduce the time to enter NREM sleep (reduce "sleep latency") and allow only limited awakenings, and most researchers assume that sleep-promoting medication should aim to reproduce the sleep EEG profile and architecture (order and length and EEG power of NREM and REM sleep) found in natural sleep. Other practical considerations are that a sleeping pill compound should occupy its receptor sites quickly and have a short half-life so that it on the one hand increases sleep time but on the other does not give "hangover effects" such as daytime sleepiness or reduced daytime alertness (Nutt and Stahl 2010). Zolpidem meets most of these requirements well. The ideal sleep-promoting compound should also have low addictive potential and not cause rebound insomnia when the drug is withdrawn. Unsurprisingly, no sedative could be yet described as "perfect". Problems with even the best sleeping medications include "confusional arousal" and ataxia and risk of injury if awakening occurs whilst on the medication (Frey et al. 2011; Mignot 2013) and rebound insomnia if use is discontinued (Mignot 2013) (see Sect. 5 below). There can also be serious side effects if sleep-promoting medication is taken with other medications that suppress neuronal activity, e.g. mixing zolpidem with narcotic pain killers or alcohol. Some people, particularly the elderly, who take zolpidem have more risk of car accidents the next day if they are driving (Booth et al. 2016). In fact, starting around 2006, there are various stories in the media of zolpidem (Ambien)-induced delirium and people acting strangely under the drug's influence during sleep walking – it is unclear if these aberrant behaviours are really due to zolpidem (see Morris 2013).

4 Activating GABAergic Neurons Generally Promotes Sleep: $GABA_A$ Receptor Positive Allosteric Modulators (PAMs) and GABA Agonists Induce Sleep

γ-Aminobutyric acid (GABA), the main inhibitory transmitter in the mammalian brain, works on the ionotropic ($GABA_A$) and metabotropic ($GABA_B$) receptor classes. The natural sleep-promoting circuitry in the brain uses sleep-active GABAergic neurons to inhibit wake-active neurons in wake-promoting circuitry (Chung et al. 2017; Lin et al. 1989; Nitz and Siegel 1996; Sherin et al. 1998; Uygun et al. 2016) (Fig. 1). Hence, it might seem intuitively obvious that enhancing GABAergic transmission throughout the brain with positive allosteric modulators (PAMs) of $GABA_A$ receptors, and thus enhancing network inhibition, will induce sedation. And indeed, with a few interesting exceptions, this is the case.

Most effective hypnotics/sedatives and anaesthetics developed to date enhance GABA's action at $GABA_A$ receptors, working as PAMs at the receptor. Most drugs were developed and marketed without knowledge of their $GABA_A$ receptor targets, and this knowledge was gained often only decades after the drugs were first in use (Franks 2008). PAM compounds do not usually work on the $GABA_A$ receptors by themselves but require GABA to bind to the receptor as well. By causing GABA to prolong the duration of the inhibitory postsynaptic currents through $GABA_A$ receptors, PAMs enhance ongoing GABAergic transmission. These drugs, which have varied molecular structures and come from multiple classes of compound, include amobarbital (barbiturates); compounds acting as agonists at the benzodiazepine site, e.g. zolpidem (an imidazopyridine), zaleplon (a pyrazolopyrimidine) and zopiclone (a cyclopyrrolone); and the benzodiazepines, e.g. diazepam, flurazepam, quazepam, temazepam and triazolam (Bertisch et al. 2014; Gottesmann et al. 1998; Lancel and Steiger 1999; Nutt and Stahl 2010). Two current major intravenous anaesthetics used during hospital operations to induce and maintain general anaesthesia, etomidate and propofol, are also selective PAMs at $GABA_A$ receptors (Franks 2008). At low concentrations, general anaesthetics produce sedation. As the drug concentrations increase, the difference in principle between propofol and zolpidem, why the first can induce and maintain general anaesthesia, whilst the other is only a sleeping pill which cannot induce more than sleep, is that probably propofol prolongs IPSCs more than zolpidem and it is a more potent PAM. In addition, propofol works on all $GABA_A$ receptor types, whereas benzodiazepines and zolpidem are more restricted in which receptors they can activate.

Hans Selye, who discovered and conceptualized the physiological responses to stress, also first observed that the endogenous steroid progesterone could cause sedation (Gunn et al. 2015; Herd et al. 2007; Lancel and Steiger 1999). Decades later it was discovered that reduced versions of progesterone, i.e. some of its metabolites, are potent PAMs of $GABA_A$ receptors (Harrison and Simmonds 1984; Lan et al. 1990; Lancel et al. 1999; Majewska et al. 1986) and these progesterone metabolites (e.g. allopregnanolone) produce behavioural effects, e.g. sedation/sleep and anxiolysis, broadly similar to other PAMs of $GABA_A$ receptors except that steroids work on a wider range of subunit combinations and

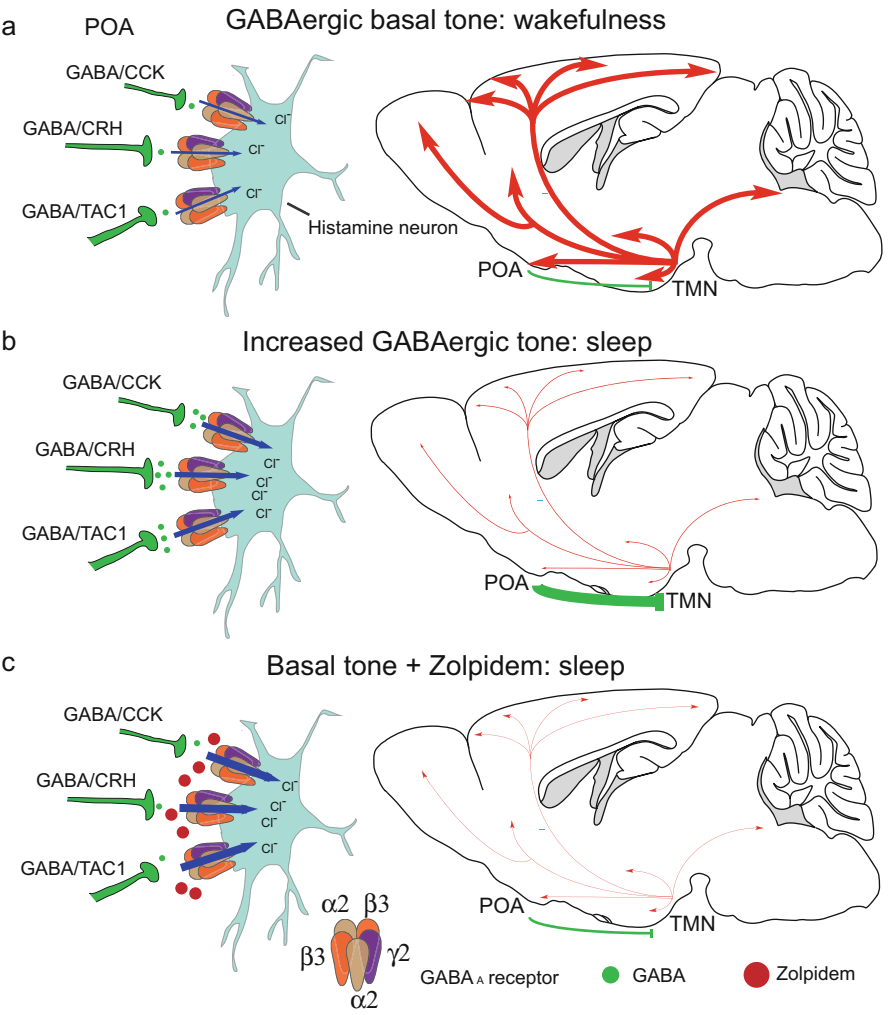


Fig. 1 Schematic of how GABAergic afferents from the preoptic area (POA) of the hypothalamus inhibit wake-promoting histamine neurons and how zolpidem enhances this natural inhibitory pathway to induce sleep. (**a**) During wakefulness, there is a basal GABAergic tone from the POA afferents (*green*) onto the histamine neurons in the tuberomammillary area (TMN). These wake-active neurons project widely (*red* arrows) to release histamine to promote aspects of arousal. In addition to GABA, the GABAergic afferents release a range of peptides (e.g. CCK, CRH, TAC1) into the tuberomammillary area (Chung et al. 2017). The $GABA_A$ receptors located postsynaptically on the histamine neurons are probably mixtures of α1βγ2 and α2βγ2 receptors. The γ1 and ε subunits are also expressed in some histamine neurons (May et al. 2013). (**b**) At the onset of NREM sleep and during NREM sleep, the GABAergic afferents have increased activity (thicker *green* line), hence depressing histaminergic activity (thin *red* arrows). Similar scenarios would also be happening at other ascending aminergic centres, e.g. the locus coeruleus and dorsal raphe. (**c**) After taking zolpidem, the drug will act as a PAM on the postsynaptic $GABA_A$ receptors to enhance the GABA afferent input onto histamine neurons and inhibit them, effectively mimicking the situation occurring in natural NREM sleep shown in panel (**a**) (Uygun et al. 2016)

are not just "dependent" on γ2-subunit-containing $GABA_A$ receptors (Herd et al. 2007) (see Sect. 6 for details on the subunits). Allopregnanolone ($t_{1/2}$ = just 35 min) was improved to give ganaxolone with better oral bioavailability and a longer half-life. This drug is highly sedative and could, in principle, be used to treat insomnia, as well as epilepsy and anxiety. In fact, allopregnanolone is a potent sedative before anything else, and therefore drug developers have concentrated on reducing its sedative properties so that it can be used for other treatments. The synthetic steroid-like derivative UCI-50027 is more anxiolytic than sedative as assessed by its effect on movement (Hogenkamp et al. 2014). This illustrates the difficulty for drug developers interested in new sleeping medications. The intellectual property space for these drugs is crowded, and there are no strong commercial advantages in having a steroid-based sedative, as opposed to benzodiazepines or zolpidem.

5 Addiction and Rebound Insomnia

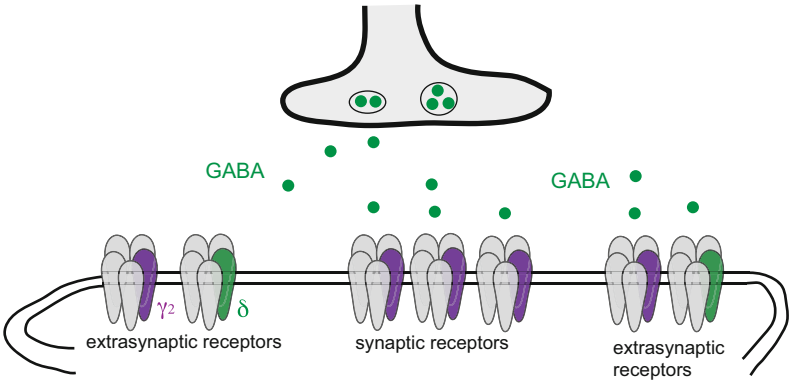
Barbiturates, the original sedatives in use since the beginning of the twentieth century, and benzodiazepines have generally fallen out of use as sleep-promoting agents. Barbiturates are simply dangerous because of the risk of overdose and lethal if taken at too high a dose because they can directly gate the $GABA_A$ receptors and depress breathing and cardiac regulation centres. Hence, barbiturates are now only used in specialist medical applications and until recently on "death row". Similarly, the benzodiazepine drugs Librium and Valium, household names in the 1960s, were used decades before it was understood that they were PAMs of $GABA_A$ receptors (Mohler 2015; Randall 1961). The benzodiazepine triazolam (Halcion) ran into legal trouble when patients committed criminal offences and had accidents whilst having this drug prescribed for sleep (Mignot 2013). Consequently, in the USA triazolam is prescribed at a lower dose, and in the UK it is not available, having been banned as a "sleeping pill" in 1991. Both classes of drugs are addictive, and tolerance emerges, and rebound insomnia appears when the drugs are withdrawn, so that the patient sleeps even less well than before. Rebound insomnia is an interesting phenomenon and presumably indicates that $GABA_A$ receptors on neurons in the sleep regulatory circuitry have become downregulated with continuous PAM use; without the drug, the relevant neurons are not sufficiently inhibited by endogenous GABAergic transmission. The circuitry and neurons involved are unknown, but it would be good to know what these are as we may then have a more precise understanding of the mechanisms involved. It could well be that PAMs down-modulate $GABA_A$ receptors on neurons that control the homeostatic sleep circuitry – the receptors on these neurons (e.g. orexin and melanin-concentrating hormone neurons) are dynamically modulated by the sleep drive (Toossi et al. 2016).

6 $GABA_A$ Receptors: A Family of Subunits Assembled to Make Many Subtypes of Receptor (Opportunities to Discover PAMs That Selectively Promote Sleep and Anxiolysis)

The $GABA_A$ receptor drugs in clinical use have been discovered pragmatically without using knowledge about the molecular structures of the receptors. We will now look at the receptors on which these drugs act, because pharmacologists hope to design more precise drugs based on the structure of the receptors. $GABA_A$ receptors were first cloned in 1987 and are now fairly well understood at the structural level (Levitan et al. 1988; Miller and Aricescu 2014; Mohler 2015; Olsen 2015; Pritchett et al. 1989; Puthenkalam et al. 2016; Schofield et al. 1987; Seeburg et al. 1990; Shivers et al. 1989; Sieghart 2015; Sigel and Steinmann 2012). They are GABA-gated chloride channels, in the same gene superfamily as glycine receptors, nicotinic acetylcholine receptors and serotonin 5-HT_3 receptors. There are six alpha (α1–α6) subunit genes, three beta (β1–β3) subunit genes, three gamma (γ1–γ3) subunit genes and one delta (δ) subunit gene; additionally, there are also epsilon (ε), theta (θ) and pi (π) subunit genes and three rho (ρ) subunit genes. The subunit genes are differentially expressed throughout the brain and enteric nervous system (Fritschy and Mohler 1995; Moragues et al. 2002; Pirker et al. 2000; Seifi et al. 2014; Sinkkonen et al. 2000; Wisden et al. 1988, 1992). Because the subunits can assemble in different combinations, there are many subtypes of $GABA_A$ receptor. We still do not know precisely how many.

Five subunits are arranged in a ring with the chloride channel at the centre. For the best studied types of $GABA_A$ receptor, the ring usually contains two α subunits, two β subunits and a γ or δ subunit. Two GABA molecules activate the receptor, binding at the α and β subunit interfaces. There are many mechanistic possibilities for PAM drugs to enhance (or sometimes decrease) GABA's action at the receptor complex. The receptor complex contains binding sites for allosteric modulators, which bind within the membrane helices, such as the propofol binding site at the interface between the extracellular domain and transmembrane domain 2 (Franks 2015; Yip et al. 2013), or the benzodiazepine and z-drug binding site between the α and γ2 subunit interfaces (Ogris et al. 2004; Puthenkalam et al. 2016). A few drugs work at the GABA binding site – between the α and β subunits – these drugs are known as orthosteric ligands (THIP/gaboxadol and muscimol) and mimic GABA's agonist actions (Puthenkalam et al. 2016).

For current drugs the important target combinations of receptor subunits are α1βγ2, α2βγ2, α3βγ2, α4βγ2, α4βδ and α5β1/2/3γ2 receptors. Benzodiazepines and the "z-drugs" (e.g. zolpidem, zopiclone) require an α and γ2 subunit in the receptor complex (Cope et al. 2004; Ogris et al. 2004; Pritchett and Seeburg 1990; Pritchett et al. 1989; Seeburg et al. 1990); for these drugs the type of β subunit is less important. Receptors that contain the γ2 subunit are enriched in the postsynaptic area but are also present extrasynaptically (Fig. 2). Synaptic locations would also be expected for receptors that contain the γ1 or the γ3 subunits, but receptors with these subunits have been relatively little studied (Herb et al. 1992) and are



Effects of drugs on sleep and other parameters:	$GABA_A$ sub-type modulated by sedative-hypnotics: GABA & BZ & Z-drugs → γ2-subtype; GABA agonists → δ-subtype
Sleep onset latency:	↓
Sleep continuity:	↑
Total sleep time:	↑
REM sleep:	↓
REM sleep latency:	↑
EEG power:	↓ in frequency< 10 Hz; ↑ in spindle frequency
Residual effects:	sedation
Effects on cognitive performance:	cognitive impairment & amnesic effects
Development of tolerance and/or dependance:	YES
Rebound insomnia:	YES

Effects of drugs on sleep and other parameters:	$GABA_A$ sub-type modulated by sedative-hypnotics: GABA agonists → δ-subtype
Sleep onset latency:	—
Sleep continuity:	↑
Total sleep time:	↑
REM sleep:	—
REM sleep latency:	—
EEG power:	↑ in frequency< 10 Hz; ↓ in spindle frequency
Residual effects:	none reported
Effects on cognitive performance:	not altered & sustain attention during sleep restriction
Development of tolerance and/or dependance:	NO
Rebound insomnia:	NO

Fig. 2 Summary of how sleep-promoting drugs work on synaptic (αβγ2 type) and extrasynaptic (αβγ2 and αβδ) $GABA_A$ receptors. The differing types of sleep produced by benzodiazepines (BSz), z-drugs such as zolpidem and GABA agonists such as gaboxadol are summarized in the table. This figure and table have been adapted from (Winsky-Sommerer 2009)

expressed in just a few cell types. Notably the γ1 gene is expressed in the medial preoptic-hypothalamic area, a sleep regulatory centre (Wisden et al. 1992). The synaptic receptors with the γ2 subunits convey fast synaptic inhibition (Brickley and Mody 2012). Receptors that contain the δ subunit do not get targeted to the postsynaptic area and are extrasynaptic (Brickley and Mody 2012) (Fig. 2). Activation of extrasynaptic $GABA_A$ receptors by GABA diffusing from the synapse, or being released non-synaptically, produces "tonic inhibition", which is a sustained

(hence the word "tonic") activation of $GABA_A$ receptors, with no precise temporal resolution (Brickley and Mody 2012). Agonists (GABA mimetics) such as 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (*THIP* – also known as gaboxadol) act preferentially on δ subunit-containing receptors (Fig. 2). The αβγ2-containing $GABA_A$ receptors outside synapses also contribute to tonic inhibition.

7 Benzodiazepines and "Z-Drug"-Induced Sleep/Sedation: Mechanisms and Circuitry

Benzodiazepines such as diazepam and the z-drugs (e.g. zolpidem) reduce the time to sleep (decrease sleep latency), increase sleep continuity and total sleep time, decrease REM sleep and increase REM sleep latency (Lancel and Steiger 1999; Winsky-Sommerer 2009) (Fig. 2). In terms of the EEG, these drugs induce a NREM-like state, with a higher delta-to-theta ratio, but the power of the EEG in this range is reduced (relative to the power in natural NREM sleep) (Fig. 3). Spindle frequency power (12–14 Hz) is also increased relative to the power of spindles in natural sleep. Lower power in the NREM range induced by these drugs might mean a less deep sleep or a less restorative sleep. Zolpidem-induced sleep has decreased EEG power in the 5–16 HZ range, relative to that in natural sleep, and benzodiazepines depress the EEG power even further (Winsky-Sommerer 2009) (Fig. 3). It is not clear what these changes in power relative to natural sleep really mean for efficacy. It could be perfectly adequate sleep, sufficient to treat insomnia, especially because these drugs are not intended to be taken permanently. For example, the benzodiazepine triazolam substantially depresses NREM EEG power but does not interfere with synaptic plasticity during sleep, whereas zolpidem does actually interfere with plasticity, but the EEG power reduction compared with normal sleep is less (Seibt et al. 2008). This work was done on the *developing* visual cortex of kittens; Seibt et al. conclude: "Our findings demonstrate that alterations in sleep architecture do not necessarily lead to impairments in sleep function. Conversely, hypnotics that produce more "physiological" sleep based on polysomnography may impair critical brain processes, depending on their pharmacology" (Seibt et al. 2008).

Diazepam and the other benzodiazepines work as PAMs on α1βγ2, α2βγ2, α3βγ2 and α5βγ2 receptors and do not differentiate between them, whereas zolpidem enhances GABA's action mainly on three $GABA_A$ receptor subtypes: α1βγ2, α2βγ2 and α3βγ2 (Pritchett and Seeburg 1990; Pritchett et al. 1989); zolpidem has the highest binding affinity at α1βγ2-containing receptors but only by 20-fold, meaning that in practice at normal doses in vivo zolpidem also acts as a PAM at α2βγ2 and α3βγ2 receptors (Pritchett and Seeburg 1990; Uygun et al. 2016), and zolpidem cannot really be called an "α1-selective compound", even though this term is frequently used. Some benzodiazepines and zolpidem will also, to some extent, enhance GABA's action at αβγ1-type receptors (Khom et al. 2006).

The $GABA_A$ receptor targets for benzodiazepines and zolpidem are widespread, and so these drugs will influence nearly all aspects of brain function and do cause

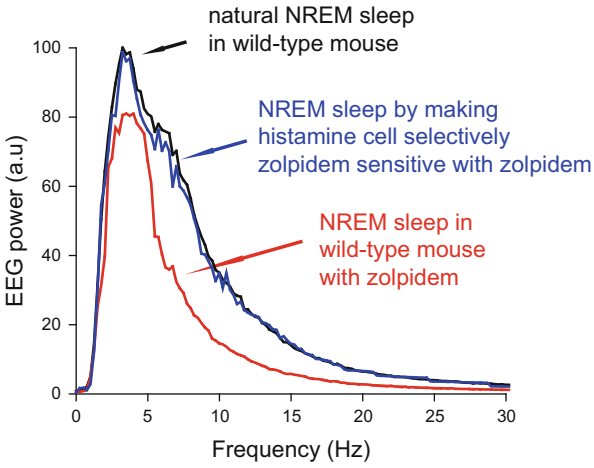


Fig. 3 Schematic of power spectra of zolpidem-evoked NREM sleep. EEG power in the 5–16 Hz range evoked by zolpidem in NREM sleep is less than that found in natural NREM sleep, whereas selectively enhancing $GABA_A$ receptors with zolpidem on histaminergic neurons produces a type of NREM sleep resembling natural NREM in terms of EEG power. This is not actual data but redrawn from (Uygun et al. 2016)

many side effects. An important advance for potentially improving drug selectivity at $GABA_A$ receptors came with the discovery that one particular histidine (H) in the extracellular domain of the alpha subunits, H101, determined if benzodiazepines could bind to the receptor complex (Wieland et al. 1992). This discovery was made because it was noticed that two of the alpha subunits, α4 and α6, formed benzodiazepine-insensitive $GABA_A$ receptors when expressed as recombinant α4βγ2 or α6βγ2 subunits combinations (Luddens et al. 1990; Wisden et al. 1991) and that this was because the α4 and α6 subunits contained an arginine R101 and not H101 at their presumed benzodiazepine binding sites (Wieland et al. 1992). When this residue in α1, α2, α3 or α5 was mutated to an R, benzodiazepines could no longer potentiate the GABA response at recombinant α(H101R)βγ2 receptors (Wieland et al. 1992). This mutation was exploited to make a series of knock-in mice where the α1, α2, α3 and α5 subunit genes were systematically mutated in the codon encoding the key histidine H101, so that R101 was incorporated into the subunit instead. These "αH101R" mice were used to behaviourally dissect the roles of the different $GABA_A$ receptor subtypes in responding to benzodiazepines and z-drugs such as zolpidem (Mohler 2015; Rudolph et al. 1999), whereby particular $GABA_A$ receptor subtypes had lost the ability to be modulated by diazepam (a pharmacogenetic loss of function experiment). For example, it was found that benzodiazepines do not reduce anxiety in α2H101R mice (Low et al. 2000) and that α1H101R do longer get sedated by benzodiazepines or zolpidem (the mice keep moving after drug injection) (Crestani et al. 2000; McKernan et al. 2000; Rudolph et al. 1999). Thus αβγ2-type $GABA_A$ receptors with α2 receptors were suggested to contribute to the anxiety-reducing effects of benzodiazepines; those receptors with α1 subunits contribute to the sedative effects of zolpidem and benzodiazepines (Rudolph et al. 1999). In recent experiments, all four lines of αH101R point mutant mice have been bred together to generate either HRRR (order is α1, α2, α3 and α5), RHRR, RRHR or RRRH mice (a pharmacogenetic restriction of function method), so that $GABA_A$ receptors with only one of the α subunits are now enhanced by

diazepam (Ralvenius et al. 2015). With this series of mouse lines, it was found that $GABA_A$ receptors with the α5 subunit, and not the α2 subunit, are the main receptors regulating anxiolysis in response to diazepam (Behlke et al. 2016). In the original, and even new, experiments with "α1H101R" and HRRR mice on "sedation", the EEG was not measured. But even in the new HRRR experiments, α1-containing $GABA_A$ receptors seem to be the only target for diazepam to reduce movement and hence cause "sedation" (Ralvenius et al. 2015).

In a separate series of studies from those on sedation (reduced movement) discussed above, the single H to R point mutant α subunit mice were used to explore the effects of diazepam and zolpidem on the EEG and sleep latency. For diazepam, the α1H101R mutation did not alter the sleep latency or sleep length, so α1-containing receptors are not involved in diazepam's hypnotic actions (Tobler et al. 2001). Similarly, α3 H to R point mutant mice had unchanged hypnotic responses to diazepam (Kopp et al. 2003). Instead, it seemed to be the α2 subunit that was partially required (Kopp et al. 2004a). Similarly, the α1-containing GABA receptors are not responsible for zolpidem's ability to promote sleep. In mice with α1 subunits made insensitive to zolpidem by the H101R mutation, zolpidem reduces latency to NREM sleep and prolongs NREM sleep time as well as it does in wild-type mice (Kopp et al. 2004b). Thus, although this seems to contradict the sedation data mentioned above (based on inhibiting movement), where zolpidem acts through the α1-type receptors to induce "sedation", zolpidem's sleep-promoting effects must come from enhancing GABA's actions at $GABA_A$ receptors with α2 and/or α3 subunits (Kopp et al. 2004b). [Note: there has been quite a lot of work done to find α2- and α3-selective anxiolytic PAMs, but it would be surprising if such drugs were not also sedatives]. However, the α1H101R mice show that α1-containing receptors are needed for zolpidem to produce its characteristic decrease in the EEG power (frequencies between 5 and 16 Hz of zolpidem-induced NREM sleep relative to that found in natural NREM sleep (Kopp et al. 2004b). Zolpidem might induce NREM sleep in part by potentiating histamine neurons in the posterior hypothalamus (see Sect. 11). In the end, it is still curious why there is a disconnect between the α1-type $GABA_A$ receptors as sedative promoting and the α2-type receptors as sleep promoting. With hindsight, both types of receptor are involved in promoting different subcomponents of sleep.

The reticular thalamus and layer VI of the neocortex are enriched in their expression of α1β2γ2- and α3β2γ2-type receptors (Wisden et al. 1988, 1992). These sites are expected to control thalamocortical oscillations such as the δ oscillation in NREM sleep. However, the global α3 knockout mouse had no obvious sleep-wake phenotype, although possibly another $GABA_A$ receptor subtype was upregulated on the reticular thalamic neurons, as the α3 KO cells still had IPSCs (Winsky-Sommerer et al. 2008). The α3 H to R (loss of function for diazepam) mice were studied to provide more information. However, the effects of diazepam given to these mice on sleep and EEG were no different to mice with diazepam-sensitive α3 subunits (Kopp et al. 2003). Given the recent discovery of the excitatory GABA projection from the lateral hypothalamus to the reticular thalamus, one might speculate that some of the reticular thalamic α3βγ2 receptors would contribute to

net excitation and wakefulness, whereas α3-containing receptors on, e.g. the locus coeruleus, could contribute to sedation (see Sect. 11).

8 Wake-Promoting GABAergic Pathways: Zolpidem Can Promote Transient Arousal from a Small Subset of Patients with Disorders of Consciousness

Some GABAergic pathways, especially some originating in the hypothalamus and brainstem, are actually wake promoting (Chung et al. 2017; Herrera et al. 2016; Venner et al. 2016), and so enhancing the synaptic transmission at their projection sites selectively would be expected to promote wakefulness. For example, some lateral hypothalamic GABAergic neurons project to the GABAergic reticular thalamus neurons (Herrera et al. 2016). Selectively activating these lateral hypothalamic GABAergic neurons produces wakefulness and even emergence from general anaesthesia (Herrera et al. 2016). Thus, selectively enhancing this feedforward inhibition with a $GABA_A$ receptor PAM would probably produce wakefulness. But in practice, current sleep-promoting drugs work all over the brain; this wake-promoting effect of the PAM at this synapse is probably swamped by the net inhibitory effect at most other synapses where PAMs enhance GABAergic inhibition. In cats and juvenile ferrets, benzodiazepines and zolpidem can promote arousal (Hsu et al. 2009; Lancel and Steiger 1999). There are some spectacular findings that zolpidem can promote transient arousal from rare types of disorders of consciousness (coma) – but the circuit mechanisms remain unclear (Chatelle et al. 2014; Williams et al. 2013). Perhaps in some types of disorders of consciousness, selective pathways, such as the lateral hypothalamic-reticular thalamus route mentioned above, are stimulated by zolpidem, and that this is possible because the normal sleep-promoting GABAergic pathways are damaged. Only 5% of patients with disorders of consciousness respond to zolpidem by transiently awakening, and the effect is small when done double-blind and placebo controlled (Thonnard et al. 2013).

9 Extrasynaptic $GABA_A$ Receptors, δ Subunits and Sleep

An important class of $GABA_A$ receptors exists exclusively outside the synapse and contains δ subunits (Brickley and Mody 2012). Delta-containing receptors are involved in tonic inhibition, sensing extrasynaptic GABA. This type of inhibition may be used physiologically as a gain control system. In the forebrain, the δ subunit is paired primarily with the α4 and α1 subunits (Fig. 4). In thalamic relay neurons, which have some of the highest expression of α4 and δ subunits in the brain and which also co-express the α1 and β2 subunits, the receptor could be α4β2δ or α1α4β2δ (Shivers et al. 1989; Wisden et al. 1991, 1992). The α1βδ subunit combination also exists in the neocortex and hippocampus on selective GABAergic

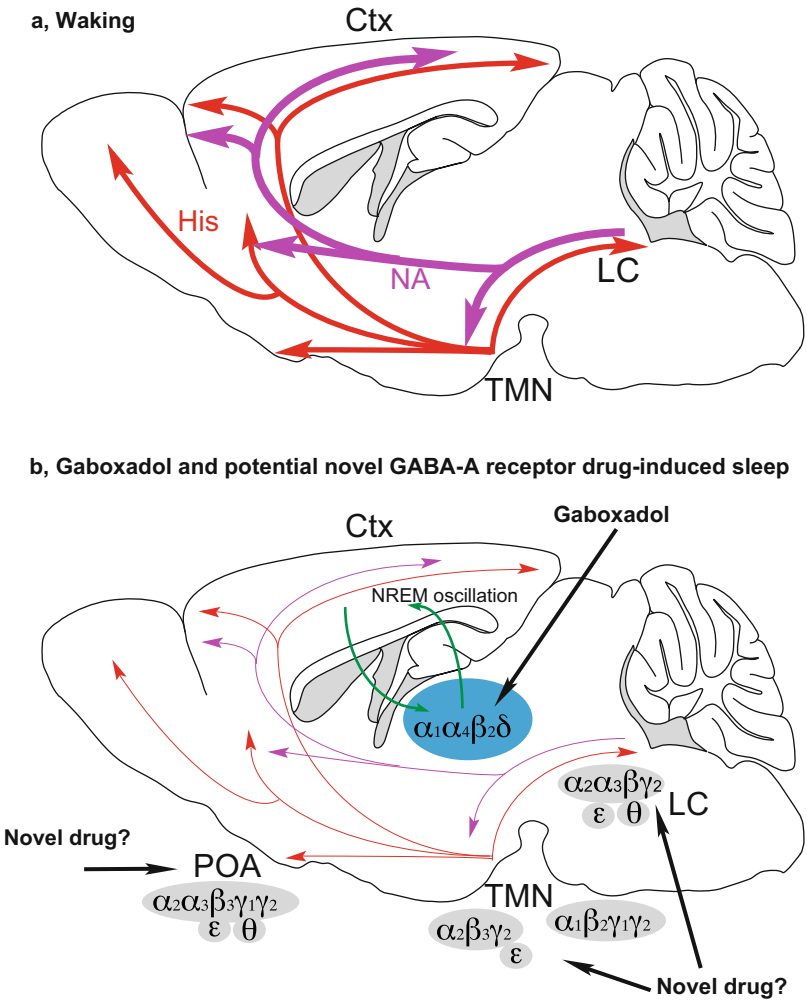


Fig. 4 Possible novel $GABA_A$ receptor targets in the hypothalamus and ascending arousal neurons. (**a**) Wakefulness is sustained in part by ascending aminergic neurons such as the histaminergic neurons in the tuberomammillary nucleus (TMN) and the noradrenergic neurons in the locus coeruleus (LC). These release histamine (His) and noradrenaline (NA) widely throughout the neocortex (Ctx) and other regions. (**b**) Gaboxadol activates α1α4βδ receptors in the thalamus (T) to induce NREM sleep by promoting δ-type oscillations. Speculative novel targets for yet undiscovered sleep-promoting drugs that work via $GABA_A$ receptors could be the ascending arousal neurons and neurons on the preoptic area (POA) of the hypothalamus. These areas express various $GABA_A$ receptor subunit gens such as the γ1, ε and θ subunit genes. The predominant α subunit genes expressed in these areas tend to be the α2 and α3 subunit genes. The receptor subunit combinations in these areas, apart from the obvious one of α1β3β2 and α2β3γ2 in histamine neurons, have not been clearly elucidated. Some drugs could work to supress wakefulness by dampening down the histamine and noradrenaline systems. Other drugs might interfere with wake-promoting GABAergic projections (not shown) in the POA area

interneuron types, especially the parvalbumin types (Ferando and Mody 2015; Milenkovic et al. 2013).

Gaboxadol (or THIP) is a direct GABA mimetic ligand at δ-containing receptors. Mice with genetically deleted δ subunits are unresponsive to muscimol and THIP (Winsky-Sommerer et al. 2007). The δ-containing receptors are insensitive to benzodiazepines, but their GABA-activated currents are potentiated by steroids and propofol, and this depends on the level of extrasynaptic GABA (Houston et al. 2012). It was suggested that GABA acting at δ-containing $GABA_A$ receptors at thalamic extrasynaptic $GABA_A$ receptors on thalamic relay cells might induce slow wave activity – the delta oscillations of NREM sleep (Wafford and Ebert 2006) (Fig. 4), although δ receptors are certainly dispensable for this, as global δ knockout mice have normal sleep-wake cycles and vigilance states (Winsky-Sommerer et al. 2007). But THIP does increase the tonic conductance on mouse thalamic relay neurons, and this could in theory promote delta NREM-like oscillations (Belelli et al. 2005), although clearly there are other δ-containing $GABA_A$ receptors in the mouse forebrain (perhaps on GABA interneurons) where THIP acts to counteract sleep and instead promotes agitation and actually delays sleep.

In mice, THIP definitely would not be regarded as a sleep-promoting drug (Alexandre et al. 2008; Winsky-Sommerer et al. 2007) – in fact, quite the opposite. A similar acting drug, muscimol, in mice promoted catalepsy and delayed sleep onset (Vyazovskiy et al. 2007). But there are big species differences in the way this drug works. Studies on rats suggested THIP would be a good drug to promote sleep (Lancel and Langebartels 2000). In rats, THIP increased the amount and depth of NREM sleep (Lancel and Langebartels 2000). Based on the results in rats, THIP/gaboxadol went into human clinical trials to test if the drug promoted sleep (the drug had already been used many times before in human trials for treating tardive dyskinesia and cancer pain, and many patients had previously reported sleep as a side effect). It was generally well tolerated and seemed to promote sleep as well as zolpidem (Fig. 2). In contrast to zolpidem, gaboxadol enhances delta power in NREM sleep in humans (Dijk et al. 2010; Lundahl et al. 2007, 2012). Also in contrast to zolpidem, gaboxadol caused no rebound insomnia on drug withdrawal (Hajak et al. 2009). But in 2007 gaboxadol failed in Phase III for sleep studies, although it is not quite clear why. Studies with the drug's efficacy in sleep on primary insomnia outpatients gave conflicting results. Some groups found at 15 mg/kg it promotes sleep, decreasing latency and increased the feeling of wakefulness after sleep in studies conducted on patients using sleep diaries ($N = 742$) (Hajak et al. 2009). As the size of the samples were scaled up, other studies on humans found that gaboxadol (THIP) at 10 mg/kg had no effect on sleep (Roth et al. 2010). At 15 mg/kg, it failed to reduce sleep latency consistently in clinical trials with, for example, one patient group reporting sleep-promoting effects, another not (Roth et al. 2010). Some inconsistent effects were reported for the prolongation of sleep duration, with the drug having effects in females but not males. Some people complained of nausea and dizziness and some severe adverse effects as the dose was increased (Lundahl et al. 2007). This could be because another target of THIP/

gaboxadol is the α6βδ receptor, which is expressed on cerebellar granule cells where it generates a tonic inhibitory GABA-activated current (Brickley et al. 2001; Luddens et al. 1990; Shivers et al. 1989; Wisden et al. 1992). Thus, on cerebellar granule cells, THIP is likely to stimulate the tonic conductance mediated by extrasynaptic GABA and acutely modulating gain control of motor systems and the vestibular reflex – hence dizziness. An improved sleep-promoting drug for humans might need to target only α4βδ-type receptors – these are not expressed in the cerebellum. Gaboxadol is not sold in the USA and has no FDA approval. It is now unlikely to ever appear as a sleep-promoting drug commercially. A PAM, DS-2, that works selectively at δ-containing $GABA_A$ receptors (Wafford et al. 2009) does not seem to have been tested for its effects on sleep promotion. Strangely, THIP/gaboxadol still seems like it would be an excellent sleep-promoting drug for some. Hamilton Morris' compelling account in Harper's Magazine of private gaboxadol ingestion (which took place in 2013 after clinical trials had ceased) is recommended reading (Morris 2013) .

10 $GABA_B$ Receptors and Sleep Promotion

$GABA_B$ receptors are dimeric G protein-coupled receptors that produce slow metabotropic inhibition and are essential to prevent over excitation and the emergence of seizures (Gassmann and Bettler 2012; Pin and Bettler 2016). Functionally, the slow $GABA_B$-mediated inhibition is quite similar to the tonic conductance inhibition arising from δ-type $GABA_A$ receptors. $GABA_B$ receptors are located extrasynaptically, often on presynaptic terminals of, e.g. glutamate or GABA neurons. Activating $GABA_B$ receptors opens K^+ channels or closes voltage-gated Ca^{2+} channels, reducing transmitter release from terminals. At the cellular level, activation of $GABA_B$ receptors is inhibitory, but at the network level, activating these receptors can produce either net excitatory or inhibitory effects. $GABA_B$ receptors acting presynaptically to decrease GABA release could be excitatory or inhibitory at the network level, depending on whether the GABAergic neuron is inhibiting another GABAergic terminal or a glutamatergic excitatory terminal.

Compared with $GABA_A$ receptors, there is no substantial receptor diversity for $GABA_B$ receptors. All $GABA_B$ receptors are made from two subunits, $GABA_{B1}$ and $GABA_{B2}$. There are two subtypes of $GABA_B$ receptor because of two splice versions of the $GABA_{B1}$ gene, $GABA_{B1a}$ and $GABA_{B1b}$, that pair with $GABA_{B2}$. Modulator proteins or auxiliary subunits (e.g. potassium channel tetramerization (KCTD) proteins KCTD8, KCDT12, KCTD12b and KCTD16), which are expressed cell type selectively, may increase the functional diversity of $GABA_B$ receptors and allow more flexibility for developing drugs (Pin and Bettler 2016). Given that the two $GABA_B$ receptor subunits are widely expressed in the brain forming receptors that contribute to every aspect of brain function, it seems unlikely that conventional specific $GABA_B$ receptor agonists will selectively interfere with sleep and not have profound side effects. Indeed, $GABA_B$ receptor agonists cannot be used for treating primary insomnia.

Drugs active at $GABA_B$ receptors do promote sleep/sedation, but some of the sedation could result from off-target effects. The best established $GABA_B$ agonist is baclofen. But in mice with $GABA_B$ receptors deleted, baclofen still promoted a delayed NREM sleep (Vienne et al. 2010). In mice and rats, the drug GHB (γ-hydroxybutyric acid, the sodium salt is called sodium oxybate) promotes sedation: hypo-locomotion, NREM sleep with increased delta power in the EEG and decreased body temperature (Kaupmann et al. 2003; Vienne et al. 2012; Wisor et al. 2006) – the drug also increases delta power during wakefulness (Vienne et al. 2012). The drug needs to be used at a high dose to get these effects (50–150 mg/kg) (Vienne et al. 2010) or 300 mg/kg (Wisor et al. 2006). In mice with deleted $GABA_B$ receptors, GHB no longer induces sedation but still binds with high affinity to neuronal membranes (Kaupmann et al. 2003). Thus, GHB causes sedation by activating $GABA_B$ receptors. GHB has a low affinity at these receptors – typically 3 mM GHB is needed to activate endogenous $GABA_B$ receptor responses on neurons (Connelly et al. 2013). The function and identity of the high-affinity GHB binding site remain a mystery – GHB does not activate αβδ $GABA_A$ receptors, so these are not the target (Connelly et al. 2013). In spite of its unclean pharmacology, GHB can help reduce the symptoms of patients suffering from narcolepsy with cataplexy (Black et al. 2014). In mice, baclofen similarly reduces the symptoms of narcolepsy with cataplexy, in part by promoting high delta power NREM sleep (Black et al. 2014); again, it is unclear if this is due to $GABA_B$ receptor activation or due to an off-target effect.

$GABA_B$ receptor inhibition is embedded throughout the sleep-wake circuitry. Mice with genetically deleted $GABA_B$ receptors (global knockouts) are unhealthy and lose weight and have seizures and disrupted sleep, with sleep being fragmented and with reduced power across all EEG frequencies (Vienne et al. 2010). If we try to look more selectively, mice with $GABA_B$ receptors selectively removed from wake-promoting orexin neurons in the mouse lateral hypothalamus have a strongly fragmented sleep-wake pattern, with many more transitions between wake and NREM sleep, and upregulation of $GABA_A$ receptors on these neurons (Matsuki et al. 2009). The overall amount of sleep and wake in these mice was unaffected by the $GABA_B$ receptor deletion – the increased fragmentation was due to more transitions between all vigilance states but no sign of cataplexy – there were no abnormal transitions from wake to REM sleep, for example (Matsuki et al. 2009). But in contrast, selectively deleting $GABA_B$ receptors from histaminergic neurons does not alter any parameter of the sleep-wake cycle, so $GABA_B$ receptor modulation of the histamine system is dispensable (Zecharia et al. 2012).

11 $GABA_A$ Receptors Expressed on Nodal Points of the Sleep-Wake Circuitry as Drug Targets for Sleep?

Some $GABA_A$ receptors are highly expressed in nodal points of circuitries that control vigilance states. So, activating $GABA_A$ receptors on these nodes will influence activity in many other parts of the brain. Injecting barbiturates, muscimol

($GABA_A$ receptor agonist) or propofol into a small area of the rat brain stem, the mesopontine tegmental area, induces anaesthesia, presumably because some key hub neurons (whose identity is unknown) that have far-reaching projections have been inhibited (Abulafia et al. 2009; Minert and Devor 2016). Similarly, injecting GABA agonist drugs (muscimol) into the posterior hypothalamus of awake cats produces NREM sleep, presumably because of the inhibition of wake-promoting histamine neurons, as these are hub neurons that promote wakefulness and influence many brain areas simultaneously (Lin et al. 1989; Nelson et al. 2002; Uygun et al. 2016) (Fig. 1).

We saw previously that based on global α2H101R mice, zolpidem is most likely acting to promote sleep through α2βγ2 and/or α3βγ2 $GABA_A$ receptors (Kopp et al. 2004b). The α2 subunits are expressed in hypothalamic areas (Wisden et al. 1992), such as on histaminergic neurons (Fritschy and Mohler 1995; Sergeeva et al. 2005). By using a genetic γ2 swap method, whereby zolpidem-sensitive γ2F77 subunits are swapped cell type selectively into a γ2F77I mouse, which is insensitive to zolpidem (Cope et al. 2004), selectively augmenting the active GABA input onto hypothalamic histamine neurons by systemic zolpidem administration decreased NREM sleep latency and enhanced sleep time but notably without reducing the power of the oscillations in the 5–16 HZ range of the EEG, so more resembling natural NREM sleep (Uygun et al. 2016) (Fig. 3). This suggests that zolpidem could in part induce sleep by enhancing GABA's actions on histamine neurons.

Some of the most obscure (or, at least, functionally less well studied) and therefore potentially interesting $GABA_A$ receptor subunits are significantly expressed in areas of the brain that regulate homeostatic functions such as sleep. In particular, the γ1, ε and θ subunits have enriched expression in preoptic-hypothalamic and some brain stem areas (May et al. 2013; Moragues et al. 2002; Sergeeva et al. 2005; Sinkkonen et al. 2000; Wisden et al. 1992) (Fig. 4). A significant feature is that these hypothalamic and brainstem areas frequently use α2 and α3 subunit expression. Triazolam is effective as a PAM at α1βγ1 receptors, as to some extent is zolpidem (Khom et al. 2006). This work could be developed further to look for, e.g. α2βγ1-selective ligands. Similarly, a drug selectively working at $GABA_A$ receptors containing α3, ε and θ subunits would be interesting to investigate for its sleep-promoting properties. Expressing recombinant ε and θ subunits with the α3 and β1 subunit reduces sensitivity to many PAMs (propofol, etomidate, pregnenolone and flurazepam) but increases GABA and gaboxadol sensitivity 100-fold (Ranna et al. 2006). The θ-containing receptors could still be potentiated by etomidate (Ranna et al. 2006). It is possible that the ε subunit could function as a β-like subunit (Jones and Henderson 2007). Compared with the αβγ2 and α1/4βδ class of $GABA_A$ receptors, not enough work has been done on the ε and θ subunits to give us a full understanding of the $GABA_A$ receptors to which they contribute.

In rodents, $GABA_A$ receptors assembled from α2, α3, ε and θ subunits could be targets for drugs that promote sleep or anxiolysis (Belujon et al. 2009) (Fig. 4). Similar to the histaminergic and other ascending aminergic groups, noradrenergic neurons in the locus coeruleus (LC) project widely throughout the brain and promote

arousal and enhanced cognitive function. Adrenergic LC neurons fire selectively during wakefulness, and thus inhibiting their activity will enhance anxiolysis and sleep. In particular, although lesions of the LC do not influence the amounts of wakefulness, rats with LC lesions do go to sleep more quickly in a novel environment (Gompf et al. 2010). A similar anxiolytic effect was found with selective deletions of γ2-containing $GABA_A$ receptors from histaminergic neurons – no change in the overall sleep-wake cycle was produced, but the mice went to sleep more quickly in a novel environment (Zecharia et al. 2012). Thus, a (novel) drug that reduces LC or histamine function by enhancing $GABA_A$ receptors on these neurons would be anxiolytic and sleep promoting (Fig. 4). The α2 and α3 subunits are found in separate clusters on the cell bodies and dendrites of rat noradrenergic LC neurons (Corteen et al. 2011). These neurons also express the unusual ε and θ subunits (Belujon et al. 2009; Sinkkonen et al. 2000), and histamine neurons also express the ε gene (Sergeeva et al. 2005). So, receptors assembled from either α2, ε and θ or α3, ε and θ might be interesting as potential targets for sleep- or anxiolytic-promoting drugs (Fig. 4). A caveat is that in humans, but not rodents, noradrenergic neurons express the γ2 subunit (Hellsten et al. 2010). This would mean that human LC neurons would be targets for PAMs that selectively modulate α2βγ2- and α3βγ2-type receptors and these α2- or α3-preferring drugs would probably be sedative too (Hellsten et al. 2010). It is unclear what the functional contribution of ε and θ subunit contributions would be if the γ2 subunit is also present on LC neurons – presumably a complex mix of receptors is present. The native subunit compositions of ε- and θ-containing receptors in human LC neurons and human hypothalamic neurons would need to be established – e.g. α2β3θε or α2θε or α2β3γ2 combinations. Again, given the differences in the way different species respond to some drugs (see in particular the section above on gaboxadol – Sect. 9), we would need to be cautious in using mice as a system to work up new sleep-promoting drugs.

12 Perspectives

The most "natural" way to induce sleep is sleep deprivation, even if this is not a practical therapy and has frequently been used as a form of torture. The drive to sleep increases in proportion to the amount of time spent awake, until the drive becomes so overwhelming that sleep is unpreventable. Nevertheless, the type of sleep that emerges through this route of sleep deprivation is physiological and is termed recovery sleep. This recovery sleep represents the process of sleep homeostasis: catching up on lost sleep with a longer and deeper sleep (as defined by enhanced EEG δ power for the first stage of NREM sleep) (Landolt et al. 2000). The circuitry controlling sleep homeostasis is not understood in detail, although it does require the preoptic hypothalamus (Zhang et al. 2015), but the mechanisms underlying sleep homeostasis could be one future route to inducing a natural sleep artificially. Current GABAergic medications, such as zolpidem, all discovered without any appreciation of how sleep-promoting circuitry works, do not induce a natural type of recovery sleep and thus are likely to use different mechanisms from

natural sleep (Landolt et al. 2000; Wisor et al. 2006). It seems we were on a new threshold for GABAergic sleep medication with gaboxadol, which actually enhances NREM-type δ power in humans, but the drug failed in clinical trials. On the other hand, the α2 adrenergic agonist dexmedetomidine seems to use the same circuitry in the lateral preoptic hypothalamus to that activated in recovery sleep, albeit with marked hypothermia and changes in blood pressure, and so compounds based on this drug mechanism, targeting the recovery sleep aspect, might be further developed to promote sedation (Zhang et al. 2015). Not enough is known about sleep circuitry to understand if more precise medications will be possible. In the meantime, hypnotic drugs such as zolpidem will continue to be widely used.

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References

Abulafia R, Zalkind V, Devor M (2009) Cerebral activity during the anesthesia-like state induced by mesopontine microinjection of pentobarbital. J Neurosci 29:7053–7064

Alexandre C, Dordal A, Aixendri R, Guzman A, Hamon M, Adrien J (2008) Sleep-stabilizing effects of E-6199, compared to zopiclone, zolpidem and THIP in mice. Sleep 31:259–270

Behlke LM, Foster RA, Liu J, Benke D, Benham RS, Nathanson AJ, Yee BK, Zeilhofer HU, Engin E, Rudolph U (2016) A pharmacogenetic 'restriction-of-function' approach reveals evidence for anxiolytic-like actions mediated by alpha5-containing GABAA receptors in mice. Neuropsychopharmacology 41:2492–2501

Belelli D, Peden DR, Rosahl TW, Wafford KA, Lambert JJ (2005) Extrasynaptic GABAA receptors of thalamocortical neurons: a molecular target for hypnotics. J Neurosci 25:11513–11520

Belujon P, Baufreton J, Grandoso L, Boue-Grabot E, Batten TF, Ugedo L, Garret M, Taupignon AI (2009) Inhibitory transmission in locus coeruleus neurons expressing GABAA receptor epsilon subunit has a number of unique properties. J Neurophysiol 102:2312–2325

Bertisch SM, Herzig SJ, Winkelman JW, Buettner C (2014) National use of prescription medications for insomnia: NHANES 1999–2010. Sleep 37:343–349

Black SW, Morairty SR, Chen TM, Leung AK, Wisor JP, Yamanaka A, Kilduff TS (2014) GABAB agonism promotes sleep and reduces cataplexy in murine narcolepsy. J Neurosci 34:6485–6494

Booth JN 3rd, Behring M, Cantor RS, Colantonio LD, Davidson S, Donnelly JP, Johnson E, Jordan K, Singleton C, Xie F, McGwin G Jr (2016) Zolpidem use and motor vehicle collisions in older drivers. Sleep Med 20:98–102

Brickley SG, Mody I (2012) Extrasynaptic GABA(A) receptors: their function in the CNS and implications for disease. Neuron 73:23–34

Brickley SG, Revilla V, Cull-Candy SG, Wisden W, Farrant M (2001) Adaptive regulation of neuronal excitability by a voltage-independent potassium conductance. Nature 409:88–92

Chatelle C, Thibaut A, Gosseries O, Bruno MA, Demertzi A, Bernard C, Hustinx R, Tshibanda L, Bahri MA, Laureys S (2014) Changes in cerebral metabolism in patients with a minimally conscious state responding to zolpidem. Front Hum Neurosci 8:917

Chung S, Weber F, Zhong P, Tan CL, Nguyen TN, Beier KT, Hormann N, Chang WC, Zhang Z, Do JP, Yao S, Krashes MJ, Tasic B, Cetin A, Zeng H, Knight ZA, Luo L, Dan Y (2017)

Identification of preoptic sleep neurons using retrograde labelling and gene profiling. Nature 545:477–481
Coenen AM, van Luijtelaar EL (1991) Pharmacological dissociation of EEG and behavior: a basic problem in sleep-wake classification. Sleep 14:464–465
Connelly WM, Errington AC, Crunelli V (2013) Gamma-Hydroxybutyric acid (GHB) is not an agonist of extrasynaptic GABAA receptors. PLoS One 8:e79062
Cope DW, Wulff P, Oberto A, Aller MI, Capogna M, Ferraguti F, Halbsguth C, Hoeger H, Jolin HE, Jones A, McKenzie AN, Ogris W, Poeltl A, Sinkkonen ST, Vekovischeva OY, Korpi ER, Sieghart W, Sigel E, Somogyi P, Wisden W (2004) Abolition of zolpidem sensitivity in mice with a point mutation in the GABAA receptor gamma2 subunit. Neuropharmacology 47:17–34
Corteen NL, Cole TM, Sarna A, Sieghart W, Swinny JD (2011) Localization of GABA-A receptor alpha subunits on neurochemically distinct cell types in the rat locus coeruleus. Eur J Neurosci 34:250–262
Crestani F, Martin JR, Mohler H, Rudolph U (2000) Mechanism of action of the hypnotic zolpidem in vivo. Br J Pharmacol 131:1251–1254
Dijk DJ, James LM, Peters S, Walsh JK, Deacon S (2010) Sex differences and the effect of gaboxadol and zolpidem on EEG power spectra in NREM and REM sleep. J Psychopharmacol 24:1613–1618
Ferando I, Mody I (2015) In vitro gamma oscillations following partial and complete ablation of delta subunit-containing GABAA receptors from parvalbumin interneurons. Neuropharmacology 88:91–98
Franks NP (2008) General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nat Rev Neurosci 9:370–386
Franks NP (2015) Structural comparisons of ligand-gated ion channels in open, closed, and desensitized states identify a novel propofol-binding site on mammalian gamma-aminobutyric acid type A receptors. Anesthesiology 122:787–794
Franks NP, Lieb WR (1994) Molecular and cellular mechanisms of general anaesthesia. Nature 367:607–614
Frey DJ, Ortega JD, Wiseman C, Farley CT, Wright KP Jr (2011) Influence of zolpidem and sleep inertia on balance and cognition during nighttime awakening: a randomized placebo-controlled trial. J Am Geriatr Soc 59:73–81
Fritschy JM, Mohler H (1995) GABAA-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. J Comp Neurol 359:154–194
Garcia PS, Kolesky SE, Jenkins A (2010) General anesthetic actions on GABA(A) receptors. Curr Neuropharmacol 8:2–9
Gassmann M, Bettler B (2012) Regulation of neuronal GABA(B) receptor functions by subunit composition. Nat Rev Neurosci 13:380–394
Gompf HS, Mathai C, Fuller PM, Wood DA, Pedersen NP, Saper CB, Lu J (2010) Locus ceruleus and anterior cingulate cortex sustain wakefulness in a novel environment. J Neurosci 30:14543–14551
Gottesmann C, Gandolfo G, Arnaud C, Gauthier P (1998) The intermediate stage and paradoxical sleep in the rat: influence of three generations of hypnotics. Eur J Neurosci 10:409–414
Greenblatt DJ, Roth T (2012) Zolpidem for insomnia. Expert Opin Pharmacother 13:879–893
Gunn BG, Cunningham L, Mitchell SG, Swinny JD, Lambert JJ, Belelli D (2015) GABAA receptor-acting neurosteroids: a role in the development and regulation of the stress response. Front Neuroendocrinol 36:28–48
Hajak G, Hedner J, Eglin M, Loft H, Storustovu SI, Lutolf S, Lundahl J, Gaboxadol Study 99775 Group (2009) A 2-week efficacy and safety study of gaboxadol and zolpidem using electronic diaries in primary insomnia outpatients. Sleep Med 10:705–712
Harrison NL, Simmonds MA (1984) Modulation of the GABA receptor complex by a steroid anaesthetic. Brain Res 323:287–292

Hellsten KS, Sinkkonen ST, Hyde TM, Kleinman JE, Sarkioja T, Maksimow A, Uusi-Oukari M, Korpi ER (2010) Human locus coeruleus neurons express the GABA(A) receptor gamma2 subunit gene and produce benzodiazepine binding. Neurosci Lett 477:77–81

Herb A, Wisden W, Luddens H, Puia G, Vicini S, Seeburg PH (1992) The third gamma subunit of the gamma-aminobutyric acid type A receptor family. Proc Natl Acad Sci U S A 89:1433–1437

Herd MB, Belelli D, Lambert JJ (2007) Neurosteroid modulation of synaptic and extrasynaptic GABA(A) receptors. Pharmacol Ther 116:20–34

Herrera CG, Cadavieco MC, Jego S, Ponomarenko A, Korotkova T, Adamantidis A (2016) Hypothalamic feedforward inhibition of thalamocortical network controls arousal and consciousness. Nat Neurosci 19:290–298

Hogenkamp DJ, Tran MB, Yoshimura RF, Johnstone TB, Kanner R, Gee KW (2014) Pharmacological profile of a 17beta-heteroaryl-substituted neuroactive steroid. Psychopharmacology 231:3517–3524

Houston CM, McGee TP, Mackenzie G, Troyano-Cuturi K, Rodriguez PM, Kutsarova E, Diamanti E, Hosie AM, Franks NP, Brickley SG (2012) Are extrasynaptic GABAA receptors important targets for sedative/hypnotic drugs? J Neurosci 32:3887–3897

Hsu N, Jha SK, Coleman T, Frank MG (2009) Paradoxical effects of the hypnotic Zolpidem in the neonatal ferret. Behav Brain Res 201:233–236

Jones BL, Henderson LP (2007) Trafficking and potential assembly patterns of epsilon-containing GABAA receptors. J Neurochem 103:1258–1271

Karalis N, Dejean C, Chaudun F, Khoder S, Rozeske RR, Wurtz H, Bagur S, Benchenane K, Sirota A, Courtin J, Herry C (2016) 4-Hz oscillations synchronize prefrontal-amygdala circuits during fear behavior. Nat Neurosci 19:605–612

Kaupmann K, Cryan JF, Wellendorph P, Mombereau C, Sansig G, Klebs K, Schmutz M, Froestl W, van der Putten H, Mosbacher J, Brauner-Osborne H, Waldmeier P, Bettler B (2003) Specific gamma-hydroxybutyrate-binding sites but loss of pharmacological effects of gamma-hydroxybutyrate in GABA(B)(1)-deficient mice. Eur J Neurosci 18:2722–2730

Khom S, Baburin I, Timin EN, Hohaus A, Sieghart W, Hering S (2006) Pharmacological properties of GABAA receptors containing gamma1 subunits. Mol Pharmacol 69:640–649

Kopp C, Rudolph U, Keist R, Tobler I (2003) Diazepam-induced changes on sleep and the EEG spectrum in mice: role of the alpha3-GABA(A) receptor subtype. Eur J Neurosci 17:2226–2230

Kopp C, Rudolph U, Low K, Tobler I (2004a) Modulation of rhythmic brain activity by diazepam: GABA(A) receptor subtype and state specificity. Proc Natl Acad Sci U S A 101:3674–3679

Kopp C, Rudolph U, Tobler I (2004b) Sleep EEG changes after zolpidem in mice. Neuroreport 15:2299–2302

Lan NC, Chen JS, Belelli D, Pritchett DB, Seeburg PH, Gee KW (1990) A steroid recognition site is functionally coupled to an expressed GABA(A)-benzodiazepine receptor. Eur J Pharmacol 188:403–406

Lancel M, Langebartels A (2000) Gamma-aminobutyric Acid(A) (GABA(A)) agonist 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol persistently increases sleep maintenance and intensity during chronic administration to rats. J Pharmacol Exp Ther 293:1084–1090

Lancel M, Steiger A (1999) Sleep and its modulation by drugs that affect GABA(A) receptor function. Angew Chem Int Ed Engl 38:2852–2864

Lancel M, Faulhaber J, Holsboer F, Rupprecht R (1999) The GABA(A) receptor antagonist picrotoxin attenuates most sleep changes induced by progesterone. Psychopharmacology 141:213–219

Landolt HP, Finelli LA, Roth C, Buck A, Achermann P, Borbely AA (2000) Zolpidem and sleep deprivation: different effect on EEG power spectra. J Sleep Res 9:175–183

Levitan ES, Schofield PR, Burt DR, Rhee LM, Wisden W, Kohler M, Fujita N, Rodriguez HF, Stephenson A, Darlison MG et al (1988) Structural and functional basis for GABAA receptor heterogeneity. Nature 335:76–79

Lin JS, Sakai K, Vanni-Mercier G, Jouvet M (1989) A critical role of the posterior hypothalamus in the mechanisms of wakefulness determined by microinjection of muscimol in freely moving cats. Brain Res 479:225–240

Low K, Crestani F, Keist R, Benke D, Brunig I, Benson JA, Fritschy JM, Rulicke T, Bluethmann H, Mohler H, Rudolph U (2000) Molecular and neuronal substrate for the selective attenuation of anxiety. Science 290:131–134

Luddens H, Pritchett DB, Kohler M, Killisch I, Keinanen K, Monyer H, Sprengel R, Seeburg PH (1990) Cerebellar GABAA receptor selective for a behavioural alcohol antagonist. Nature 346:648–651

Lundahl J, Staner L, Staner C, Loft H, Deacon S (2007) Short-term treatment with gaboxadol improves sleep maintenance and enhances slow wave sleep in adult patients with primary insomnia. Psychopharmacology 195:139–146

Lundahl J, Deacon S, Maurice D, Staner L (2012) EEG spectral power density profiles during NREM sleep for gaboxadol and zolpidem in patients with primary insomnia. J Psychopharmacol 26:1081–1087

Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM (1986) Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232:1004–1007

Matsuki T, Nomiyama M, Takahira H, Hirashima N, Kunita S, Takahashi S, Yagami K, Kilduff TS, Bettler B, Yanagisawa M, Sakurai T (2009) Selective loss of GABA(B) receptors in orexin-producing neurons results in disrupted sleep/wakefulness architecture. Proc Natl Acad Sci U S A 106:4459–4464

May AC, Fleischer W, Kletke O, Haas HL, Sergeeva OA (2013) Benzodiazepine-site pharmacology on GABAA receptors in histaminergic neurons. Br J Pharmacol 170:222–232

McKernan RM, Rosahl TW, Reynolds DS, Sur C, Wafford KA, Atack JR, Farrar S, Myers J, Cook G, Ferris P, Garrett L, Bristow L, Marshall G, Macaulay A, Brown N, Howell O, Moore KW, Carling RW, Street LJ, Castro JL, Ragan CI, Dawson GR, Whiting PJ (2000) Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor alpha1 subtype. Nat Neurosci 3:587–592

Mignot E (2013) Physiology. The perfect hypnotic? Science 340:36–38

Milenkovic I, Vasiljevic M, Maurer D, Hoger H, Klausberger T, Sieghart W (2013) The parvalbumin-positive interneurons in the mouse dentate gyrus express GABAA receptor subunits alpha1, beta2, and delta along their extrasynaptic cell membrane. Neuroscience 254:80–96

Miller PS, Aricescu AR (2014) Crystal structure of a human GABAA receptor. Nature 512:270–275

Minert A, Devor M (2016) Brainstem node for loss of consciousness due to GABA(A) receptor-active anesthetics. Exp Neurol 275(Pt 1):38–45

Mohler H (2015) The legacy of the benzodiazepine receptor: from flumazenil to enhancing cognition in Down syndrome and social interaction in autism. Adv Pharmacol 72:1–36

Moragues N, Ciofi P, Tramu G, Garret M (2002) Localisation of GABA(A) receptor epsilon-subunit in cholinergic and aminergic neurones and evidence for co-distribution with the theta-subunit in rat brain. Neuroscience 111:657–669

Morris H (2013) Gaboxadol. Harper's Magazine, New York. August issue: https://harpers.org/archive/2013/08/gaboxadol/3/

Nelson LE, Guo TZ, Lu J, Saper CB, Franks NP, Maze M (2002) The sedative component of anesthesia is mediated by GABA(A) receptors in an endogenous sleep pathway. Nat Neurosci 5:979–984

Nitz D, Siegel JM (1996) GABA release in posterior hypothalamus across sleep-wake cycle. Am J Phys 271:R1707–R1712

Nutt DJ, Stahl SM (2010) Searching for perfect sleep: the continuing evolution of GABAA receptor modulators as hypnotics. J Psychopharmacol 24:1601–1612

Ogris W, Poltl A, Hauer B, Ernst M, Oberto A, Wulff P, Hoger H, Wisden W, Sieghart W (2004) Affinity of various benzodiazepine site ligands in mice with a point mutation in the GABA (A) receptor gamma2 subunit. Biochem Pharmacol 68:1621–1629

Olsen RW (2015) Allosteric ligands and their binding sites define gamma-aminobutyric acid (GABA) type A receptor subtypes. Adv Pharmacol 73:167–202

Pin JP, Bettler B (2016) Organization and functions of mGlu and GABAB receptor complexes. Nature 540:60–68

Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G (2000) GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience 101:815–850

Pritchett DB, Seeburg PH (1990) Gamma-aminobutyric acidA receptor alpha 5-subunit creates novel type II benzodiazepine receptor pharmacology. J Neurochem 54:1802–1804

Pritchett DB, Sontheimer H, Shivers BD, Ymer S, Kettenmann H, Schofield PR, Seeburg PH (1989) Importance of a novel GABAA receptor subunit for benzodiazepine pharmacology. Nature 338:582–585

Puthenkalam R, Hieckel M, Simeone X, Suwattanasophon C, Feldbauer RV, Ecker GF, Ernst M (2016) Structural studies of GABAA receptor binding sites: which experimental structure tells us what? Front Mol Neurosci 9:44

Qiu MH, Chen MC, Lu J (2015) Cortical neuronal activity does not regulate sleep homeostasis. Neuroscience 297:211–218

Ralvenius WT, Benke D, Acuna MA, Rudolph U, Zeilhofer HU (2015) Analgesia and unwanted benzodiazepine effects in point-mutated mice expressing only one benzodiazepine-sensitive GABAA receptor subtype. Nat Commun 6:6803

Randall LO (1961) Pharmacology of chlordiazepoxide (Librium). Dis Nerv Syst 22(Suppl 7):7–15

Ranna M, Sinkkonen ST, Moykkynen T, Uusi-Oukari M, Korpi ER (2006) Impact of epsilon and theta subunits on pharmacological properties of alpha3beta1 GABAA receptors expressed in Xenopus oocytes. BMC Pharmacol 6:1

Reynolds DS, Rosahl TW, Cirone J, O'Meara GF, Haythornthwaite A, Newman RJ, Myers J, Sur C, Howell O, Rutter AR, Atack J, Macaulay AJ, Hadingham KL, Hutson PH, Belelli D, Lambert JJ, Dawson GR, McKernan R, Whiting PJ, Wafford KA (2003) Sedation and anesthesia mediated by distinct GABA(A) receptor isoforms. J Neurosci 23:8608–8617

Roth T, Lines C, Vandormael K, Ceesay P, Anderson D, Snavely D (2010) Effect of gaboxadol on patient-reported measures of sleep and waking function in patients with Primary Insomnia: results from two randomized, controlled, 3-month studies. J Clin Sleep Med 6:30–39

Rudolph U, Crestani F, Benke D, Brunig I, Benson JA, Fritschy JM, Martin JR, Bluethmann H, Mohler H (1999) Benzodiazepine actions mediated by specific gamma-aminobutyric acid (A) receptor subtypes. Nature 401:796–800

Schofield PR, Darlison MG, Fujita N, Burt DR, Stephenson FA, Rodriguez H, Rhee LM, Ramachandran J, Reale V, Glencorse TA et al (1987) Sequence and functional expression of the GABA A receptor shows a ligand-gated receptor super-family. Nature 328:221–227

Schroeck JL, Ford J, Conway EL, Kurtzhalts KE, Gee ME, Vollmer KA, Mergenhagen KA (2016) Review of safety and efficacy of sleep medicines in older adults. Clin Ther 38:2340–2372

Seeburg PH, Wisden W, Verdoorn TA, Pritchett DB, Werner P, Herb A, Luddens H, Sprengel R, Sakmann B (1990) The GABAA receptor family: molecular and functional diversity. Cold Spring Harb Symp Quant Biol 55:29–40

Seibt J, Aton SJ, Jha SK, Coleman T, Dumoulin MC, Frank MG (2008) The non-benzodiazepine hypnotic zolpidem impairs sleep-dependent cortical plasticity. Sleep 31:1381–1391

Seifi M, Brown JF, Mills J, Bhandari P, Belelli D, Lambert JJ, Rudolph U, Swinny JD (2014) Molecular and functional diversity of GABA-A receptors in the enteric nervous system of the mouse colon. J Neurosci 34:10361–10378

Sergeeva OA, Andreeva N, Garret M, Scherer A, Haas HL (2005) Pharmacological properties of GABAA receptors in rat hypothalamic neurons expressing the epsilon-subunit. J Neurosci 25:88–95

Sherin JE, Elmquist JK, Torrealba F, Saper CB (1998) Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. J Neurosci 18:4705–4721

Shivers BD, Killisch I, Sprengel R, Sontheimer H, Kohler M, Schofield PR, Seeburg PH (1989) Two novel GABAA receptor subunits exist in distinct neuronal subpopulations. Neuron 3:327–337

Sieghart W (2015) Allosteric modulation of GABAA receptors via multiple drug-binding sites. Adv Pharmacol 72:53–96

Sigel E, Steinmann ME (2012) Structure, function, and modulation of GABA(A) receptors. J Biol Chem 287:40224–40231

Sinkkonen ST, Hanna MC, Kirkness EF, Korpi ER (2000) GABA(A) receptor epsilon and theta subunits display unusual structural variation between species and are enriched in the rat locus ceruleus. J Neurosci 20:3588–3595

Thonnard M, Gosseries O, Demertzi A, Lugo Z, Vanhaudenhuyse A, Bruno MA, Chatelle C, Thibaut A, Charland-Verville V, Habbal D, Schnakers C, Laureys S (2013) Effect of zolpidem in chronic disorders of consciousness: a prospective open-label study. Funct Neurol 28:259–264

Tobler I, Kopp C, Deboer T, Rudolph U (2001) Diazepam-induced changes in sleep: role of the alpha 1 GABA(A) receptor subtype. Proc Natl Acad Sci U S A 98:6464–6469

Toossi H, Del Cid-Pellitero E, Jones BE (2016) GABA receptors on orexin and melanin-concentrating hormone neurons are differentially homeostatically regulated following sleep deprivation. eNeuro 3. https://doi.org/10.1523/ENEURO.0077-16.2016

Uygun DS, Ye Z, Zecharia AY, Harding EC, Yu X, Yustos R, Vyssotski AL, Brickley SG, Franks NP, Wisden W (2016) Bottom-up versus top-down induction of sleep by zolpidem acting on histaminergic and neocortex neurons. J Neurosci 36:11171–11184

Venner A, Anaclet C, Broadhurst RY, Saper CB, Fuller PM (2016) A novel population of wake-promoting GABAergic neurons in the ventral lateral hypothalamus. Curr Biol 26:2137–2143

Vienne J, Bettler B, Franken P, Tafti M (2010) Differential effects of GABAB receptor subtypes, {gamma}-hydroxybutyric Acid, and Baclofen on EEG activity and sleep regulation. J Neurosci 30:14194–14204

Vienne J, Lecciso G, Constantinescu I, Schwartz S, Franken P, Heinzer R, Tafti M (2012) Differential effects of sodium oxybate and baclofen on EEG, sleep, neurobehavioral performance, and memory. Sleep 35:1071–1083

Vyazovskiy VV, Kopp C, Bosch G, Tobler I (2005) The GABAA receptor agonist THIP alters the EEG in waking and sleep of mice. Neuropharmacology 48:617–626

Vyazovskiy VV, Tobler I, Winsky-Sommerer R (2007) Alteration of behavior in mice by muscimol is associated with regional electroencephalogram synchronization. Neuroscience 147:833–841

Wafford KA, Ebert B (2006) Gaboxadol – a new awakening in sleep. Curr Opin Pharmacol 6:30–36

Wafford KA, Ebert B (2008) Emerging anti-insomnia drugs: tackling sleeplessness and the quality of wake time. Nat Rev Drug Discov 7:530–540

Wafford KA, van Niel MB, Ma QP, Horridge E, Herd MB, Peden DR, Belelli D, Lambert JJ (2009) Novel compounds selectively enhance delta subunit containing GABA A receptors and increase tonic currents in thalamus. Neuropharmacology 56:182–189

Wieland HA, Luddens H, Seeburg PH (1992) A single histidine in GABAA receptors is essential for benzodiazepine agonist binding. J Biol Chem 267:1426–1429

Williams ST, Conte MM, Goldfine AM, Noirhomme Q, Gosseries O, Thonnard M, Beattie B, Hersh J, Katz DI, Victor JD, Laureys S, Schiff ND (2013) Common resting brain dynamics indicate a possible mechanism underlying zolpidem response in severe brain injury. elife 2: e01157

Winsky-Sommerer R (2009) Role of GABAA receptors in the physiology and pharmacology of sleep. Eur J Neurosci 29:1779–1794

Winsky-Sommerer R, Vyazovskiy VV, Homanics GE, Tobler I (2007) The EEG effects of THIP (Gaboxadol) on sleep and waking are mediated by the GABA(A)delta-subunit-containing receptors. Eur J Neurosci 25:1893–1899

Winsky-Sommerer R, Knapman A, Fedele DE, Schofield CM, Vyazovskiy VV, Rudolph U, Huguenard JR, Fritschy JM, Tobler I (2008) Normal sleep homeostasis and lack of epilepsy phenotype in GABA A receptor alpha3 subunit-knockout mice. Neuroscience 154:595–605

Wisden W, Morris BJ, Darlison MG, Hunt SP, Barnard EA (1988) Distinct GABAA receptor alpha subunit mRNAs show differential patterns of expression in bovine brain. Neuron 1:937–947

Wisden W, Herb A, Wieland H, Keinanen K, Luddens H, Seeburg PH (1991) Cloning, pharmacological characteristics and expression pattern of the rat GABAA receptor alpha 4 subunit. FEBS Lett 289:227–230

Wisden W, Laurie DJ, Monyer H, Seeburg PH (1992) The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. J Neurosci 12:1040–1062

Wisor JP, Morairty SR, Huynh NT, Steininger TL, Kilduff TS (2006) Gene expression in the rat cerebral cortex: comparison of recovery sleep and hypnotic-induced sleep. Neuroscience 141:371–378

Wulff P, Goetz T, Leppa E, Linden AM, Renzi M, Swinny JD, Vekovischeva OY, Sieghart W, Somogyi P, Korpi ER, Farrant M, Wisden W (2007) From synapse to behavior: rapid modulation of defined neuronal types with engineered GABAA receptors. Nat Neurosci 10:923–929

Yip GM, Chen ZW, Edge CJ, Smith EH, Dickinson R, Hohenester E, Townsend RR, Fuchs K, Sieghart W, Evers AS, Franks NP (2013) A propofol binding site on mammalian GABAA receptors identified by photolabeling. Nat Chem Biol 9:715–720

Zecharia AY, Yu X, Gotz T, Ye Z, Carr DR, Wulff P, Bettler B, Vyssotski AL, Brickley SG, Franks NP, Wisden W (2012) GABAergic inhibition of histaminergic neurons regulates active waking but not the sleep-wake switch or propofol-induced loss of consciousness. J Neurosci 32:13062–13075

Zhang Z, Ferretti V, Guntan I, Moro A, Steinberg EA, Ye Z, Zecharia AY, Yu X, Vyssotski AL, Brickley SG, Yustos R, Pillidge ZE, Harding EC, Wisden W, Franks NP (2015) Neuronal ensembles sufficient for recovery sleep and the sedative actions of alpha2 adrenergic agonists. Nat Neurosci 18:553–561

Advances of Melatonin-Based Therapies in the Treatment of Disturbed Sleep and Mood

Megan Alston, Sean W. Cain, and Shantha M. W. Rajaratnam

Contents

Abstract

Melatonin and melatonin agonists offer novel treatments for sleep and mood disorders, particularly where circadian misalignment is also present. The therapies offer both phase-shifting and sleep-promoting effects and have shown potential to treat advanced and delayed sleep-wake phase disorder, non-24-h sleep-wake cycle, jetlag, shift work disorder, insomnia, seasonal affective disorder and major depressive disorder.

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Agomelatine · Circadian rhythm · Circadin · Major depressive disorder · Melatonin · Melatonin agonist · Mood · Ramelteon · Sleep · Tasimelteon

1 Introduction

There is increasing interest in the potential of melatonin and melatonin agonists to treat sleep and mood disorders. Melatonin has phase-shifting (Arendt and Rajaratnam 2008) and sleep-promoting (Wyatt et al. 2006) effects and has shown potential in treating sleep disorders, particularly where circadian misalignment is also present (Arendt and Rajaratnam 2008). It has also shown promise for treating seasonal affective disorder (SAD) (Lewy et al. 2006), a mood disorder characterised by circadian misalignment. Recent evidence shows melatonin and melatonin agonists also offer potential to treat other sleep and mood disorders such as some types of insomnia and major depressive disorder. However, the mechanisms underlying these effects are unclear. We review the role of melatonin in the circadian regulation of sleep and mood and the phase-shifting and sleep-promoting properties of exogenous melatonin and melatonin agonists and outline how melatonin and melatonin agonists might be used for treatment of various sleep and mood disorders.

2 Endogenous Melatonin and the Sleep-Wake Cycle

First isolated by Lerner et al. (1958), melatonin (*N*-acetyl-5-methoxytryptamine) is a hormone produced in the pineal gland primarily during darkness (Lynch et al. 1975). The timing of melatonin secretion is controlled by the circadian pacemaker, in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus, via GABAergic inhibition and glutamatergic stimulation of the paraventricular nucleus and sympathetic noradrenergic stimulation of the pineal gland (Klein and Moore 1979; Perreau-Lenz et al. 2003; Perreau-Lenz et al. 2004; Reppert et al. 1981). Measurement of melatonin concentration (through the dim light melatonin onset, DLMO) is regularly used by researchers to determine a person's biological night (Lewy et al. 2006; Lewy et al. 1985), as the production of the hormone rises in the evening and falls during the morning hours (Arendt and Rajaratnam 2008). While the role of melatonin is not fully understood, its role in modulating the circadian system and controlling the sleep-wake system is increasingly recognised (Rajaratnam et al. 2009a). Prior to melatonin onset is the time of day referred to as the wake maintenance zone when sleep propensity is lowest (Lavie 1986; Strogatz et al. 1987). At the same time endogenous melatonin rises, there is an abrupt transition from increased wakefulness to high sleep propensity (Dijk and Cajochen 1997; Lavie 1997). Peak melatonin concentration is associated with low core body temperature, alertness, mental performance, metabolic functions and maximum sleep propensity (Rajaratnam and Arendt 2001). Endogenous melatonin appears to support sleep by inhibiting the circadian system's wake-promoting drive (Lavie 1997; Sack et al. 1997).

The circadian effects of melatonin are mediated by melatonin receptors MT_1 and MT_2 located within the SCN (Reppert et al. 1996). Animal models have shown some evidence for divergent roles of MT_1 and MT_2 receptors. Studies in knockout mice demonstrate that activation of the MT_1 receptor appears to inhibit SCN neuronal firing, while the MT_2 receptor is more important in mediating the phase-shifting effect of melatonin (Liu et al. 1997), although this is not widely agreed in humans and the exact role of the receptors is not yet understood (Liu et al. 2016).

3 Circadian Timing and Amplitude of Melatonin and Their Relationship to Mood

Disruptions in circadian rhythms, including endogenous melatonin production, appear to underlie not only disturbances in sleep but also disturbances in mood. For example, disruptions in the rhythms of the release of melatonin have been found in major depressive disorder (MDD) (Li et al. 2013), and depression severity has been found to correlate with delayed melatonin secretion; the more delayed, the more severe the depressive symptoms (Emens et al. 2009). In delayed sleep-wake phase disorder, circadian misalignment has been found to be associated with increased depressive symptoms (Murray et al. 2017). For patients suffering from SAD, adjusting phase to a more ideal time improves mood (Lewy et al. 2005a). Compared with patients in earlier stages of affective disorders, patients in later stages have been found to have reduced levels of melatonin secretion and melatonin onsets closer to sleep onset (shorter "phase angles"), lower subjective sleepiness and poorer performance on neuropsychological tests of verbal memory (Naismith et al. 2012). Reduced amplitude has also been found during depression (Avery et al. 1982; Buckley and Schatzberg 2010; Souêtre et al. 1989), and amplitude reduction has been found to correlate with symptom severity (Souêtre et al. 1989). Meanwhile, recovery has been associated with normalising amplitude (Avery et al. 1982; Szuba et al. 1997).

4 Physiological Mechanisms Behind Effects of Melatonin and Melatonin Agonists

The physiological mechanisms behind the sleep-facilitating and phase-shifting effects of melatonin and melatonin agonists remain to date unclear. Sleep-facilitating effects may be related to melatonin inhibiting SCN multiunit activity (Shibata et al. 1989; Stehle et al. 1989). As SCN electrical activity is high in the day and low at night, melatonin-induced inhibition may create a "night-like" SCN state during the day (Colwell 2011). In humans, this may be especially important in promoting sleep during the "wake maintenance zone", when the signal for wakefulness from the clock appears strongest (Shekleton et al. 2013). Thermoregulatory effects may also provide an explanation. For example, melatonin-induced sleepiness has been found to occur in parallel with a reduction in the thermoregulatory set point, and heat loss is

linked to increased sleepiness (Hughes and Badia 1997; Krauchi et al. 2006). The phase-shifting effects of melatonin may be explained by the activation of melatonin receptors in the SCN, which both inhibit neuronal activity and phase shift circadian firing rhythms (Dubocovich 2007). The phase-shifting effects of melatonin have been found to be mediated by activation of melatonin receptors in the SCN in rodent studies (Hunt et al. 2001).

5 Exogenous Melatonin and Melatonin Agonists

The therapeutic potential offered by exogenous melatonin utilises its phase-shifting and sleep-promoting effects. Exogenous melatonin phase shifts circadian rhythms according to a phase response curve (PRC); the direction and magnitude of the phase shift depend on the circadian time at which melatonin is administered (Burgess et al. 2008, 2010; Lewy 2010; Redman 1997; Sharkey and Eastman 2002). In addition to its phase-shifting properties, exogenous melatonin appears to support sleep by attenuating the wake-promoting drive from the circadian system (Lavie 1997; Sack et al. 1997). This sleep-promoting property means it could be used to enhance sleep scheduled out of phase with endogenous melatonin production, a treatment useful for shift workers, individuals suffering from jet lag and patients with advanced or delayed sleep phase syndrome (Wyatt et al. 2006). Indeed, exogenous melatonin may only be efficacious in promoting sleep if taken where endogenous melatonin is not present (Wyatt et al. 2006).

Exogenous melatonin and melatonin agonists have shown potential for treating circadian rhythm sleep-wake disorders, including non-24-h sleep-wake disorder, insomnia and delayed sleep phase disorder and mood disorders including seasonal affective disorder and major depressive disorder. Table 1 sets out the potential treatments offered by each compound, including the dose for each indication and current regulatory approval in Australia, the USA and Europe.

6 Potential Treatments

6.1 Circadian Rhythm Sleep-Wake Disorders

In circadian rhythm sleep-wake disorders, sleep disruption is likely to be due to an altered circadian system or misalignment between the endogenous circadian system and an individual's required sleep-wake schedule (American Psychiatric Association 2013). Those most at risk of suffering include shift workers, people travelling across time zones (Rajaratnam et al. 2009b) and individuals suffering from chronic circadian rhythm disorders such as advanced or delayed sleep-wake phase disorder (Dahlitz et al. 1991). Melatonin is a potentially effective treatment for circadian rhythm sleep-wake disorder because of its ability to phase-shift the circadian clock, including the circadian rhythm of sleep-wakefulness and multiple endogenous rhythms (Rajaratnam et al. 2003; Rajaratnam et al. 2004; Vandewalle et al. 2007).

Table 1 Potential treatments for melatonin and melatonin agonists

Compound	Primary mechanism	Indication	Dose for indication	Regulatory approval		
				FDA	EMA	TGA
Melatonin	High affinity for MT_1 and MT_2 receptors	Circadian rhythm sleep-wake disorder	1.5 mg at beginning of sleep opportunity (Rajaratnam et al. 2003)	No	No	Yes
		Non-24-h sleep-wake disorder in blind	0.3 mg or less per day (Lewy et al. 2005c)			
		Transient insomnia induced by shifted sleep-wake cycle (e.g. jet lag, shift work, advanced or delayed sleep phase disorder)	0.3 mg and 5.0 mg – efficacious only if taken when endogenous melatonin absent, i.e. during a person's biological day (Wyatt et al. 2006)			
		Seasonal affective disorder (SAD)	Up to 0.3 mg/day – optimal administration when plasma DLMO and mid-sleep are 6 h apart. Evening administration for prototypical patients (Lewy et al. 2005b)			
		Delayed sleep-wake phase disorder	0.3–5 mg – optimal treatment 3–6 h before DLMO, lower doses preferred (van Geijlswijk et al. 2010)			
Tasimelteon	Melatonin analogue High affinity for MT_1 and MT_2	Non-24-h sleep-wake disorder in blind	20 mg 1 hour before desired sleep time (Lockley et al. 2015)	Yes	Yes	No
		Transient insomnia induced by shifted sleep-wake cycle in healthy individuals (e.g. jet lag, shift work)	50–100 mg 30 min before bedtime (Rajaratnam et al. 2009b)			
Ramelteon	Melatonin analogue High affinity for MT_1 and MT_2 and low affinity for MT_3	Insomnia in older adults	4–8 mg taken nightly (Roth et al. 2006)	Yes	No	No
Beta-methyl-6-chloromelatonin (LY156735)	Melatonin analogue High-affinity agonist for MT_1 and MT_2 receptors	Transient insomnia from shift in sleep time	5 mg per day (Nickelsen et al. 2002)	No	No	No
		Primary insomnia	20–100 mg (Zemlan et al. 2005)			
Circadin®	Slow-release melatonin application High affinity for MT_1 and MT_2 receptors	Insomnia in adults over 55	2 mg taken 1–2 h before bed (Lemoine et al. 2007b)	No	Yes	Yes

(continued)

Table 1 (continued)

Compound	Primary mechanism	Indication	Dose for indication	Regulatory approval		
				FDA	EMA	TGA
Agomelatine	Melatonin analogue	Major depressive disorder (moderate to severe)	25–50 mg per day (Olié and Kasper 2007)	No	Yes[a]	Yes
	Selective agonist at MT_1 and MT_2 receptors					
	Antagonist at the serotonergic receptor subtypes 5-HT2B and 5-HT2C					

FDA US Food and Drug Administration, *EMA* European Medicines Agency, *TGA* Therapeutic Goods Administration (Australia)
[a]EMA warns against use in ages over 75 because of risk of liver toxicity

The size and direction of the shift will depend on the internal circadian time of administration, but in general, melatonin taken in the afternoon/early evening will advance the circadian clock, and taken in the morning, it will delay it (Khalsa et al. 2003). Administration of 1.5 mg of melatonin has been found to advance the timing of sleep and endogenous circadian rhythms in healthy adults (Rajaratnam et al. 2003). Melatonin has been found to advance the timing of sleep so that it occurs during the wake maintenance zone, without affecting the percentage of REM sleep. The percentage of stage 2 sleep and sleep spindle activity does increase, and the percentage of stage 3 sleep decreases with melatonin (Rajaratnam et al. 2004).

6.2 Non-24-h Sleep-Wake Disorder

Melatonin is the preferred treatment for totally blind people, many of whom suffer from non-24-h sleep-wake disorder, a disorder characterised by an inability to synchronise to the 24-h day through light exposure. A common complaint of non-24-h sleep-wake disorder is sleep-wake disruption including insomnia and daytime sleepiness (Lockley et al. 2015). A number of studies have shown melatonin to be effective at entraining blind people suffering from non-24-h sleep-wake disorder (Sack et al. 1999a; Sack et al. 1999b; Sack et al. 2000; Sack et al. 1990), and entrainment has been reported at low dosages that do not exceed typical physiological levels, with most participants entraining with 0.3 mg or less (Lewy et al. 2005c). In addition to melatonin, tasimelteon (VEC-162), a compound with high affinity for the MT_1 and MT_2 receptors, has been able to entrain free-running blind people and improve sleep when 20 mg is taken once per day 1 h before target bedtime (Lockley et al. 2015) (Fig. 1). Continued use is required to maintain improvements. Tasimelteon is the first circadian drug to be approved by the Food and Drug Administration (FDA) in the USA and the European Medicines Agency (EMA) (Lockley et al. 2015).

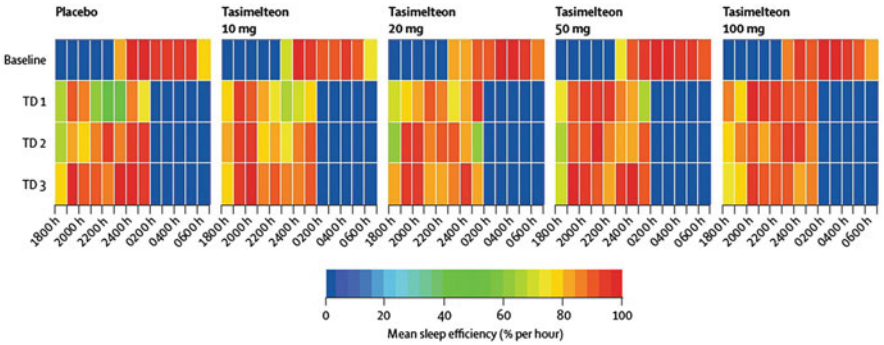

Fig. 1 The pattern of subjective night-time and daytime sleep and acrophases from serial 48-h measurements of urinary 6-sulphatoxymelatonin (aMT6s) for four totally blind patients. Measures of aMT6s acrophase are indicated by red stars. Sequential study days during the screening phase and randomisation phase (starting day 0) are shown on the *y*-axis, and clock time is double-plotted on the *x*-axis. The length of each sleep episode is indicated by black lines, and measures of aMT6s acrophase are indicated by red stars. Tasimelteon was able to produce 24-h entrainment (left), while placebo was not (right) (Modified from Lockley et al. 2015, Lancet)

6.3 Transient Insomnia Induced by Shifted Sleep-Wake Cycle

The sleep-promoting effects of melatonin (Lavie 1997; Sack et al. 1997) suggest that the hormone could be used to enhance sleep scheduled out of phase with endogenous melatonin production, a treatment potentially useful for shift workers, individuals suffering from jet lag and patients with advanced or delayed sleep-wake phase disorder (Wyatt et al. 2006). In the study by Wyatt et al. (2006), both physiologic (0.3 mg) and pharmacologic (5.0 mg) doses of exogenous melatonin improved sleep efficiency when the sleep opportunity occurred out of phase with endogenous melatonin production. However, the benefit of exogenous melatonin was not seen if endogenous melatonin was present, making melatonin an unsuitable treatment for promoting sleep in a person's biological night (Wyatt et al. 2006).

The melatonin agonist tasimelteon may be beneficial for sufferers of transient insomnia brought about by an abrupt shift in the sleep-wake cycle, such as is experienced with jet lag and shift work. Transient insomnia is experienced as impaired sleep initiation, sleep maintenance or both. Rajaratnam et al. (2009b) tested tasimelteon for treatment of insomnia associated with shifted sleep and wake time in healthy individuals (Fig. 2). Following an abrupt advance in sleep time, doses of 10, 20, 50 and 100 mg tasimelteon improved sleep initiation and maintenance and shifted endogenous circadian rhythms. A 50 mg dose of tasimelteon taken 30 min before bedtime was consistently efficacious in improving polysomnographic and self-reported sleep initiation and maintenance outcome variables, while maximal circadian phase shift was found with a 100 mg dose.

Other melatonin agonists have also shown potential as treatments for insomnia. Randomised controlled trials show ramelteon, an agonist approved by the FDA, to be a well-tolerated treatment option for insomnia (Borja and Daniel 2006). In a study

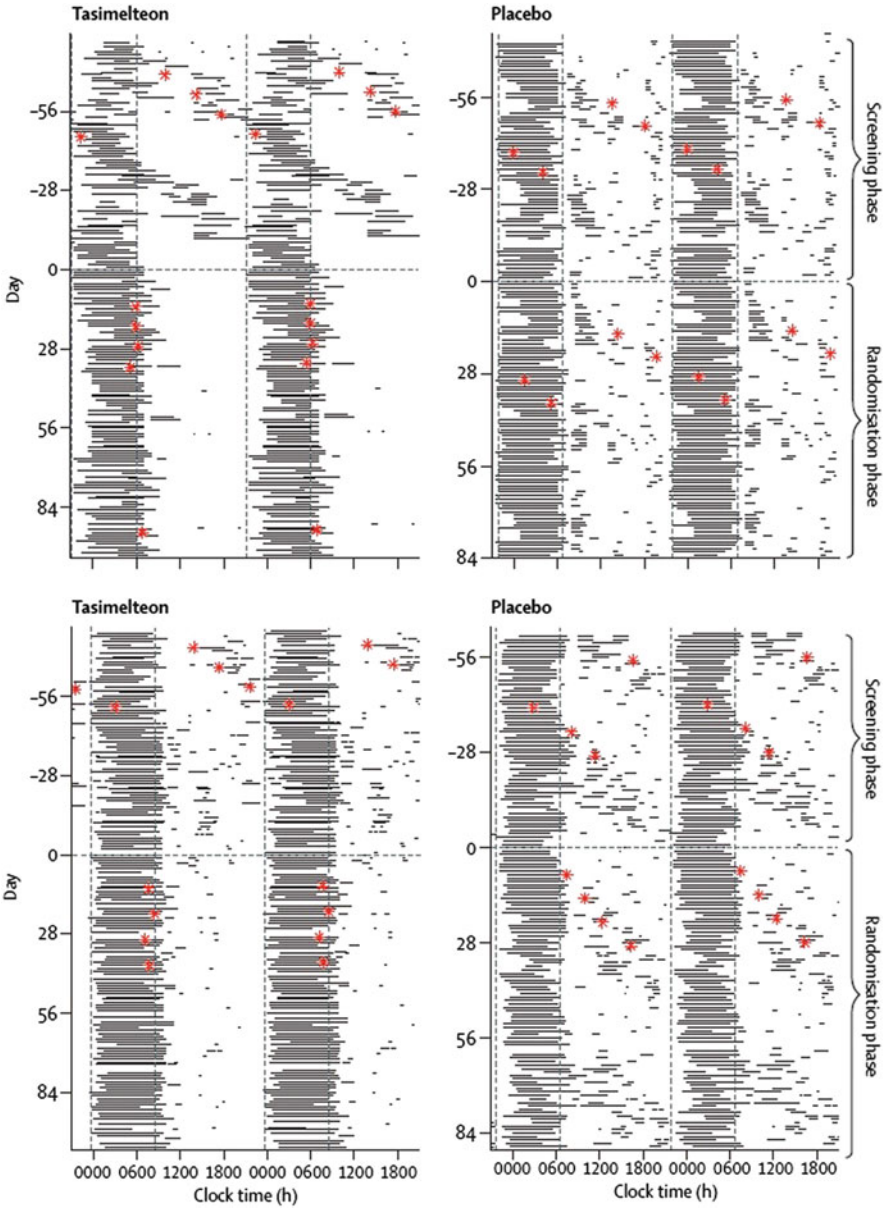

Fig. 2 Sleep-promoting and circadian rhythm phase-shifting effects of tasimelteon. Sleep efficiency data (mean values at hourly intervals) are presented as colour-contour plots, with 0% sleep efficiency shown in dark blue and 100% in red, for inpatient day baseline, treatment days 1, 2 and 3. The baseline sleep episode was from 2300 to 0700 h; sleep episodes during treatment days were from 1800 to 0200 h. *TD* treatment day. Tasimelteon at all doses resulted in greater sleep efficiency when the circadian clock promoted wakefulness (Modified from Rajaratnam et al. 2009, Lancet)

of older adults, nightly doses of 4 mg or 8 mg of ramelteon over a period of 5 weeks reduced patient reports of sleep-onset latency (Roth et al. 2006). Another treatment option for those suffering from sleep problems associated with a shift in sleep time is the agonist beta-methyl-6-chloromelatonin (LY156735). Healthy participants have been shown to adapt to a simulated time shift after taking 5 mg of the compound (Nickelsen et al. 2002). Meanwhile, 20, 50 and 100 mg of LY156735 were found to decrease subjective and objective measures of sleep latency in participants with primary insomnia (Zemlan et al. 2005), and 2 mg of the prolonged-released melatonin application Circadin® taken 1–2 h before bed has been found to reduce sleep latency and improve sleep quality and morning alertness in insomnia patients aged over 55 years (Lemoine et al. 2007b).

6.4 Delayed Sleep-Wake Phase Disorder

Meta-analyses have concluded that melatonin is an effective treatment for delayed sleep-wake phase disorder (DSWPD), with melatonin advancing sleep-wake timing and endogenous melatonin in patients with DSWPD (Buscemi et al. 2005; van Geijlswijk et al. 2010). DSWPD is characterised by the inability to fall asleep and to wake at conventional times (American Psychiatric Association 2013), with sleep onset usually after midnight and wake after midday (Rahman et al. 2010). Individuals at risk of DSWPD are more likely to report absenteeism and disruption to school, work, social and family life (Rajaratnam et al. 2015). Reported biomarkers of DSWPD include delayed plasma melatonin concentration and core body temperature (Oren et al. 1995). The meta-analysis conducted by van Geijlswijk et al. (2010) examined five studies in adults and four in children. The adult trials showed melatonin advanced mean endogenous melatonin onset by 1.18 h and clock hour of sleep onset by 0.67 h. Sleep-onset latency was decreased by 23.27 min. The dose administered to adults and children was generally 5 mg per day; one study by Mundey et al. (2005) compared 0.3 mg with 3 mg and found both doses effective at advancing the circadian phase of endogenous melatonin. Based on their meta-analysis, van Geijlswijk et al. (2010) suggest melatonin treatments for DSWPD be administered 3–6 h before DLMO using small doses to avoid high melatonin levels during late night or early morning. While early administration is recommended, the optimum timing of administration is yet to be determined.

6.5 Seasonal Affective Disorder (SAD)

Patients with SAD tend to have delayed endogenous rhythms, including DLMO and temperature rhythm (Avery et al. 1997; Dahl et al. 1993), and moving from an ideal circadian phase is related to symptom severity in patients with SAD (Lewy et al. 2005a). In such cases, melatonin-induced phase shift may help. Patients with

SAD may benefit from treatment that involves DLMO testing and melatonin administration to cause an optimal phase angle. In SAD patients, between 0.075 and 0.1 mg of melatonin was given every 2 h from either the early morning or afternoon, with no more than 0.3 mg per day. Optimal mood was found when plasma DLMO (defined as time after plasma melatonin levels continuously exceed 10 pg/ml threshold) and mid-sleep were 6 h apart (Lewy et al. 2005b). The ideal timing between DLMO and sleep is associated with optimal mood, before and after treatment with melatonin (Lewy et al. 2006). Importantly, timing of the melatonin dosage is crucial for SAD patients and depends on whether the patient is phase-delayed or phase-advanced. For example, morning melatonin (which induces a phase delay) can make a prototypical phase-delayed SAD patient more depressed, and afternoon/evening melatonin can worsen the mood of an atypical phase-advanced SAD patient (Lewy et al. 2005b).

6.6 Major Depressive Disorder

Although melatonin may assist SAD patients, it does not effectively treat other types of depression (Carman et al. 1976; Dalton et al. 2000), except perhaps in combination with existing antidepressant treatments (Hickie and Rogers 2011).

However, the melatonin agonist agomelatine (S20098), which also binds to the serotonergic receptor subtypes 5-HT_{2B} and 5-HT_{2C} (Arendt and Rajaratnam 2008), does offer potential as an antidepressant drug (Hickie and Rogers 2011). Agomelatine has chronobiotic, antidepressant and anxiolytic effects and, in the short term, has been found to be as effective as antidepressant drugs venlafaxine, fluoxetine and sertraline, with the added benefit of improved subjective sleep (Hickie and Rogers 2011). Depressed patients taking the compound have reported improved sleep quality and reduced waking after sleep onset (Lemoine et al. 2007a). Objective measures also show sleep improvements in these individuals. Following doses of 25/50 mg per day of agomelatine, improvements in sleep efficiency, slow-wave sleep and the distribution of delta activity throughout the night following have been measured using polysomnography (Quera-Salva et al. 2010). A randomised trial of agomelatine (25–50 mg) in patients with moderate to severe major depressive disorder reported significant improvement in depression severity using the Hamilton Rating Scale for Depression compared to controls (Olié and Kasper 2007). In addition to the sleep improvement benefits, because the compound does not increase serotonin levels, there appear to be fewer side effects than traditional depression treatments (Hickie and Rogers 2011). However, the EMA does warn that further measures should be put in place to minimise the risk of liver toxicity and warns against its use in people aged over 75 (European Medicines Agency 2014).

7 Administration, Dosage and Safety Profile

The most common form of melatonin administration is oral tablets and capsules, although intravenous solutions, intranasal sprays, transbuccal patches and transdermal creams have also been used (Rajaratnam et al. 2009a).

The doses administered in melatonin studies have varied. Deacon and Arendt (1995) found a significant dose response when comparing 0.05, 0.5 and 5 mg, and Sharkey and Eastman (2002) found a dose-dependent phase shift when comparing 0.5 and 3 mg. However, generally researchers conclude there is no dose-response effect (Mundey et al. 2005; Wyatt et al. 2006). In some cases, smaller phase shifts seen from higher doses of melatonin have been explained by high doses spilling over into the wrong zone of the melatonin phase response curve (Lewy et al. 2006).

Doses required for effective treatment with melatonin agonists are comparatively higher than the natural compound (Arendt and Rajaratnam 2008). Improvements in depressive severity have been found with doses of 25–50 mg of agomelatine (Olié and Kasper 2007), and ramelteon improves insomnia at daily doses of 4–8 mg (Roth et al. 2006). Transient insomnia can be alleviated with doses of between 10 and 100 mg tasimelteon (Rajaratnam et al. 2009b), and 20 mg daily has been effective at entraining free-running blind people (Lockley et al. 2015).

Melatonin and melatonin agonists have been found to be safe during occasional, short-term use (Rajaratnam et al. 2009b). However, caution should still be taken, particularly as the regulation of these products varies across the USA, Europe and Australia. Potential side effects of melatonin and melatonin agonists include increased sleepiness and impaired neurobehavioural performance (Cajochen et al. 1997; Deacon and Arendt 1995; Graw et al. 2001) emphasising that the timing of administration is important to consider. Agonists appear to offer a good safety profile (Arendt and Rajaratnam 2008). However, currently there is insufficient data to show if agonists are preferable to the natural compound (Arendt and Rajaratnam 2008). The effects of long-term use are also unknown.

8 Conclusion

The phase-shifting and sleep-promoting effects of melatonin plus additional effects of melatonin agonists on melatonin and serotonin receptors have shown promise for novel treatments for a variety of circadian, sleep and mood disorders. Importantly, the main advantage melatonin and its agonists offer over traditional sleep and depression treatments is that they assist to restore circadian function which is often misaligned in these disorders (Rajaratnam et al. 2009b) and which is increasingly thought to be a causal mechanism and part of the aetiology of sleep and mood disorders. Treatments that fail to address the misaligned circadian system present in sleep and mood disorders may not fully address the underlying causes, and for this reason, further investigation on the potential for melatonin-based treatments should be undertaken.

References

American Psychiatric Association (2013) DSM 5. American Psychiatric Association, Washington, DC

Arendt J, Rajaratnam SMW (2008) Melatonin and its agonists: an update. Br J Psychiatry 193 (4):267–269. https://doi.org/10.1192/bjp.bp.108.050955

Avery DH, Wildschiødtz G, Rafaelsen OJ (1982) Nocturnal temperature in affective disorder. J Affect Disord 4(1):61–71

Avery D, Dahl K, Savage M, Brengelmann G, Larsen L, Kenny M, Eder DN, Vitiello MV, Prinz P (1997) Circadian temperature and cortisol rhythms during a constant routine are phase-delayed in hypersomnic winter depression. Biol Psychiatry 41:1109–1123. https://doi.org/10.1016/S0006-3223(96)00210-7

Borja NL, Daniel KL (2006) Ramelteon for the treatment of insomnia. Clin Ther 28:1540–1555. https://doi.org/10.1016/j.clinthera.2006.10.016

Buckley TM, Schatzberg AF (2010) A pilot study of the phase angle between cortisol and melatonin in major depression – a potential biomarker? J Psychiatr Res 44(2):69–74. https://doi.org/10.1016/j.jpsychires.2009.06.012

Burgess HJ, Revell VL, Eastman CI (2008) A three pulse phase response curve to three milligrams of melatonin in humans. J Physiol 586(2):639–647. https://doi.org/10.1113/jphysiol.2007.143180

Burgess HJ, Revell VL, Eastman CI (2010) Human phase response curves to three days of daily melatonin: 0.5 mg versus 3.0 mg. J Clin Endocrinol Metab 95(7):3325–3331. https://doi.org/10.1210/jc.2009-2590

Buscemi N, Vandermeer B, Hooton N, Pandya R, Tjosvold L, Hartling L, Baker G, Klassen TP, Vohra S (2005) The efficacy and safety of exogenous melatonin for primary sleep disorders: a meta-analysis. J Gen Intern Med 20(12):1151–1158. https://doi.org/10.1111/j.1525-1497.2005.0243.x

Cajochen C, Kräuchi K, Wirz-Justice A (1997) The acute soporific action of daytime melatonin administration: effects on the EEG during wakefulness and subjective alertness. J Biol Rhythms 12:636–643. https://doi.org/10.1177/074873049701200619

Carman JS, Post RM, Buswell R, Goodwin FK (1976) Negative effects of melatonin on depression. Am J Psychiatry 133:1181–1186

Colwell CS (2011) Linking neural activity and molecular oscillations in the SCN. Nat Rev Neurosci 12(10):553–569. https://doi.org/10.1038/nrn3086

Dahl K, Avery DH, Lewy AJ, Savage M, Brengelmann G, Larsen L, Vitiello MV, Prinz P (1993) Dim light melatonin onset and circadian temperature during a constant routine in hypersomnic winter depression. Acta Psychitar Scand 88:60–66

Dahlitz MJ, Alvarez B, Vignau J, English J, Arendt J, Parkes JD (1991) Delayed sleep-phase syndrome: response to melatonin. Lancet 337:1121–1124

Dalton EJ, Rotondi D, Levitan RD, Kennedy SH, Brown GM (2000) Use of slow-release melatonin in treatment-resistant depression. J Psychiatry Neurosci 25:48–52

Deacon S, Arendt J (1995) Melatonin-induced temperature suppression and its acute phase-shifting effects correlate in a dose-dependent manner in humans. Brain Res 688:77–85

Dijk DJ, Cajochen C (1997) Melatonin and the circadian regulation of sleep initiation, consolidation, structure, and the sleep EEG. J Biol Rhythms 12(6):627–635

Dubocovich ML (2007) Melatonin receptors: role on sleep and circadian rhythm regulation. Sleep Med 8(3):34–42. https://doi.org/10.1016/j.sleep.2007.10.007

Emens J, Lewy AJ, Mark J, Arntz D, Rough J (2009) Circadian misalignment in major depressive disorder. Psychiatry Res 168:259–261. https://doi.org/10.1016/j.psychres.2009.04.009

European Medicines Agency (2014) EMA confirms positive benefit-risk for antidepressant Valdoxan/Thymanax (agomelatine) [Press release]. http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2014/09/news_detail_002173.jsp&mid=WC0b01ac058004d5c1

van Geijlswijk IM, Korzilius HPLM, Smits MG (2010) The use of exogenous melatonin in delayed sleep phase disorder: a meta-analysis. Sleep 33(12):1605–1614

Graw P, Werth E, Krauchi K, Gutzwiller F, Cajochen C, Wirz-Justice A (2001) Early morning melatonin administration impairs psychomotor vigilance. Behav Brain Res 121(1–2):167–172

Hickie IB, Rogers NL (2011) Novel melatonin-based therapies: potential advances in the treatment of major depression. Lancet 378:621–631. https://doi.org/10.1016/S0140-6736(11)60095-0

Hughes RJ, Badia P (1997) Melatonin and sleep: sleep-promoting and hypothermic effects of daytime melatonin administration in humans. Sleep 20(2):124–131

Hunt AE, Al-Ghoul WM, Gillette MU, Dubocovich ML (2001) Activation of MT(2) melatonin receptors in rat suprachiasmatic nucleus phase advances the circadian clock. Am J Physiol Cell Physiol 280(1):C110–C118. https://doi.org/10.1152/ajpcell.2001.280.1.C110

Khalsa SB, Jewett ME, Cajochen C, Czeisler CA (2003) A phase response curve to single bright light pulses in human subjects. J Physiol (Lond) 549(Pt 3):945–952

Klein DC, Moore RY (1979) Pineal N-acetyltransferase and hydroxyindole-O-methyltransferase: control by the retinohypothalamic tract and the suprachiasmatic nucleus. Brain Res 174:245–262

Krauchi K, Cajochen C, Pache M, Flammer J, Wirz-Justice A (2006) Thermoregulatory effects of melatonin in relation to sleepiness. Chronobiol Int 23(1–2):475–484. https://doi.org/10.1080/07420520500545854

Lavie P (1986) Ultrashort sleep-waking schedule. III. 'Gates' and 'forbidden zones' for sleep. Electroencephalogr Clin Neurophysiol 63(5):414–425

Lavie P (1997) Melatonin: role in gating nocturnal rise in sleep propensity. J Biol Rhythms 12:657–665

Lemoine P, Guilleminault C, Alvarez E (2007a) Improvement in subjective sleep in major depressive disorder with a novel antidepressant, agomelatine: randomized, double-blind comparison with venlafaxine. J Clin Psychiatry 68:1723–1732

Lemoine P, Nir T, Laudon M, Zisapel N (2007b) Prolonged-release melatonin improves sleep quality and morning alertness in insomnia patients aged 55 years and older and has no withdrawal effects. J Sleep Res 16:372–380

Lerner AB, Case JD, Takahashi Y, Lee TH, Mori W (1958) Isolation of melatonin, the pineal gland factor that lightens melanocytes. J Am Chem Soc 80(10):2587

Lewy A (2010) Clinical implications of the melatonin phase response curve. J Clin Endocrinol Metab 95(7):3158–3160. https://doi.org/10.1210/jc.2010-1031

Lewy AJ, Sack RL, Singer CM (1985) Immediate and delayed effects of bright light on human melatonin production: shifting "dawn" and "dusk" shifts the dim light melatonin onset (DLMO). Ann N Y Acad Sci 453:253–259

Lewy A, Lefler BJ, Emens J, Bauer VK (2005a) The circadian basis of winter depression. Proc Natl Acad Sci 103(19):7414–7419. https://doi.org/10.1073/pnas.0602425103

Lewy AJ, Emens JS, Lefler BJ, Bauer VK (2005b) In winter depression (SAD), the sweet spot for the 10pg/mL plasma dim light melatonin onset (DLMO) is six hours before mid-sleep. Neuropsychopharmacology 30:562–563

Lewy AJ, Emens JS, Lefler BJ, Yuhas K, Jackman AR (2005c) Melatonin entrains free-running blind people according to a physiological dose-response curve. Chronobiol Int 22:1093–1106. https://doi.org/10.1080/07420520500398064

Lewy AJ, Emens J, Jackman A, Yuhas K (2006) Circadian uses of melatonin in humans. Chronobiol Int 23(1–2):403–412. https://doi.org/10.1080/07420520500545862

Li SX, Liu LJ, Xu LZ, Gao L, Wang XF, Zhang JT, Lu L (2013) Diurnal alterations in circadian genes and peptides in major depressive disorder before and after escitalopram treatment. Psychoneuroendocrinology 38:2789–2799. https://doi.org/10.1016/j.psyneuen.2013.07.009

Liu C, Weaver DR, Jin X, Shearman LP, Pieschl RL, Gribkoff VK, Reppert SM (1997) Molecular dissection of two distinct actions of melatonin on the suprachiasmatic circadian clock. Neuron 19(1):91–102

Liu C, Clough SJ, Hutchinson AJ, Adamah-Biassi EB, Popovska-Gorevski M, Dubocovich ML (2016) MT1 and MT2 melatonin receptors: a therapeutic perspective. Annu Rev Phamacol Toxicol 56:361–383. https://doi.org/10.1146/annurev-pharmtox-010814-124742

Lockley SW, Dressman MA, Licamele L, Xiao C, Fisher DM, Flynn-Evans EE, Hull JT, Torres R, Polymeropoulos MH (2015) Tasimelteon for non-24-hour sleep–wake disorder in totally blind people (SET and RESET): two multicentre, randomised, double-masked, placebo-controlled phase 3 trials. Lancet 386(10005):1754–1764. https://doi.org/10.1016/s0140-6736(15)60031-9

Lynch H, Wurtman R, Moskowitz M, Archer M, Ho M (1975) Daily rhythm in human urinary melatonin. Science 187:169–171

Mundey K, Benloucif S, Harsanyi K, Dubocovich ML, Zee P (2005) Phase-dependent treatment of delayed sleep phase syndrome with melatonin. Sleep 28(10):1271–1278

Murray JM, Sletten T, Magee M, Gordon C, Lovato N, Bartlett DJ, Kennaway D, Lack LC, Grunstein RR, Lockley SW, Rajaratnam SM, Delayed Sleep on Melatonin (DelSoM) Study Group (2017) Prevalence of circadian misalignment and its association with depressive symptoms in delayed sleep phase disorder. Sleep 40(1). https://doi.org/10.1093/sleep/zsw002

Naismith SL, Hermens DF, Ip TK, Bolitho S, Scott E, Rogers NL, Hickie IB (2012) Circadian profiles in young people during the early stages of affective disorder. Transl Psychiatry 2:e123. https://doi.org/10.1038/tp.2012.47

Nickelsen T, Samel A, Vejvoda M, Wenzel J, Smith B, Gerzer R (2002) Chronobiotic effects of the melatonin agonist LY 156735 following a simulated 9h time shift: results of a placebo-controlled trial. Chronobiol Int 19(5):915–936

Olié JP, Kasper S (2007) Efficacy of agomelatine, a MT1/MT2 receptor agonist with 5-HT2C antagonistic properties, in major depressive disorder. Int J Neuropsychopharmacol 10(5):661–673. https://doi.org/10.1017/S1461145707007766

Oren DA, Turner EH, Wehr TA (1995) Abnormal circadian rhythm of plasma melatonin and body temperature in the delayed sleep phase syndrome. J Neurol Neurosurg Psychiatry 58:379

Perreau-Lenz S, Kalsbeek A, Garidou ML, Wortel J, van der Vliet J, van Heijningen C, Simonneaux V, Pévet P, Buijs RM (2003) Suprachiasmatic control of melatonin synthesis in rats: inhibitory and stimulatory mechanisms. Eur J Neurosci 17(2):221–228

Perreau-Lenz S, Kalsbeek A, Pevet P, Buijs RM (2004) Glutamatergic clock output stimulates melatonin synthesis at night. Eur J Nuerosci 19:318–324

Quera-Salva MA, Lemoine P, Guilleminault C (2010) Impact of the novel antidepressant agomelatine on disturbed sleep-wake cycles in depressed patients. Hum Psychopharmacol 25 (3):222–229. https://doi.org/10.1002/hup.1112

Rahman SA, Kayumov L, Shapiro CM (2010) Antidepressant action of melatonin in the treatment of delayed sleep phase syndrome. Sleep Med 11(2):131–136. https://doi.org/10.1016/j.sleep.2009.07.013

Rajaratnam SMW, Arendt J (2001) Health in the 24-hour society. Lancet 358:999–1005. https://doi.org/10.1016/S0140-6736(01)06108-6

Rajaratnam SMW, Dijk DJ, Middleton B, Stone BM, Arendt J (2003) Melatonin phase-shifts human circadian rhythms with no evidence of changes in the duration of endogenous melatonin secretion or the 24-h production of reproductive hormones. J Clin Endocrinol Metab 88:4303–4309. https://doi.org/10.1210/jc.2003-030460

Rajaratnam SMW, Middleton B, Stone BM, Arendt J, Dijk DJ (2004) Melatonin advances the circadian timing of EEG sleep and directly facilitates sleep without altering its duration in extended sleep opportunities in humans. J Physiol 561(Pt 1):339–351. https://doi.org/10.1113/jphysiol.2004.073742

Rajaratnam SMW, Cohen DA, Rogers NL (2009a) Melatonin and melatonin analogues. Sleep Med Clin 4:179–193. https://doi.org/10.1016/j.jsmc.2009.02.007

Rajaratnam SMW, Polymeropoulos MH, Fisher DM, Roth T, Scott C, Birznieks G, Klerman EB (2009b) Melatonin agonist tasimelteon (VEC-162) for transient insomnia after sleep-time shift: two randomised controlled multicentre trials. Lancet 373(9662):482–491. https://doi.org/10.1016/s0140-6736(08)61812-7

Rajaratnam SMW, Licamele L, Birznieks G (2015) Delayed sleep phase disorder risk is associated with absenteeism and impaired functioning. Sleep Health 1(2):121–127. https://doi.org/10.1016/j.sleh.2015.03.001

Redman JR (1997) Circadian entrainment and phase shifting in mammals with melatonin. J Biol Rhythms 12(6):581–587

Reppert SM, Perlow MJ, Ungerleider LG et al (1981) Effects of damage to the suprachiasmatic area of the anterior hypothalamus on the daily melatonin and cortisol rhythms in the rhesus monkey. J Neurosci 1:1414–1425

Reppert SM, Weaver DR, Godson C (1996) Melatonin receptors step into the light: cloning and classification of subtypes. Trends Pharmacol Sci 17(3):100–102

Roth T, Seiden D, Sainati S, Wang-Weigand S, Zhang J, Zee P (2006) Effects of ramelteon on patient-reported sleep latency in older adults with chronic insomnia. Sleep Med 7(4):312–318. https://doi.org/10.1016/j.sleep.2006.01.003

Sack RL, Stevenson J, Lewy AJ (1990) Entrainment of a previously free-running blind human with melatonin administration. Sleep Res 19:404

Sack RL, Hughes RJ, Edgar DM, Lewy AJ (1997) Sleep-promoting effects of melatonin: at what dose, in whom, under what conditions, and by what mechanisms? Sleep 20(10):908–915

Sack RL, Brandes RL, DeJongh EA, Pen S, Nordstrom S, Lewy AJ (1999a) Melatonin entrains free-running circadian rhythms in a totally blind person. Sleep 22:S138

Sack RL, Brandes RL, Lewy AJ (1999b) Totally blind people with free-running circadian rhythms can be normally entrained with melatonin. Sleep Res Online 2:624

Sack RL, Brandes RW, Kendall AR, Lewy AJ (2000) Entrainment of freerunning circadian rhythms by melatonin in blind people. N Engl J Med 343:1070–1077. https://doi.org/10.1056/NEJM200010123431503

Sharkey KM, Eastman CI (2002) Melatonin phase shifts human circadian rhythms in a placebo-controlled simulated night-work study. Am J Physiol Regul Integr Comp Physiol 282(2):R454–R463. https://doi.org/10.1152/ajpregu.00135.2001

Shekleton JA, Rajaratnam SMW, Gooley JJ, Van Reen E, Czeisler CA, Lockley SW (2013) Improved neurobehavioral performance during the wake maintenance zone. J Clin Sleep Med 9(4):353–362. https://doi.org/10.5664/jcsm.2588

Shibata S, Cassone VM, Moore RY (1989) Effects of melatonin on neuronal activity in the rat suprachiasmatic nucleus in vitro. Neurosci Lett 97(1–2):140–144

Souêtre E, Salvati E, Belugou JL, Pringuey D, Candito M, Krebs B, Ardisson JL, Darcourt G (1989) Circadian rhythms in depression and recovery: evidence for blunted amplitude as the main chronobiological abnormality. Psychiatry Res 28:263–278

Stehle J, Vanecek J, Vollrath L (1989) Effects of melatonin on spontaneous electrical activity of neurons in rat suprachiasmatic nuclei: an in vitro iontophoretic study. J Neural Transm 78 (2):173–177

Strogatz SH, Kronauer RE, Czeisler CA (1987) Circadian pacemaker interferes with sleep onset at specific times each day: role in insomnia. Am J Physiol 253(1):R172–R178

Szuba MP, Guze BH, Baxter LRJ (1997) Electroconvulsive therapy increases circadian amplitude and lowers core body temperature in depressed subjects. Biol Psychiatry 42(12):1130–1137

Vandewalle G, Middleton B, Rajaratnam SMW, Stone BM, Thorleifsdottir B, Arendt J, Dijk DJ (2007) Robust circadian rhythm in heart rate and its variability: influence of exogenous melatonin and photoperiod. J Sleep Res 16(2):148–155. https://doi.org/10.1111/j.1365-2869.2007.00581.x

Wyatt JK, Dijk D, Ritz-De Cecco A, Ronda JM, Czeisler C (2006) Sleep-facilitating effect of exogenous melatonin in healthy young men and women is circadian-phase dependent. Sleep 29 (5):609–618

Zemlan FP, Mulchahey JJ, Scharf MB, Mayleben DW, Rosenberg R, Lankford A (2005) The efficacy and safety of the melatonin agonist beta-methyl-6-chloromelatonin in primary insomnia: a randomized, placebo-controlled, crossover clinical trial. J Clin Psychiatry 66(3):384–390

Dopamine and Wakefulness: Pharmacology, Genetics, and Circuitry

Jonathan P. Wisor

Contents

Abstract

Over the period of decades in the mid to late twentieth century, arousal-promoting functions were attributed to neuromodulators including serotonin, hypocretin, histamine, and noradrenaline. For some time, a relatively minor role in regulating sleep and wake states was ascribed to dopamine and the dopamine-producing cells of the ventral tegmental area, despite the fact that dopaminergic signaling is a major target, if not the primary target, for wake-promoting agents. In recent years, due to observations from human genetic studies, pharmacogenetic studies in animal models, and the increasingly sophisticated methods used to manipulate the nervous systems of experimental animals, it has become clear that dopaminergic signaling is central to the regulation of arousal. This chapter reviews this central role of dopaminergic signaling, and in particular its antagonistic interaction with adenosinergic signaling, in maintaining vigilance and in the response to wake-promoting therapeutics.

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Dopamine and Wakefulness: Key Concepts

- Genetic variation in dopaminergic signaling pathways influences the negative impacts of sleep loss and the response to wake-promoting therapeutics used to mitigate those negative impacts.
- Dopaminergic signaling from the ventral tegmental area varies across vigilance states and regulates sleep/wake state timing.
- Dopamine/adenosine interactions in the striatum are positioned as a key mediator of the impacts of sleep loss on cortical arousal state.

The regulation of sleep and wakefulness is a function of the interactions of widely projecting sleep-promoting and wake-promoting systems. The serotonergic cells of the raphe nuclei, noradrenergic cells of the locus coeruleus (LC), hypocretinergic cells of the lateral hypothalamus/perifornical area (LH/PFA), and histaminergic cells of the tuberomammillary nucleus (TMN) are among the most well-recognized wake-promoting neuromodulatory systems (reviewed in McGinty and Szymusiak 2011). The activity of raphe, LC, LH/PFA, and TMN cells is greatest in wakefulness, and drops precipitously at sleep onset, as do extracellular levels of the neuromodulators they produce in the central nervous system. Pharmacological perturbations or lesioning of these systems results in disruption of sustained wakefulness. Thus, the concerted activity of these systems is regarded as a critical mediator of sustained wakefulness and the performance of waking activities.

Dopaminergic signaling did not play a central role in the model of wake-promoting systems that emerged in the late twentieth century, and not without reason. The activity of cells in dopaminergic nuclei (substantia nigra, SN and ventral tegmental area, VTA) was not found to be consistently state-dependent across studies (Miller et al. 1983; Steinfels et al. 1983; Trulson and Preussler 1984). Extracellular dopamine concentration in the CNS was also not found to vary as a function of state across studies (de Saint Hilaire et al. 2000; Shouse et al. 2000; Trulson and Preussler 1984). Lesioning of dopaminergic cell body-containing regions did not alter sleep timing (Jones et al. 1973). Yet it has become clear over the last couple of decades that dopaminergic regulation of cortical arousal was a missing component in this conceptual model. Relatively recent work demonstrates that (1) genetic variation in dopaminergic signaling pathways influences the negative impacts of sleep loss and the response to wake-promoting therapeutics used to mitigate those negative impacts; (2) dopaminergic signaling from the VTA varies across vigilance states and regulates sleep/wake state timing; and (3) dopamine/adenosine interactions in the striatum are positioned as a key regulator of cortical

arousal state and arousal-dependent functions. This chapter reviews these three concepts with the intention of giving the reader insights into how they might be applied to improve functioning in an increasingly sleep-deprived world.

1 Genetic Insights into Dopaminergic Regulation of Sleep and Wakefulness

Polymorphisms in loci involved in dopamine metabolism or dopaminergic signaling have been associated with sleep/wake timing, or vigilance-related phenotypes (reviewed in Dauvilliers et al. 2015; Holst et al. 2016). Catechol-O-methyltransferase (COMT) eliminates dopamine by converting it to 3-methoxytyramine. A single-nucleotide polymorphism in *COMT* results in a valine for methionine substitution that increases dopamine metabolism by roughly four-fold (Lachman et al. 1996). This polymorphism associates with (1) time-on-task effects in the psychomotor vigilance task, a measure of fatigue during sustained wakefulness (Lim et al. 2012), (2) the severity of EEG changes indicative of increased sleep drive during partial sleep restriction (4 h time in bed per night) (Goel et al. 2011), and (3) the therapeutic efficacy of the widely prescribed wake-promoting therapeutic, modafinil during conditions of sleep deprivation (SD) (Bodenmann et al. 2008).

The D_2 dopamine receptor (DRD2) is located both on dopaminergic terminals as an autoreceptor and on postsynaptic cells as a neuromodulatory receptor. This G protein-coupled receptor reduces neuronal excitability by reducing cyclic AMP concentration and suppressing voltage-gated Ca^{2+} currents (Sanders-Bush and Hazelwood 2011). A non-coding polymorphism (C957T; one that changes the codon, but not the resulting amino acid sequence in the protein) in the *DRD2* gene was found to associate with (1) deficits in reversal learning in association with 62-h total SD (Van Dongen et al. 2017; a measure of cognitive flexibility) and (2) the severity of PVT lapses emerging with SD (Holst et al. 2017). Another non-coding (in this case, intronic) polymorphism was found to associate with self-reported habitual sleep duration (Cade et al. 2016). It is difficult to interpret the significance of these associations, as the functional consequences of the noncoding *DRD2* polymorphisms are unknown. It is possible that the *DRD2* locus is in close proximity to the relevant functional polymorphism. One of the three above-mentioned studies linking *DRD2* to a sleep phenotype (Holst et al. 2017) also found an interaction of the *DRD2* polymorphism with a polymorphism in gene encoding the cell membrane dopamine transporter (*DAT*) in affecting the severity of PVT lapses during SD. The DAT transports dopamine from the extracellular milieu into dopaminergic terminals for repackaging into vesicles. A variable number tandem repeat (VNTR) polymorphism in the 3′ untranslated region of the *DAT* locus, of which the most common alleles are a 9-repeat or 10-repeat, confers differential expression: individuals harboring the 9-repeat allele express DAT at higher levels than those harboring the 10-repeat, according to a meta-analysis of in vivo imaging data with labeled DAT ligands (Faraone et al. 2014). Individuals homozygous for the 10-repeat allele of *DAT* and the C allele of *DRD2* were more severely affected with PVT lapses after 40 h of SD than other genotypic variants (Holst et al. 2017). The *DAT* polymorphism

has further been associated with (1) variability in time on task effects in the psychomotor vigilance task (Lim et al. 2012), (2) the severity of EEG changes indicative of increased sleep drive during SD (Holst et al. 2014), and (3) the effects of modafinil on EEG markers of sleep need (Holst et al. 2014).

Also of note from the perspective of sleep regulatory mechanism is the fact that the *DAT* VNTR polymorphism predicts the effects of the most widely used (non-prescription) wake-promoting agent, caffeine (Holst et al. 2014) on EEG markers of sleep need. Caffeine is dependent on the adenosine 2a receptor (ADORA2a), where it acts as an inverse agonist [Fernandez-Duenas et al. 2014 #7141] for its wake-promoting effect (Huang et al. 2005). This fact coupled with the considerable body of evidence implicating adenosine in mediating the accumulation of sleep need over time (reviewed in Basheer et al. 2004) suggest that adenosinergic signaling via ADORA2a in the brain is a key mediator of sleep need. The genetic linkage of dopamine to caffeine sensitivity points to the striatum, where dopamine and adenosine interact antagonistically (see below), as a possible nexus for mediating the effects of sleep loss on vigilance. In fact, polymorphisms in *ADORA2a* are known to confer sleep-related phenotypes (reviewed in Landolt 2011). It is worth mentioning this genetic linkage here, before we consider more mechanistic studies on the dopaminergic/adenosinergic axis of sleep regulation. Much like the *DAT* VNTR polymorphism, both a single nucleotide polymorphism in *ADORA2a* (Retey et al. 2007) and *ADORA2a* haplotype (Bodenmann et al. 2012) predict sensitivity to caffeine at EEG and behavioral levels. The single nucleotide polymorphism also predicted resiliency to sleep restriction (seven consecutive nights of restriction to 3 h in bed) (Rupp et al. 2013). The parallels between adenosine-associated loci and dopamine-related loci in modulating the response to sleep loss and wake-promoting agents do not indicate a mechanistic relationship between the two systems at the cellular level in regulating vigilance. However, experimental and theoretical considerations described in the remainder of this chapter make a compelling case for this mechanistic relationship.

2 Experimental Insights into Dopaminergic Regulation of Sleep and Wakefulness

The above summarized human genetic studies that link dopaminergic signaling, and potentially its interactions with adenosinergic signaling, to sleep regulation have been complemented by increasingly sophisticated studies in animal models. Collectively, the two bodies of work converge on dopaminergic/adenosinergic interactions as a mediator of manifestations of sleepiness during protracted wake. The importance of dopaminergic groups, and in particular the VTA in promoting wakefulness was neglected for many years, because while electrolytic lesions of VTA suppressed behavioral arousal, they did not alter the timing or quality of electroencephalographically defined arousal (Jones et al. 1973). Nor did dopamine-specific neurochemical lesion of the VTA (hypocretin-saporin; Gerashchenko et al. 2006) alter the timing or quality of electroencephalographically defined arousal. Electrophysiological

recordings of the activity of these cells also failed to detect modulation of the activity of presumed dopaminergic cells in either the VTA or SN across sleep wake states (Miller et al. 1983; Steinfels et al. 1983; Trulson and Preussler 1984). Thus, authors were led to conclude that the dopaminergic cell groups of the VTA and SN, in contrast to their monoaminergic kindred in the raphe, TMN, LC, and the hypocretinergic cells of the LH/PFA, do not serve a wake-promoting function within the CNS.

However, more recent work in animal models, in addition to the genetic data cited above, causes a rethinking of this role for dopaminergic signaling. In vivo pharmacological studies, including work on the mechanism of action of modafinil (reviewed in Wisor 2013), have ascertained the arousal-promoting role of dopaminergic signaling. The collective observations that modafinil binds to the dopamine transporter at physiologically relevant concentrations (Loland et al. 2012; Mignot et al. 1994; Schmitt and Reith 2011), that systemic modafinil administration elevates extracellular dopamine concentration in the striatum (Murillo-Rodriguez et al. 2007; Wisor et al. 2001; Zolkowska et al. 2009), and that genetic inactivation of the dopamine transporter nullifies its wake-promoting effect (Wisor et al. 2001) indicate that its action as a dopamine transporter inhibitor underlies its wake-promoting effect. Other putative actions [including modulation of gap junctional communication (Urbano et al. 2007; Garcia-Rill et al. 2007)] may be secondary to its action at the dopamine transporter. Any effect hypothetically mediated by a target other than DAT must be verified by assessing this effect in DAT-deficient animals.

If an agent such as modafinil increases arousal by elevating extracellular dopamine, then dopamine receptors are a presumed target for this mechanism. In general, dopaminergic signaling is necessary for arousal, as pharmacological depletion of dopamine results in intrusions of slow wave sleep (SWS)-like EEG synchronization in awake, behaving mice (Dzirasa et al. 2006). But which receptors are involved? Pharmacological studies support an arousal-inducing effect for DRD2-based dopaminergic signaling in general, and in the response to modafinil in particular. Genetic inactivation of *Drd2* in nocturnal rodents, or pharmacological antagonism of DRD2 nullifies the arousing effect of modafinil (Qu et al. 2008). Genetic or pharmacological inactivation of *Drd2* in nocturnal rodents results in a failure to sustain wake in contexts wherein wake normally predominates (in the hours after dark onset or immediately after introduction to a novel environment; Qu et al. 2010). These data offered a compelling case for the role of dopaminergic signaling via DRD2 receptors in maintaining arousal. However, such studies based on either constitutive genetic manipulations or systemic pharmacological manipulations failed to offer insights into the specific circuitry underlying the arousal-promoting role of dopaminergic signaling.

Work within the last few years using sophisticated targeting of dopaminergic and dopaminoceptive cell populations has addressed this limitation of the prior work. Modern experimental tools, all of which can be applied to neurochemically defined cell populations via cell type-specific genetic manipulations involving the enzyme Cre recombinase, were critical to this achievement: (1) in vivo imaging of the activation of neuronal populations across sleep and wake states; (2) in vivo

optogenetic electrical activation of neurons with millisecond precision; and (3) in vivo chemogenetic potentiation/inhibition of neurons over periods of minutes to hours, coinciding with sleep/wake cycling (Fig. 1). The Cre recombinase enzyme, isolated from bacteria in the 1980s (Abremski and Hoess 1984), catalyzes recombination of DNA at specific recognition sites. When driven by a mammalian promoter that is expressed in a cell type-specific manner (for instance the tyrosine hydroxylase promoter that is expressed in catecholaminergic cells exclusively), Cre recombinase activity will occur in that cell type exclusively. The studies described below used mice harboring a germ line transgene (*Th-Cre*) in which the tyrosine hydroxylase (*Th*) promoter drives Cre recombinase expression. A second transgenic construct, delivered by viral transfection via brain cannulation in these studies (Fig. 1), houses an inactive coding sequence that can only be activated by Cre-mediated recombination. So viral transfection of an experimental DNA construct into the VTA of animals in which the *Th* promoter drives Cre recombinase expression, for instance,

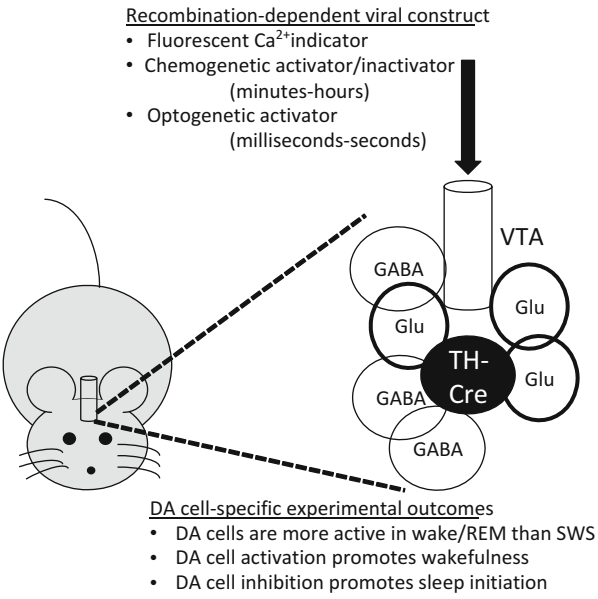

Fig. 1 Recent technologies targeting dopaminergic cells selectively in experimental rodents have yielded insights into dopaminergic regulation of sleep and wakefulness (Eban-Rothschild et al. 2016; Oishi et al. 2017). Genetically engineered viral constructs contain coding sequences for the fluorescing Ca^{2+} indicator GCamp6f, the chemogenetic designer G protein-coupled activating (hM3Dq) and inactivating (hM4Di) receptors, or the optogenetic blue light sensitive cation channel Channelrhodopsin-2. These constructs are silenced in their native state and must be unsilenced by Cre recombinase-dependent recombination. The animal receiving viral infusions via intracerebral cannulation harbors a germ line transgene in which the tyrosine hydroxylase (*Th*) promoter drives expression of Cre recombinase only in tyrosine hydroxylase-positive neurons. As a result, only TH-positive dopaminergic cells at the site of infusion (VTA) will undergo recombination-dependent activation of the viral transgene. Any experimental effects or observations can then be attributed to the dopaminergic population in the VTA and its dopaminoceptive targets

results in Cre recombinase-dependent activation of that construct in the VTA dopaminergic population. Three such experimentally useful DNA constructs have been applied to gain insights into the role of the VTA dopaminergic population in sleep/wake regulation. First, viral transfection of a construct encoding the calcium-sensing protein GCaMP6f allowed for real-time in vivo microscopic monitoring of dopaminergic neuronal activity to demonstrate state specific activation of VTA cells. Results showed both a tonic elevation of calcium concentration and more frequent calcium transients, taken as evidence of a barrage of synaptic inputs, during both REMS and wake relative to NREMS (Eban-Rothschild et al. 2016). This result is arguably more definitive than previously applied electrophysiological approaches (de Saint Hilaire et al. 2000; Shouse et al. 2000; Trulson and Preussler 1984), which made assumptions about the dopaminergic cell type based on firing properties rather than neurochemical phenotype.

The second cell type-specific manipulation of these cells, which was ultimately responsible for demonstrating a unique role for the VTA, specifically, and not the SN, in maintaining wakefulness, was the chemogenetic approach. With chemogenetics, a viral transfection is used to confer expression of a G protein-coupled receptor (known as a Designer Receptors Exclusively Activated by a Designer Drug or DREADD) to the cell type of interest. The G protein-coupled receptor has been engineered to be responsive to clozapine N-oxide (CNO) and to be unresponsive to any known endogenous ligand. Expression of this DREADD is under the control of Cre recombinase, which means in the current context, when targeted to the VTA by cannula-mediated viral transfection, that DA cells expressing Cre recombinase will express the receptor of interest. Mice are then treated systemically with CNO to activate the DREADD, resulting in profound inhibition of the activity of the VTA-DA cells exclusively. This chemogenetic silencing of VTA-DA cells profoundly disrupted behavior. When administered in a home cage environment 2 h into the dark phase of the LD 12:12 cycle, CNO triggered SWS in animals harboring the DREADD selectively in VTA-DA cells (Eban-Rothschild et al. 2016). When faced with three highly motivating stimuli (an opportunity to eat, a potential mate, or a perceived predator), the mice subjected to CNO prior to the stimulus instead transitioned into sleep. Moreover, when placed in a novel environment, CNO-treated mice engaged in nesting behavior, interpreted by the authors as a manifestation of sleep drive. A very recently published study highlights the necessity for rigorous design in experiments involving CNO: in fact, it is not CNO but its metabolite clozapine that serves as the ligand for the DREADD (Gomez et al. 2017). Because clozapine is a ligand for multiple endogenous receptors and affects both sleep timing and EEG markers for sleepiness (Gronli et al. 2016), there is the concern that the response to CNO administration might be mediated by off-target effects of clozapine. In the study reporting sleep-promoting effects of VTA inhibition (Eban-Rothschild et al. 2016), wild type control mice (mice not subjected to viral DREADD infection) in the same environmental condition as DREADD-expressing mice engaged in exploratory behaviors rather than nesting and sleep after CNO administration, as expected from mice placed acutely in a novel environment. VTA control of wakefulness seems ethologically relevant in the sense that it

maintains wakefulness, even in the face of sleep pressure, when the environmental context provides a compelling motivational force, either positive (mating and feeding opportunities) or negative (vulnerability to predation) to maintain wakefulness.

In a similar approach (Oishi et al. 2017), *Dat-Cre* mice were subjected to adenovirus transfection of a Cre recombinase-dependent chemogenetic inhibitor construct targeted exclusively to either VTA or SN. Exposure to a chemogenetic activating ligand resulted in Fos expression in vivo and firing in vitro for both VTA and SN. But only VTA transfection resulted in sustained wakefulness. This effect was blocked by DRD2 antagonist raclopride but not a DRD1 antagonist. The same effect was not seen with chemogenetic inactivation of the dopaminergic cells of the SN (Oishi et al. 2017). The latter observation addresses the previously iterated concern over off-target effects of clozapine, as it shows that anatomically restricted DREADD expression, not just exposure to CNO, is necessary for the CNO response. So the VTA population seems to be the unique source of dopaminergic fibers that promote wakefulness, and this function is dependent on DRD2.

A third experiment involving optogenetics confirmed that activation of VTA-DA cells is sufficient to promote arousal in an acute fashion (Eban-Rothschild et al. 2016). In optogenetics, a bacterially derived blue light sensitive ion channel is expressed in neurons, where it can be activated by light emitted from a microscopic fiber optic cable embedded in the brain (Deisseroth 2011). For the purpose of targeting the VTA-DA cells, a viral Cre recombinase-dependent optogenetic construct was administered into the VTA of *Th-Cre* mice. Optogenetic stimulation of the VTA, or of regions receiving its projections (nucleus accumbens, NAc, central amygdala, CeA, and dorsolateral striatum, DLS) during SWS resulted in a decrease in the latency to awaken from sleep (Eban-Rothschild et al. 2016). A 6-h period of continuous optogenetic stimulation at a time of day when rodents exhibit a high propensity to sleep under baseline conditions, the first 6 h of the daily 12-h light phase, resulted in sustained wakefulness throughout the 6-h stimulation session. Independent work verifies that VTA-DA activation is a powerful arousing stimulus: optogenetic activation of the VTA is sufficient to induce arousal of mice from isoflurane anesthesia (Taylor et al. 2016).

3 Through What Circuitry Do Ventral Tegmental Area Cells Promote Arousal?

So cell type-specific manipulations of the VTA-DA cells with optogenetic and chemogenetic approaches shows an obligatory role for these cells in driving arousal. To understand the role of these cells in context, it is first necessary to ask how VTA DA cells themselves become activated in association with wakefulness. Multiple input sources are potential mediators of the wake-related activation of these cells (Oishi and Lazarus 2017). They receive significant glutamatergic innervation from the periaqueductal gray, lateral habenula, and VTA itself (reviewed in Oishi and Lazarus 2017). Likewise, there are GABAergic inputs to the VTA from some of the same areas, the roles of which in sleep/wake regulation have not been characterized. Classic sleep regulators (raphe serotonergic cells, laterodorsal tegmentum cholinergic cells,

lateral hypothalamus) innervate the VTA, but whether these VTA-specific projections from these widely projecting sleep regulatory populations are critical in sleep regulation is unknown. The pattern of activation of these cells (elevated in both wake and REMS relative to SWS; Eban-Rothschild et al. 2016) coincides with that of acetylcholine release but not that of monoaminergic or hypocretinergic cells (Siegel 2011). Cholinergic terminals innervate (Garzon et al. 1999) and excite (Xiao et al. 2016) VTA dopaminergic cells. Thus, it seems likely that the sleep/wake cycle related activity of VTA-DA cells is influenced by cholinergic inputs.

By what projections does the VTA sustain wakefulness? Wakefulness induced by chemogenetic activation of VTA-DA cells requires DRD2 activation, as it is blocked by systemic administration of the DRD2 antagonist raclopride (Oishi et al. 2017). But DRD2 receptors and DA terminals are widespread in the brain, so this information does not implicate a specific circuit in the wake-promoting effect of dopamine. Evidence to date points to the striatum, specifically the ventral portion of the striatum, the NAc as a critical site for DRD2-mediated waking. The NAc is a critical regulatory nexus for sleep regulation (reviewed in Oishi and Lazarus 2017). In this region, extracellular dopamine levels vary in inverse proportion to cortical synchronization, a hallmark measure of sleep need (Lena et al. 2005). Optogenetic stimulation of dopaminergic terminals in the NAc, but not in other dopamine-enriched regions (medial prefrontal cortex or central nucleus of the amygdala), is sufficient to maintain wakefulness continuously over 6 h in the face of accumulating sleep need (Eban-Rothschild et al. 2016). Lesion of the NAc eliminates the wake promoting effect of modafinil (Qiu et al. 2012). Selective elimination of ADORA2a receptors in the NAc results in loss of the wake-promoting effects of caffeine (Lazarus et al. 2011).

These last two observations indicate a role for cells of the NAc in responses to the antagonistic wake-promoting dopaminergic and sleep-promoting adenosinergic signaling mechanisms. And in fact, the antagonism plays out at the cellular level. Medium spiny neurons make up the vast majority of cells in the NAc and can be divided into two classes (Kim and Palmiter 2008). Those of the basal ganglia direct path are positive for DRD1 and adenosine receptor A1. Those of the indirect path are positive for DRD2 and ADORA2a. Since the latter two have been implicated by both genetics and pharmacology in behavioral and EEG responses to sleep loss, this population is especially of interest. It is necessary to consider any putative sleep/wake regulatory function for the DRD2/ADORA2a subpopulation of medium spiny neurons of the NAc in the context of their role in the basal ganglia. The direct and indirect pathways of the basal ganglia are parallel and very much intertwined pathways (Blumenfeld 2002). The net effect of the direct pathway on the cerebral cortex is activation and the net effect of the indirect pathway on the cerebral cortex is inhibition. In this context, it is intriguing that there are both sleep-active (Tellez et al. 2012) and wake-active (Callaway and Henriksen 1992; Tellez et al. 2012) populations of cells in the NAc. As a matter of speculation, dominance of the indirect path over the direct path would provide a possible mechanism for inhibitory mechanisms to predominate throughout the cortex and promote synchronization characteristic of sleep onset. While the effect of targeting the indirect pathway on EEG-defined sleep has not been studied, behavioral quiescence is reported to occur

upon chemogenetic activation of the NAc cells that participate in the indirect pathway (Zhu et al. 2016). DRD2/ADORA2a-positive cells project widely to numerous brain areas (Zhang et al. 2013), and are thus poised to play a role in the complex orchestration of events associated with sleep and the deficits associated with sleep loss.

As a working model, we might consider their role in regulating forebrain functions via interactions with the prefrontal cortex. Prefrontal cortex dependent reward-oriented executive functions deteriorate as a result of SD (Gujar et al. 2011; Venkatraman et al. 2007, 2011) Changes in cerebral blood flow (Braun et al. 1997), glutamate concentration (Dash et al. 2009), and glucose utilization (Maquet 1997) in association with sleep are more robust in the prefrontal cortex than other regions. SD triggers a decline in glutamatergic transmission from prefrontal cells: medial prefrontal cortex (mPFC)-to-nucleus accumbens (NAc) glutamatergic transmission is selectively weakened following acute SD (Liu et al. 2016). Deterioration of prefrontal cortex-dependent, reward-oriented executive functions as a result of SD (Gujar et al. 2011; Venkatraman et al. 2007, 2011; Whitney et al. 2015) can be understood in the context of dopamine/adenosine interactions within the basal ganglia.

4 Synthesis: Dopaminergic/Adenosinergic Interactions in the Striatum Mediate Effects of Sleep Deprivation on Prefrontal Cortex-Dependent Performance

The observations summarized so far can be used to synthesize a novel conceptual model for the emergence of performance deficits during SD: performance deficits in reward-oriented executive functions are the product of increasing activation of the DRD2/ADORA2a-positive population of medium spiny neurons in the striatum, specifically, the ventral striatum (i.e., NAc) across time spent awake. The rationale for this model is as follows. *DRD2* and *ADORA2a* have been linked through genetic and pharmacological studies to EEG and behavioral markers of sleep that emerge during SD. These two receptors interact antagonistically in a unique population of striatopallidal (those that project primarily to the globus pallidus, external, GPe) medium spiny neurons of the striatum, which serve in the indirect pathway of the basal ganglia (Fig. 2). In the well-rested state, DRD2 receptors activate the G protein G_i, resulting in suppression of cyclic adenosine monophosphate (cAMP) concentration in the cell. This effect is countered (minimally in the well-rested state due to relatively low levels of adenosine) by ADORA2a-induced G_s activation, which promotes the accumulation of cAMP. The effect of DRD2 receptors becomes less prominent with time awake for two reasons: first, DRD2 receptors are down-regulated in striatum as a result of SD (Volkow et al. 2012). Second, reduced cholinergic tone as a result of increased adenosine accumulation with time awake (Basheer et al. 2004) disfacilitates dopaminergic VTA cells, reducing their activity. There is no known evidence for changes in ADORA2a availability as a function of sleep states. To the contrary, adenosinergic tone is elevated in the brain with SD (Basheer et al. 2004). Thus, the net effect of SD on the striatopallidal neurons

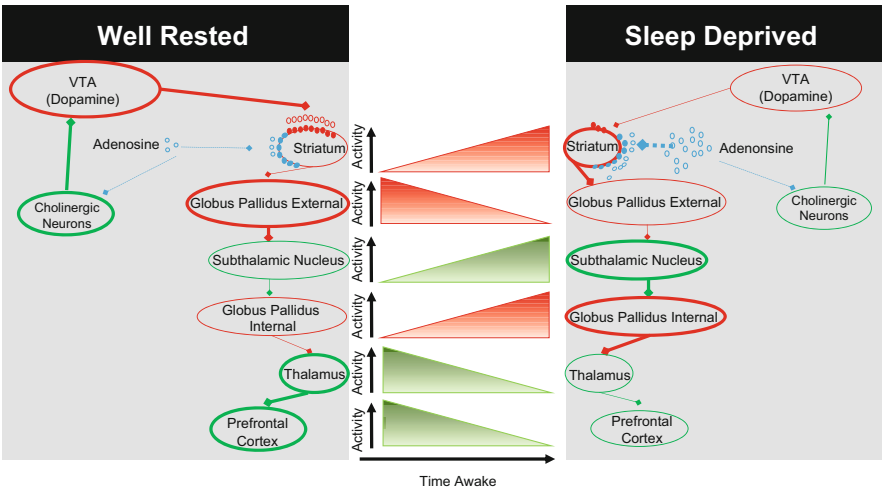

Fig. 2 Adenosinergic/dopaminergic interactions in the striatum mediate effects of sleep deprivation on prefrontal cortex. The indirect path is a multi-synaptic circuit that regulates prefrontal cortical executive functions via feedback from the basal ganglia. The indirect pathway is initiated by glutamatergic projections from the prefrontal cortex to striatum (not shown). The pathway is modulated antagonistically via dopaminergic projections to the striatum, which suppress intracellular cAMP synthesis via DRD2 receptors, and accumulated extracellular adenosine, which promotes intracellular cAMP synthesis via ADORA2a receptors on the same striatal population. From the striatum, a 5-synaptic pathway provides feedback to the prefrontal cortex. Glutamatergic neuronal populations and their primary projections are schematized in green and GABAergic neuronal populations in red. Reduced DRD2 tone and elevated ADORA2a tone over time awake drive an increase in striatal interneuron excitability. The net effect of sleep deprivation-induced striatal activation is the suppression of prefrontal activity and with it executive function

(elevated ADORA2a activation and less DRD2 activation) is to increase striatopallidal GABAergic medium spiny neuron activation. This increase leads, through a 5-synaptic pathway (Blumenfeld 2002), to a decline in prefrontal cortex activation (Fig. 2). (1) An increase in the activity of the striatopallidal GABAergic medium spiny neurons of the NAc causes a decrease in the firing of globus pallidus external (GPe) neurons. GABAergic GPe cells project to the subthalamic nucleus and inhibit their postsynaptic targets (glutamatergic cells) there. (2) The net effect of activating striatopallidal GABAergic medium spiny neurons during protracted wake is thus to disinhibit the subthalamic nucleus by withdrawal of GPe GABAergic inputs. (3) Disinhibition of the glutamatergic subthalamic nucleus facilitates the activation of its target cells, the GABAergic cells of the globus pallidus, internal (GPi). (4) Activation of the GABAergic cells of the Gpi results in inhibition of the glutamatergic thalamic cells that project to the medial prefrontal cortex, (5) decreasing medial prefrontal cortical activation. This pathway provides a means to explain why glutamatergic outputs from prefrontal cortex mPFC are selectively weakened following acute SD (Liu et al. 2016). The model could be tested in experimental animals: deterioration of performance on reward-oriented tasks should be precipitated in well-rested animals

by selective activation of the DRD2/ADORA2A positive striatopallidal cells and reversed in sleep-deprived animals by silencing the activity of these cells. Ascertainment of these predictions will identify DRD2/ADORA2A positive striatopallidal cells as a target for therapeutic intervention in the face of sleep loss or poor quality sleep. Similarly, the deleterious effects of SD on reward-oriented executive functions in humans (Gujar et al. 2011; Venkatraman et al. 2007, 2011; Whitney et al. 2015) should be reversed by inverse ADORA2a agonists such as caffeine. While somnolytic effects of caffeine are universally recognized, the efficacy of caffeine in the context of reward-oriented functions, specifically, remains to be quantified.

References

Abremski K, Hoess R (1984) Bacteriophage P1 site-specific recombination. Purification and properties of the Cre recombinase protein. J Biol Chem 259:1509–1514

Basheer R, Strecker RE, Thakkar MM, McCarley RW (2004) Adenosine and sleep-wake regulation. Prog Neurobiol 73:379–396

Blumenfeld H (2002) Basal ganglia. In: Blumenfeld H (ed) Neuroanatomy through clinical cases. Sinauer Associates, Sunderland, pp 689–735

Bodenmann S, Xu S, Luhmann U, Arand M, Berger W, Jung H, Landolt H (2008) Pharmacogenetics of modafinil after sleep loss: catechol-O-methyltransferase genotype modulates waking functions but not recovery sleep. Clin Pharmacol Ther 85:296–304

Bodenmann S, Hohoff C, Freitag C, Deckert J, Retey JV, Bachmann V, Landolt HP (2012) Polymorphisms of *ADORA2A* modulate psychomotor vigilance and the effects of caffeine on neurobehavioural performance and sleep EEG after sleep deprivation. Br J Pharmacol 165:1904–1913

Braun AR, Balkin TJ, Wesenten NJ, Carson RE, Varga M, Baldwin P, Selbie S, Belenky G, Herscovitch P (1997) Regional cerebral blood flow throughout the sleep-wake cycle. An H2(15)O PET study. Brain 120(Pt 7):1173–1197

Cade BE, Gottlieb DJ, Lauderdale DS, Bennett DA, Buchman AS, Buxbaum SG, De Jager PL, Evans DS, Fulop T, Gharib SA, Johnson WC, Kim H, Larkin EK, Lee SK, Lim AS, Punjabi NM, Shin C, Stone KL, Tranah GJ, Weng J, Yaffe K, Zee PC, Patel SR, Zhu X, Redline S, Saxena R (2016) Common variants in *DRD2* are associated with sleep duration: the CARe consortium. Hum Mol Genet 25:167–179

Callaway CW, Henriksen SJ (1992) Neuronal firing in the nucleus accumbens is associated with the level of cortical arousal. Neuroscience 51:547–553

Dash MB, Douglas CL, Vyazovskiy VV, Cirelli C, Tononi G (2009) Long-term homeostasis of extracellular glutamate in the rat cerebral cortex across sleep and waking states. J Neurosci 29:620–629

Dauvilliers Y, Tafti M, Landolt HP (2015) Catechol-O-methyltransferase, dopamine, and sleep-wake regulation. Sleep Med Rev 22:47–53

de Saint Hilaire Z, Orosco M, Rouch C, Python A, Nicolaidis S (2000) Neuromodulation of the prefrontal cortex during sleep: a microdialysis study in rats. Neuroreport 11:1619–1624

Deisseroth K (2011) Optogenetics. Nat Methods 8:26–29

Dzirasa K, Ribeiro S, Costa R, Santos LM, Lin SC, Grosmark A, Sotnikova TD, Gainetdinov RR, Caron MG, Nicolelis MA (2006) Dopaminergic control of sleep-wake states. J Neurosci 26:10577–10589

Eban-Rothschild A, Rothschild G, Giardino WJ, Jones JR, de Lecea L (2016) VTA dopaminergic neurons regulate ethologically relevant sleep-wake behaviors. Nat Neurosci 19:1356–1366

Faraone SV, Spencer TJ, Madras BK, Zhang-James Y, Biederman J (2014) Functional effects of dopamine transporter gene genotypes on in vivo dopamine transporter functioning: a meta-analysis. Mol Psychiatry 19:880–889

Fernandez-Duenas V, Gomez-Soler M, Lopez-Cano M, Taura JJ, Ledent C, Watanabe M, Jacobson KA, Vilardaga JP, Ciruela F (2014) Uncovering caffeine's adenosine A2A receptor inverse agonism in experimental parkinsonism. ACS Chem Biol 9:2496–2501

Garcia-Rill E, Heister DS, Ye M, Charlesworth A, Hayar A (2007) Novel mechanism for sleep-wake control: electrical coupling. Sleep 30:1405–1414

Garzon M, Vaughan RA, Uhl GR, Kuhar MJ, Pickel VM (1999) Cholinergic axon terminals in the ventral tegmental area target a subpopulation of neurons expressing low levels of the dopamine transporter. J Comp Neurol 410:197–210

Gerashchenko D, Blanco-Centurion CA, Miller JD, Shiromani PJ (2006) Insomnia following hypocretin2-saporin lesions of the substantia nigra. Neuroscience 137:29–36

Goel N, Banks S, Lin L, Mignot E, Dinges DF (2011) Catechol-O-methyltransferase Val158Met polymorphism associates with individual differences in sleep physiologic responses to chronic sleep loss. PLoS One 6:e29283

Gomez JL, Bonaventura J, Lesniak W, Mathews WB, Sysa-Shah P, Rodriguez LA, Ellis RJ, Richie CT, Harvey BK, Dannals RF, Pomper MG, Bonci A, Michaelides M (2017) Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. Science 357:503–507

Gronli J, Clegern WC, Schmidt MA, Nemri RS, Rempe MJ, Gallitano AL, Wisor JP (2016) Sleep homeostatic and waking behavioral phenotypes in Egr3-deficient mice associated with serotonin receptor 5-HT2 deficits. Sleep 39:2189–2199

Gujar N, Yoo SS, Hu P, Walker MP (2011) Sleep deprivation amplifies reactivity of brain reward networks, biasing the appraisal of positive emotional experiences. J Neurosci 31:4466–4474

Holst SC, Bersagliere A, Bachmann V, Berger W, Achermann P, Landolt HP (2014) Dopaminergic role in regulating neurophysiological markers of sleep homeostasis in humans. J Neurosci 34:566–573

Holst SC, Valomon A, Landolt HP (2016) Sleep pharmacogenetics: personalized sleep-wake therapy. Annu Rev Pharmacol Toxicol 56:577–603

Holst SC, Muller T, Valomon A, Seebauer B, Berger W, Landolt HP (2017) Functional polymorphisms in dopaminergic genes modulate neurobehavioral and neurophysiological consequences of sleep deprivation. Sci Rep 7:45982

Huang ZL, Qu WM, Eguchi N, Chen JF, Schwarzschild MA, Fredholm BB, Urade Y, Hayaishi O (2005) Adenosine A2A, but not A1, receptors mediate the arousal effect of caffeine. Nat Neurosci 8:858–859

Jones BE, Bobillier P, Pin C, Jouvet M (1973) The effect of lesions of catecholamine-containing neurons upon monoamine content of the brain and EEG and behavioral waking in the cat. Brain Res 58:157–177

Kim DS, Palmiter RD (2008) Interaction of dopamine and adenosine receptor function in behavior: studies with dopamine-deficient mice. Front Biosci 13:2311–2318

Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM (1996) Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 6:243–250

Landolt HP (2011) Genetic determination of sleep EEG profiles in healthy humans. Prog Brain Res 193:51–61

Lazarus M, Shen HY, Cherasse Y, Qu WM, Huang ZL, Bass CE, Winsky-Sommerer R, Semba K, Fredholm BB, Boison D, Hayaishi O, Urade Y, Chen JF (2011) Arousal effect of caffeine depends on adenosine A2A receptors in the shell of the nucleus accumbens. J Neurosci 31:10067–10075

Lena I, Parrot S, Deschaux O, Muffat-Joly S, Sauvinet V, Renaud B, Suaud-Chagny MF, Gottesmann C (2005) Variations in extracellular levels of dopamine, noradrenaline, glutamate, and aspartate across the sleep--wake cycle in the medial prefrontal cortex and nucleus accumbens of freely moving rats. J Neurosci Res 81:891–899

Lim J, Ebstein R, Tse CY, Monakhov M, Lai PS, Dinges DF, Kwok K (2012) Dopaminergic polymorphisms associated with time-on-task declines and fatigue in the psychomotor vigilance test. PLoS One 7:e33767

Liu Z, Wang Y, Cai L, Li Y, Chen B, Dong Y, Huang YH (2016) Prefrontal cortex to accumbens projections in sleep regulation of reward. J Neurosci 36:7897–7910

Loland CJ, Mereu M, Okunola OM, Cao J, Prisinzano TE, Mazier S, Kopajtic T, Shi L, Katz JL, Tanda G, Newman AH (2012) R-modafinil (armodafinil): a unique dopamine uptake inhibitor and potential medication for psychostimulant abuse. Biol Psychiatry 72:405–413

Maquet P (1997) Positron emission tomography studies of sleep and sleep disorders. J Neurol 244: S23–S28

McGinty D, Szymusiak R (2011) Neural control of sleep in mammals. In: Kryger MH, Roth T, Dement WC (eds) Principles and practice of sleep medicine. Elsevier/Saunders, Philadelphia, pp 76–91

Mignot E, Nishino S, Guilleminault C, Dement WC (1994) Modafinil binds to the dopamine uptake carrier site with low affinity. Sleep 17:436–437

Miller JD, Farber J, Gatz P, Roffwarg H, German DC (1983) Activity of mesencephalic dopamine and non-dopamine neurons across stages of sleep and walking in the rat. Brain Res 273:133–141

Murillo-Rodriguez E, Haro R, Palomero-Rivero M, Millan-Aldaco D, Drucker-Colin R (2007) Modafinil enhances extracellular levels of dopamine in the nucleus accumbens and increases wakefulness in rats. Behav Brain Res 176:353–357

Oishi Y, Lazarus M (2017) The control of sleep and wakefulness by mesolimbic dopamine systems. Neurosci Res 118:66–73

Oishi Y, Suzuki Y, Takahashi K, Yonezawa T, Kanda T, Takata Y, Cherasse Y, Lazarus M (2017) Activation of ventral tegmental area dopamine neurons produces wakefulness through dopamine D2-like receptors in mice. Brain Struct Funct 222:2907–2915

Qiu MH, Liu W, Qu WM, Urade Y, Lu J, Huang ZL (2012) The role of nucleus accumbens core/shell in sleep-wake regulation and their involvement in modafinil-induced arousal. PLoS One 7: e45471

Qu WM, Huang ZL, Xu XH, Matsumoto N, Urade Y (2008) Dopaminergic D1 and D2 receptors are essential for the arousal effect of modafinil. J Neurosci 28:8462–8469

Qu WM, Xu XH, Yan MM, Wang YQ, Urade Y, Huang ZL (2010) Essential role of dopamine D2 receptor in the maintenance of wakefulness, but not in homeostatic regulation of sleep, in mice. J Neurosci 30:4382–4389

Retey JV, Adam M, Khatami R, Luhmann UF, Jung HH, Berger W, Landolt HP (2007) A genetic variation in the adenosine A2A receptor gene (*ADORA2A*) contributes to individual sensitivity to caffeine effects on sleep. Clin Pharmacol Ther 81:692–698

Rupp TL, Wesensten NJ, Newman R, Balkin TJ (2013) *PER3* and *ADORA2A* polymorphisms impact neurobehavioral performance during sleep restriction. J Sleep Res 22:160–165

Sanders-Bush E, Hazelwood L (2011) 5-Hydroxytryptamine (serotonin) and dopamine. In: Brunton LL, Chabner BA, Knollmann BC (eds) Goodman and Gilman's: the pharmacological basis of therapeutics. McGraw-Hill, New York

Schmitt KC, Reith ME (2011) The atypical stimulant and nootropic modafinil interacts with the dopamine transporter in a different manner than classical cocaine-like inhibitors. PLoS One 6: e25790

Shouse MN, Staba RJ, Saquib SF, Farber PR (2000) Monoamines and sleep: microdialysis findings in pons and amygdala. Brain Res 860:181–189

Siegel JM (2011) REM sleep. In: Kryger MH, Roth T, Dement WC (eds) Principles and practice of sleep medicine. Elsevier/Saunders, Philadelphia, pp 92–111

Steinfels GF, Heym J, Strecker RE, Jacobs BL (1983) Behavioral correlates of dopaminergic unit activity in freely moving cats. Brain Res 258:217–228

Taylor NE, Van Dort CJ, Kenny JD, Pei J, Guidera JA, Vlasov KY, Lee JT, Boyden ES, Brown EN, Solt K (2016) Optogenetic activation of dopamine neurons in the ventral tegmental area induces reanimation from general anesthesia. Proc Natl Acad Sci U S A

Tellez LA, Perez IO, Simon SA, Gutierrez R (2012) Transitions between sleep and feeding states in rat ventral striatum neurons. J Neurophysiol 108:1739–1751

Trulson ME, Preussler DW (1984) Dopamine-containing ventral tegmental area neurons in freely moving cats: activity during the sleep-waking cycle and effects of stress. Exp Neurol 83:367–377

Urbano FJ, Leznik E, Llinas RR (2007) Modafinil enhances thalamocortical activity by increasing neuronal electrotonic coupling. Proc Natl Acad Sci U S A 104:12554–12559

Van Dongen HP, Hinson JM, Whitney P, Satterfield BC, Schmidt MA, Wisor JP (2017) Feedback blunting due to sleep deprivation is affected by dopaminergic genotype. In: Cognitive Neuroscience Society, annual meeting

Venkatraman V, Chuah YM, Huettel SA, Chee MW (2007) Sleep deprivation elevates expectation of gains and attenuates response to losses following risky decisions. Sleep 30:603–609

Venkatraman V, Huettel SA, Chuah LY, Payne JW, Chee MW (2011) Sleep deprivation biases the neural mechanisms underlying economic preferences. J Neurosci 31:3712–3718

Volkow ND, Tomasi D, Wang GJ, Telang F, Fowler JS, Logan J, Benveniste H, Kim R, Thanos PK, Ferre S (2012) Evidence that sleep deprivation downregulates dopamine D2R in ventral striatum in the human brain. J Neurosci 32:6711–6717

Whitney P, Hinson JM, Jackson ML, Van Dongen HP (2015) Feedback blunting: total sleep deprivation impairs decision making that requires updating based on feedback. Sleep 38:745–754

Wisor J (2013) Modafinil as a catecholaminergic agent: empirical evidence and unanswered questions. Front Neurol 4:139

Wisor JP, Nishino S, Sora I, Uhl GH, Mignot E, Edgar DM (2001) Dopaminergic role in stimulant-induced wakefulness. J Neurosci 21:1787–1794

Xiao C, Cho JR, Zhou C, Treweek JB, Chan K, McKinney SL, Yang B, Gradinaru V (2016) Cholinergic mesopontine signals govern locomotion and reward through dissociable midbrain pathways. Neuron 90:333–347

Zhang JP, Xu Q, Yuan XS, Cherasse Y, Schiffmann SN, de Kerchove d'Exaerde A, Qu WM, Urade Y, Lazarus M, Huang ZL, Li RX (2013) Projections of nucleus accumbens adenosine A2A receptor neurons in the mouse brain and their implications in mediating sleep-wake regulation. Front Neuroanat 7:43

Zhu X, Ottenheimer D, DiLeone RJ (2016) Activity of D1/2 receptor expressing neurons in the nucleus accumbens regulates running, locomotion, and food intake. Front Behav Neurosci 10:66

Zolkowska D, Jain R, Rothman RB, Partilla JS, Roth BL, Setola V, Prisinzano TE, Baumann MH (2009) Evidence for the involvement of dopamine transporters in behavioral stimulant effects of modafinil. J Pharmacol Exp Ther 329:738–746

Ketamine-Induced Glutamatergic Mechanisms of Sleep and Wakefulness: Insights for Developing Novel Treatments for Disturbed Sleep and Mood

Wallace C. Duncan Jr., Elizabeth D. Ballard, and Carlos A. Zarate

Contents

Abstract

Ketamine, a drug with rapid antidepressant effects and well-described effects on slow wave sleep (SWS), is a useful intervention for investigating sleep–wake mechanisms involved in novel therapeutics. The drug rapidly (within minutes to hours) reduces depressive symptoms in individuals with major depressive

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disorder (MDD) or bipolar disorder (BD), including those with treatment-resistant depression. Ketamine treatment elevates extracellular glutamate in the prefrontal cortex. Glutamate, in turn, plays a critical role as a proximal element in a ketamine-initiated molecular cascade that increases synaptic strength and plasticity, which ultimately results in rapidly improved mood. In MDD, rapid antidepressant response to ketamine is related to decreased waking as well as increased total sleep, SWS, slow wave activity (SWA), and rapid eye movement (REM) sleep. Ketamine also increases brain-derived neurotrophic factor (BDNF) levels. In individuals with MDD, clinical response to ketamine is predicted by low baseline delta sleep ratio, a measure of deficient early night production of SWS. Notably, there are important differences between MDD and BD that may be related to the effects of diagnosis or of mood stabilizers. Consistent with its effects on clock-associated molecules, ketamine alters the timing and amplitude of circadian activity patterns in rapid responders versus non-responders with MDD, suggesting that it affects mood-dependent central neural circuits. Molecular interactions between sleep homeostasis and clock genes may mediate the rapid and durable elements of clinical response to ketamine and its active metabolite.

Keywords
Brain-derived neurotrophic factor (BDNF) • Circadian • Major depressive disorder • Neuroplasticity • Slow wave sleep • Suicidality

1 Introduction

The glutamatergic *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine is a rapid-acting antidepressant with immediate clinical utility. It is also a valuable research intervention that provides insights into the development of novel treatments for mood disorders. Ketamine rapidly (within minutes to hours) reduces depressive symptoms in individuals with major depressive disorder (MDD) or bipolar disorder (BD), including in patients with treatment-resistant depression (Diazgranados et al. 2010a; Murrough et al. 2013; Zarate et al. 2012); treatment effects may last for up to 7 days in some individuals. Importantly, the drug may also reduce suicidal ideation in patients (DiazGranados et al. 2010b; Price et al. 2009; Zarate et al. 2012), suggesting an important treatment option for this population with urgent clinical needs.

Disrupted sleep patterns and circadian rhythms have long been associated with depressive disorders (Gillin et al. 1979; Wehr and Goodwin 1983). Relatedly, sleep deprivation (SD) was suggested to have rapid chronotherapeutic effects on both sleep homeostasis and mood (Borbély and Wirz-Justice 1982; Wirz-Justice and Van den Hoofdakker 1999). In addition, the mood stabilizer lithium was recently found to have beneficial molecular effects on the circadian clock; a full discussion of lithium's effects on circadian function is beyond the scope of this chapter. We refer interested readers to several excellent reviews on this subject (Gould and

Manji 2002; Lenox et al. 2002; McCarthy et al. 2012; Gould and Manji 2005). Interestingly, in addition to its antidepressant effects, ketamine has well-described effects on slow wave sleep (SWS) (Duncan et al. 2013b; Duncan and Zarate 2013), and is thus a useful intervention for investigating the sleep–wake mechanisms involved in novel therapeutics.

Recent research has identified both sleep homeostatic (sleep slow waves) and circadian components that both modulate and mediate the antidepressant and antisuicidal effects of ketamine. The relevant mechanism(s) of action is an area of active investigation, and includes release of glutamate, the major excitatory neurotransmitter in the brain. A sequence of molecular and cellular events follows this glutamate release. These include increased α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) neurotransmission, activation of the mammalian target of rapamycin (mTOR) signaling pathway, and increased brain-derived neurotrophic factor (BDNF) activity. Ketamine also activates clock-associated gene molecules (Bellet et al. 2011), including those within reward circuits of the nucleus accumbens (Zhao et al. 2014).

Taken together, the evidence gathered to date highlights a rich field of clinical and preclinical results that can be fruitfully explored to identify links between circadian/sleep wake systems and the development of novel rapid antidepressant treatments. To further understand the homeostatic process of sleep and how it relates to the development of novel therapeutics, recent investigations have focused on glutamatergic-associated molecular and SWS associations that parallel ketamine's rapid antidepressant effects. This chapter will review current insights drawn from sleep and circadian system research related to the development of novel interventions.

2 Glutamatergic Function in Sleep and Circadian Systems

Distribution of metabotropic glutamate R2/R3 receptors is widespread, including in the prefrontal cortex (PFC), hippocampus, and the reticular and sensory thalamic nuclei, all of which have been implicated in neuropsychiatric syndromes (Wright et al. 2013). Glutamate is co-localized within orexin (also known as hypocretin) neurons of the dorsal lateral hypothalamus, which also have widespread distribution throughout the brain (Peyron et al. 1998). These neurons, whose deficiency is the cause of narcolepsy/cataplexy, become hyperactive after SD and metabolic challenge (Willie et al. 2001; Sakurai 2007).

As regards circadian regulation of sleep–wake patterns, synchronization between environmental light dark cycles and internal biological circadian rhythms is mediated in part by release of glutamate from the retinohypothalamic tract (RHT). The consequent light response of light sensitive clock genes (Roecklein et al. 2009) and their downstream molecular events – which ultimately affects sleep quality – are affected by this glutamate-initiated sequence of clock-associated effects.

Ketamine treatment elevates extracellular glutamate in the PFC (Bagley and Moghaddam 1997; Homayoun and Moghaddam 2007). In addition, glutamate plays a critical role as a proximal element in a ketamine-initiated molecular cascade that increases synaptic strength and plasticity, which ultimately results in rapidly improved mood. Glutamatergic effects are common to interventions with rapid antidepressant properties such as SD, electroconvulsive therapy (ECT) (Murck et al. 2009), and ketamine. For instance, SD (extended wakefulness) increases the availability of metabotropic glutamate receptors of subtype 5 (Hefti et al. 2013) and alters glutamatergic function (Benedetti et al. 2009). Increased cortical excitability, as measured by gamma activity, has been associated with ketamine treatment (Cornwell et al. 2012) as well as with increased prior wake time (Huber et al. 2013); both have glutamatergic underpinnings.

2.1 Ketamine-Induced Glutamatergic Signaling Initiates a Cascade of Molecular, Cellular, and BDNF-Associated Events

The rapid antidepressant properties of ketamine and one of its key metabolites, (2*R*,6*R*)-hydroxynorketamine (HNK) (Zanos et al. 2016), induce altered glutamatergic signaling and upregulation of AMPA receptors, consistent with previously described findings of increased synaptic strength and plasticity (Duman and Aghajanian 2012). Interestingly, in the study by Zanos and colleagues, (2*R*,6*R*)-HNK was essential to ketamine's antidepressant effects. Furthermore, the (2*R*,6*R*)-HNK enantiomer was responsible for behavioral, EEG, electrophysiological, and cellular antidepressant-related actions in mice without inducing any ketamine-related side effects. The specific sequence from ketamine infusion to improved mood, cognition, and behavior includes: (1) increased glutamate release; (2) early activation and upregulation of AMPA receptors; and (3) activity-dependent release of BDNF (Li et al. 2010; Maeng and Zarate 2007). Ultimately, these events activate the mTOR signaling pathway, affecting downstream changes in dendritic spines and local synaptic protein synthesis, including BDNF (Duman and Aghajanian 2012). BDNF secretion, activation of the tropomyosin-receptor-kinase B (TrkB) receptor, and downstream trafficking lead to further dendritic structural complexity, spine and BDNF synthesis, synaptic plasticity, and strengthened local circuitry (Duman and Aghajanian 2012; Maeng and Zarate 2007).

Downstream to these events, polymorphisms such as BDNF Val66Met also contribute to the altered functional effects of BDNF trafficking (Chen et al. 2004; Egan et al. 2003). Enhanced synaptic plasticity and neuronal synchronization resulting from changes in critical local neuronal circuits – especially in areas involved in mood and behavior – converge to produce rapid antidepressant effects (Maeng and Zarate 2007; Zarate et al. 2006). As discussed later in Sects. 2.3, 3.1, and 4, below, changes in BDNF, SWS, and mood are present in patients who respond to ketamine.

2.2 Clues to Rapid Antidepressant Mechanisms of Ketamine and Sleep Deprivation Are Found Within Core Elements of Sleep and Circadian Systems

In mood disorders, both homeostatic and circadian processes are dysregulated, with significant impact on sleep–wake cycles, circadian rhythms of body temperature, hormones, behavior, and mood. Patients with MDD often, but not uniformly, exhibit disturbed sleep. For example, several studies have reported an absence of disturbed sleep patterns in individuals with MDD (Frey et al. 2012; Quitkin et al. 1985; Antonijevic 2006), suggesting that distinctions such as typical versus atypical subtypes, gender, or other co-morbidities may be involved. Disturbed sleep is generally characterized by decreased sleep and sleep continuity, low levels of SWS and slow wave activity (SWA, defined as EEG activity between 1 and 4 Hz in non-rapid eye movement (NREM) sleep), short rapid eye movement (REM) latency, and increased and early morning waking (Gillin et al. 1979; Duncan et al. 1979; Kupfer et al. 1985; Pillai et al. 2011; Benca et al. 1992). Many of these disturbances are consistent with abnormal function of the sleep homeostat (SWS deficiency) and central clock (temperature and hormone rhythms). Notably, many of these features have been incorporated within the two-process model of sleep–wake regulation (described next) in which a sleep homeostat (modeled by Process S) interacts with the central circadian clock (modeled by Process C) to influence human sleep behavior and mood. Numerous chronotherapeutic sleep interventions, such as partial SD and sleep phase advance have been evaluated within this model for their capacity to alter homeostatic mechanisms and to affect interactions between sleep homeostatic and circadian processes. For instance, the antidepressant properties of bright light – the major signal responsible for synchronizing internal circadian timing to the external day – are used during bright light therapy.

The two-process model of human sleep–wake regulation (Borbély 1982) has been used to measure the timing, duration, and structure of human sleep (Dijk and Czeisler 1995) with important implications for health and depression. In healthy individuals, extended prior wakefulness is associated with a homeostatic increase in the level of "S," measured by increased SWS/SWA (a surrogate marker of "S") during recovery sleep. The "S"-deficiency hypothesis of depression (Borbély 1987; Borbély and Wirz-Justice 1982) posits that both sleep disturbances and depressive symptoms are related to low levels of "S." The rapid antidepressant effects of SD therapy in depression are hypothesized to increase "S" toward normal levels, as indicated by elevated SWS/SWA during early recovery (Borbély and Wirz-Justice 1982). Key elements of this hypothesis have further provided a basis for launching promising investigations regarding the molecular effects of SD and their role in rapid antidepressant effects, with predictions based on synaptic homeostasis (Tononi and Cirelli 2006).

The mechanism of action of rapid-acting antidepressants may be complex, involving temporal and quantitative differences in BDNF and synaptic plasticity (by SD and partial SD) on the one hand, and altered timing of the central clock (e.g., by bright light therapy) on the other. The timing of these events, along with the

course of mood changes, provides important clues regarding their relative contribution to both the rapid expression of antidepressant response and the maintenance of that response (Kuhn et al. 2016). The fact that SD does not consistently elevate BDNF (Kuhn et al. 2016; Ibrahim et al. 2011) suggests that declines in BDNF after extended wakefulness might have a protective effect against excessive cortical excitability and plasticity (Kuhn et al. 2016). Both SD and bright light therapy [as well as selective serotonin reuptake inhibitors (SSRIs)] produce rapid changes (within 12 h) in both SWA levels and clock-gene expression (Cuesta et al. 2009; Uz 2005), respectively. However, the rapid antidepressant response latencies of these interventions vary, ranging from hours (in the case of ketamine, SD, and partial SD), to several days (bright light therapy and sleep phase advance), to weeks for SSRIs (Rush et al. 2006). The distinct molecular pathways activated by homeostatic versus circadian-linked interventions may contribute differently to the rapid onset of antidepressant effects, particularly compared to the persistence (durability) of any such effects.

Chronotherapies that control the duration of prior waking – and, therefore, sleep homeostatic mechanisms [e.g., dark therapy and "extended-night" interventions (Wehr et al. 1998; Wirz-Justice et al. 1999; Barbini et al. 2005)] – reduce symptoms of mania, hypomania, and rapid cycling in BD (Barbini et al. 2005; Wehr et al. 1998; Wirz-Justice et al. 1999). Thus, controlled sleep–wake schedules, regulation of sleep homeostasis, and underlying pathways of synaptic plasticity (including glutamatergic function) may inform our understanding of sleep homeostasis and SWS as modulating elements of mood and novel mechanisms of mood stabilizers. The role of interacting homeostatic and circadian systems in developing novel treatment interventions is discussed later.

2.3 Sleep Slow Waves and Evoked Potentials Are Markers of Synaptic Plasticity

Sleep EEG and evoked potentials are useful markers of altered synaptic plasticity in humans (Huber et al. 2004, 2006), and evidence from these studies is consistent with the synaptic homeostasis hypothesis (Tononi and Cirelli 2006). High-density EEG studies have shown that interventions such as rotation learning and high-frequency transcranial magnetic stimulation (TMS) associated with synaptic potentiation in local cortical circuits lead to local increases in SWA during subsequent sleep (Huber et al. 2004). Interventions such as arm immobilization, which is associated with synaptic depression, lead to a local reduction in SWA (Huber et al. 2006). Computer simulations indicate that sleep SWA directly reflects synaptic strength due to changes in neural synchronization and recruitment (Esser et al. 2007; Vyazovskiy et al. 2007).

In addition, several studies that directly examined the effects of BDNF on EEG SWS also noted a close relationship between SWA and BDNF (Faraguna et al. 2008; Huber et al. 2007). These studies found that SWA was increased by intrahemispheric infusion of BDNF, by behavioral interventions that increase

central levels of BDNF, and by the plasticity-related genes *Arc*, *Homer,* and *NGFI-A* (Huber et al. 2007). Conversely, SWA was diminished by BDNF antagonism (Faraguna et al. 2008). The fact that acoustic suppression of SWA was associated with diminished perceptual learning (Aeschbach et al. 2008) suggests that decreased SWS levels could contribute to cognitive and memory deficits in some depressed patients. Interestingly, human carriers of the BDNF Met allele of the Val66Met polymorphism produce less SWS (Bachmann et al. 2012) and also display altered mood response to ketamine (Laje et al. 2012), thus establishing a link between genetic variants of BDNF, SWA, and mood.

3 Clinical Effects of Ketamine on Sleep and Mood

As noted above, ketamine increases SWS, memory, and plasticity, and elevates slow wave production (Campbell and Feinberg 1996; Feinberg and Campbell 1993) in addition to its antidepressant effects. Ketamine's effects on SWS suggest a possible association between this agent's rapid antidepressant effects and its plasticity-related effects. These effects on both mood and sleep have been specifically explored in MDD and BD.

In MDD, rapid antidepressant response to ketamine was linked to decreased waking, as well as increased total sleep, SWS, SWA, and REM sleep (Fig. 1) (Duncan et al. 2013b). Furthermore, clinical response to ketamine was predicted in MDD by a low baseline delta sleep ratio (defined as the quotient of SWA in the first to the second NREM episode), a measure of deficient early night production of SWS (Duncan et al. 2013a), and the abnormal production of SWS during the sleep period. Normalizing the deficient early night production of SWS by increasing early production of SWS in the first non-REM period (Fig. 1, lower panel) appears critical to ketamine's rapid antidepressant effects. Notably, although increased sleep slow waves were not present on Day 1 post-ketamine infusion, increased total sleep, REM sleep, and sleep efficiency (i.e., decreased time spent awake after sleep onset) were associated with antidepressant response (Duncan et al. 2013b) on Day 2 post-ketamine treatment, indicating their association with a more prolonged antidepressant response.

Increasing the early nighttime production of SWS (Fig. 1, upper panel) may be important to ketamine's rapid antidepressant effects. Interestingly, sleep slow waves are markers of neuronal plasticity that are similarly increased using other (non-ketamine) novel therapies, further implicating sleep and circadian systems in mood response. For instance, the effects of repetitive TMS (rTMS) on plasticity (Cohen et al. 2010) involve both sleep–wake and/or circadian-dependent processes. Applying rTMS to the left dorsolateral prefrontal cortex (DLPFC) increased SWA at F3 (Saeki et al. 2013), indicating locally enhanced synaptic plasticity, similar to ketamine's plasticity-related effects on SWS (Duncan et al. 2013b). In that rTMS study, SWA production was particularly enhanced during the first half of the sleep period (Saeki et al. 2013). This result echoes the early night effects of ketamine (see Fig. 1, top panel) in which ketamine elevated the early night peak in SWA, followed

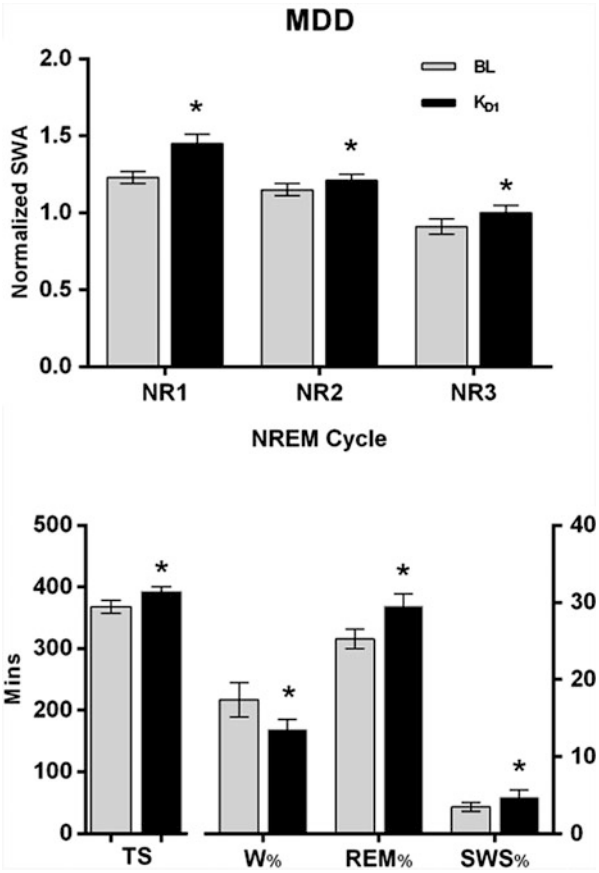

Fig. 1 Slow wave activity (SWA, *top*) during non-REM episodes (cycles) 1–3 (NR1,2,3) and selected sleep measures (*bottom*) in patients with major depressive disorder (MDD; $n = 30$) compared during baseline (BL, *grey bars*) and the first night (D1) after ketamine infusion (K_{D1}, *black bars*). All patients had severe, treatment-resistant MDD at the time of ketamine treatment. The *lower panel* shows that in patients with MDD, ketamine significantly improved sleep quality [increased Total Sleep (TS), slow wave sleep (SWS), and percent time spent in rapid eye movement sleep (REM %)], and decreased the percent of time spent awake (W%) on D1. Ketamine also increased early production of SWA during NR1, thus enhancing the nighttime decline in SWA across successive non-REM episodes. (* $p < 0.05$)

by a progressive decline in SWA levels during the remaining sleep period (Duncan et al. 2013b). This homeostatic nighttime decline, common to ketamine and to other novel therapeutics, is consistent with a pattern of synaptic downscaling that follows the gradual rise of daytime synaptic potentiation and elevated SWA at sleep onset.

Interestingly, an important discrepancy between MDD and BD was found when examining ketamine's effects on SWS in BD patients who were receiving mood stabilizer therapy. Increased SWS production in response to ketamine was not observed in this sample of BD patients, in contrast to the results obtained for MDD patients. Although rapid antidepressant effects as well as improved sleep were observed (Duncan and Zarate 2013), ketamine infusions *reduced* SWA levels in BD during non-REM episodes 1 and 3. This result has two possible interpretations. First, the finding is consistent with sleep differences between MDD and BD patients. Genetic factors influence REM sleep expression (Kupfer and Ehlers 1989) and this, in turn, indirectly affects SWS expression; the result is thus consistent with underlying genetic differences between MDD and BD. These differences may stem from complex and unstable waking patterns during mania and

hypomania (Linkowski et al. 1986) that potentially influence and confound measurement of SWS expression in BD. Second, the result is consistent with studies indicating that mood stabilizers per se influence SWS expression. The ongoing use of mood stabilizers (i.e., lithium) during ketamine infusion may thus have affected SWS and could confound any study of ketamine-induced slow wave production (Duncan and Zarate 2013).

More provocatively, this result could also suggest that the effect of mood stabilizers on SWS also contributes to their mood stabilizing properties. Controlling mood cycles by stabilizing day-to-day variations in SWS may be a property shared by dark-therapy/extended bed-rest interventions (i.e., chronotherapeutic interventions that impose fixed bed-rest schedules; these control the duration of prior wakefulness, thereby minimizing night-to-night fluctuations in SWS and ultimately stabilizing mood). This finding may echo a recent report that SWS levels correlate with mood (Eidelman et al. 2010) such that low SWS levels are associated with reduced future mania. Thus, a property of mood stabilizers might be their ability to minimize night-to-night fluctuations in SWS.

3.1 Ketamine's Rapid Effects on Mood, Sleep Slow Waves, and BDNF Levels Are Consistent with Increased Synaptic Plasticity

Response to ketamine infusions also suggest links between neuroplasticity, SWS, and mood on the one hand, and the neurotrophin BDNF on the other. Increased BDNF levels have been associated with several rapid-acting antidepressant interventions (Duncan et al. 2013b; Giese et al. 2014; Haile et al. 2014; Gorgulu and Caliyurt 2009), underscoring its key role in the rapid response mechanism. Beyond the association with rapid mood response, the magnitude of BDNF and SWS levels were correlated in ketamine responders (Duncan et al. 2013b), a finding consistent with the preclinical effects of BDNF on SWS production (Huber et al. 2007). Furthermore, the magnitude of BDNF levels measured 4 h post-ketamine infusion predicted mood response 3 days post-infusion (Haile et al. 2014), suggesting that these acute levels might provide a marker of durable response.

3.2 Ketamine's Rapid Antisuicidal Effects Reverse Late-Night Waking

An area of increasing interest is ketamine's ability to rapidly (within minutes to hours) reduce suicidal thoughts in individuals with treatment-resistant depression (DiazGranados et al. 2010b; Murrough et al. 2015; Price et al. 2009, 2014; Zarate et al. 2012). No FDA-approved medications presently exist for acute suicidal thoughts, and rapid-acting treatments are critically needed for this psychiatric emergency. Ketamine's impact on suicidal thoughts has been shown to occur independently of its antidepressant effects (Ballard et al. 2014) and has been

associated with specific neuroimaging biomarkers, specifically glucose metabolism in the infralimbic cortex (BA 25) (Ballard et al. 2015). This suggests that investigations of ketamine use in suicidal individuals could identify potential biomarkers of antisuicidal response and elucidate the neurobiological underpinnings of acute suicide risk.

Interestingly, suicide risk has also been linked to sleep difficulties. Self-reported sleep difficulties are associated with subsequent death by suicide (Bernert et al. 2014; Gunnell et al. 2013), and the association between subjective sleep difficulties and suicidal thoughts and behaviors has been found to occur independently of depressive symptoms (Pigeon et al. 2012).

More objective measures of suicidal thoughts or past behaviors have been associated with polysomnography-defined increased REM sleep percentage, REM activity, REM duration, and decreased sleep efficiency (Agargun and Cartwright 2003; Sabo et al. 1991). Evidence of seasonality and suicidal behavior, including altered circadian psychomotor activity in suicidal individuals, suggests altered chronobiological activity in suicidal patients (Hiltunen et al. 2011; Verkes et al. 1996). Chronotherapeutics – including SD in conjunction with lithium, sleep phase advance, and bright light therapy (triple chronotherapy), as well as cognitive behavioral therapy with SD (Breitstein et al. 2014) – have been associated with rapid reductions in suicidal thinking (Benedetti et al. 2014; Sahlem et al. 2014), suggesting that sleep may be a particularly important modifiable risk factor for acutely suicidal patients.

Interestingly, ketamine's antisuicidal effects may also involve the circadian sleep–wake system. First, the hours of 12:00 a.m. to 5:00 a.m. may be an especially high-risk time for suicide deaths (Perlis et al. 2016), suggesting a particular circadian interval of risk. Second, in initial investigations of this time period across EEG sleep, wakefulness over the course of the night, particularly in the 4:00–4:59 a.m. hour, was associated with suicidal thoughts the next day in a sample of depressed inpatients (Ballard et al. 2016); this effect was independent of age, gender, diagnosis (MDD vs. BD), or depressive symptom severity. Third, in evaluating wakefulness after ketamine administration, antisuicidal response (that is, complete next-day remission of suicidal thoughts) was associated with reduced wakefulness, even when adjusting for baseline sleep (Vande Voort et al. 2016). Taken together, these results point to the potential importance of sleep and circadian rhythms in ketamine's antisuicidal effects and indicate the need for further evaluation of BDNF, clock-gene expression, and cortisol on this process.

4 Ketamine's Interactive Effects on Sleep and Circadian Systems

While some ketamine studies indicate a relationship between BDNF levels and SWS amplitude, other studies describe a diurnal and/or circadian component to plasticity-evoked changes that implicate both sleep homeostatic/plasticity mechanisms and circadian-related processes. For example, effective rapid

antidepressant interventions appear related to BDNF regulation as well as circadian and homeostatic sleep systems. The presence of a diurnal rhythm for BDNF in partial SD responders (Giese et al. 2014) suggests a similarity between circadian system variations in BDNF and the circadian effects of other rapid-acting treatment interventions. Similar to SD and sleep homeostatic challenges on clock-gene expression [for a review, see (Franken and Dijk 2009)], other novel therapeutics (such as rTMS) affect clock and circadian function. Plasticity associated with rTMS was found to correlate with cortisol awakening response, a circadian biomarker related to peripheral circadian *CLOCK* genes (Clow et al. 2014); in addition, rTMS treatment increased REM latency, another circadian marker, also consistent with linkage between rTMS antidepressant effects and circadian rhythms (Cohrs et al. 1998).

5 Ketamine Alters Circadian Amplitude and Timing in MDD

Interactions between molecular and neural elements of the circadian clock and the sleep homeostat regulate the expression and timing of the human sleep–wake pattern. Chronotherapies are hypothesized to alter mood, in part by altering the timing and expression of this central clock (Benedetti et al. 2007a). Bright light therapy (Wirz-Justice and Terman 2012), low-dose melatonin (Lewy et al. 2002), and the novel therapeutic drug agomelatine (Kasper and Hamon 2009; Kasper et al. 2010) share the common property of altering the timing and expression of the central clock. The fact that glutamate is a key transmitter that functions to resynchronize the timing of the central clock with the external light–dark cycle suggests that it may also alter central circadian timing during clinical intervention.

As noted above, glutamate levels are altered by ketamine infusion. As regards the circadian clock in particular, ketamine alters the timing and amplitude of circadian activity patterns in MDD rapid responders versus non-responders (Fig. 2). While consistent with preclinical studies of ketamine's effects on circadian clock-gene-related molecules, the finding indicates that the effect is mood-dependent. Specifically, on day 1, the day after ketamine infusion (Fig. 2, left panel) rapid responders to ketamine showed phase-advanced (early) timing of the 24-h of activity pattern (as evidenced by higher AM and lower PM activity counts) relative to non-responders. Also, 2 days after the infusion (D3, right panel), responders showed an increased amplitude of the 24-h pattern of motor activity whereas non-responders did not, suggesting that the amplitude of the rhythm is more likely to be associated with durability of the mood response (given that the amplitude increases in day 3 responders) than with acute response – note, for instance, the similar amplitudes in day 1 responders versus non-responders. The mood-dependent properties may incorporate effects on clock-gene pathways, possibly within the expression of mood-associated circuits that contribute to durable clinical response. The early timing of the activity pattern on day 1 is also consistent with the beneficial effects of SD, which are associated with advanced timing of the circadian system (Benedetti et al. 2007b).

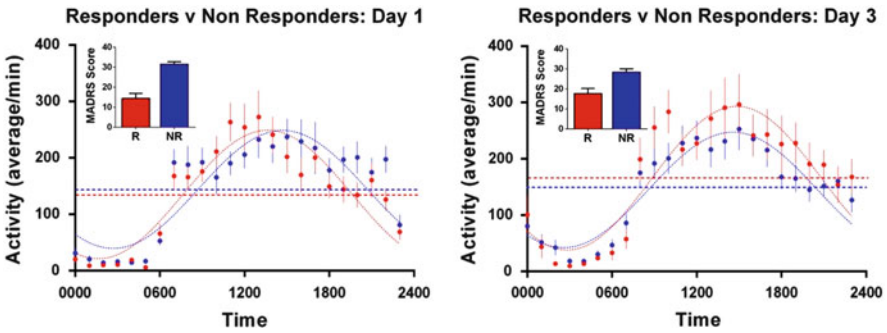

Fig. 2 Day 1 and Day 3 patterns of wrist activity for ketamine-treated patients who responded (>50% decrease in Montgomery-Asberg Depression Rating Scale (MADRS) score) within 1 day of ketamine infusion compared with non-responders. (*Left panel*) Responders (R, *red*) compared with non-responders (NR, *blue*) 1 day after ketamine infusion (Day 1). (*Right panel*) Responders (R, *red*) who maintained the 50% decrease in MADRS score compared with patients who did not meet response criteria (NR, *blue*) on Day 3. The raw MADRS scores for each group are shown as inserts for each day. On Day 1, the phase of the 24-h pattern of activity in ketamine responders differed from non-responders ($p = 0.0038$). On Day 3 the amplitude ($p = 0.0488$) of the 24-h pattern significantly differed between responders and non-responders. Group sizes are: D1 (R = 21, NR = 30) and D3 (R = 13, NR = 35)

6 Do Homeostatic and Circadian Systems Interact to Affect Ketamine's Antidepressant Properties?

SWS-associated neural plasticity (Duncan et al. 2013b) as well as clock genes and their associated molecules (Bunney and Bunney 2012, 2013) have both been linked to ketamine's rapid antidepressant effects. Clear delineation of the temporal relationship between clock-gene activation versus sleep homeostatic mechanisms can inform our understanding of how these processes contribute to rapid antidepressant response. This requires close examination of day-to-day clinical mood changes on the one hand, and specific changes in clock genes and sleep homeostasis on the other.

In the case of sleep homeostasis, clinical studies have found that rapid antidepressant response and SWS-related changes in neural plasticity both occur within 12 h of ketamine infusion (Duncan et al. 2013b; Duncan and Zarate 2013). In contrast to conventional antidepressant treatments, rapid-acting interventions such as SD and ketamine infusion quickly increase SWS production, highlighting the role of increased "S" and SWS as markers of rapid mood response. Underlying the role of SWS-associated processes in the onset of rapid response are neurotrophin release and neuronal plasticity; these are produced both by the rapid-acting effects of SD and the rapid-acting antidepressant ketamine.

Clinical studies have not directly examined ketamine's effects on clock-gene expression. In contrast, preclinical studies have shown rapid clock-gene-associated effects after ketamine infusion but, as discussed here, their role in mediating rapid

antidepressant response is unclear. For example, like ketamine, SSRIs and bright light exposure produce rapid clock-gene related effects in preclinical studies (Cuesta et al. 2009; Uz 2005), as well as demonstrated effects in MDD (Li et al. 2013; Rush et al. 2006). However, unlike ketamine, SSRIs often require 5 weeks for their antidepressant effects to manifest (Posternak and Zimmerman 2005; Rush et al. 2006), which is inconsistent with a rapidly acting antidepressant clock-gene mechanism; nevertheless, a chronic mechanism involving clock gene effects might be relevant (Li et al. 2013). Similarly, bright light therapy has rapid effects on clock-gene expression but requires several days for an antidepressant response to manifest (Benedetti et al. 2007a; Duncan 2009). Thus, the extent to which clock-gene effects are both necessary and sufficient for a rapid and/or durable antidepressant response is an important question that requires further examination of the specific circadian phase-dependent elements that both moderate and mediate rapid and durable response effects.

The fact that sleep homeostasis and clock genes interact at the molecular level (Franken 2013; Franken and Dijk 2009) allows for interacting effects that mediate clinical response. Subsequent to the initial events that trigger the cascade of molecular and plasticity-associated events – for which SWS is an important marker – the interaction between homeostatic and circadian clock processes (Archer et al. 2014) could be critical for sustaining antidepressant response.

7 Strategies for Prolonging Ketamine's Mood Response

Although ketamine provides rapid relief from depressive symptoms – with a response rate of approximately 70% within 24 h (Machado-Vieira et al. 2010) – its effects are often short-lived, and most patients relapse within 7 days. Therefore, an important goal of ongoing research is to identify mechanisms of durable response to ketamine. This approach might include pairing activation of: (a) the acute rapid response mechanism with, (b) a response maintenance mechanism. The fact that repeated chronotherapies, or chronotherapies combined with conventional treatments, extend acute rapid antidepressant effects (e.g. of single SD or partial SD interventions), suggests that repeat chronotherapies might be effectively used for extending ketamine treatment, or that incorporating their mechanisms of action may extend beneficial effects, but this remains to be evaluated.

7.1 Sleep, Naps, and Post-ketamine Relapse

For an individual who has experienced a rapid antidepressant response to ketamine, the night of sleep that follows the infusion is not predictably followed by relapse. This contrasts with the fact that few SD responders (5–10%) remain euthymic after recovery sleep (Benedetti et al. 1999; Benedetti and Colombo 2011). Furthermore, the rapid clinical benefits of both SD and partial SD are: (a) reduced by naps and microsleeps during extended waking and prior to remission itself, and (b) reduced

by naps and recovery sleep during remission (Benedetti and Colombo 2011; Hemmeter et al. 2010; Wiegand et al. 1987). The fact that relapses can be limited (or response extended) by repeated episodes of sleep phase advance (Benedetti et al. 2001; Berger et al. 1997) indicates that sleep per se is not sufficient for relapse, but that sleep stage, circadian phase, or other factors influence relapse (Wiegand et al. 1986, 1987, 1993) [see also (Hemmeter et al. 2007)]. To our knowledge, whether or not the extended antidepressant effects of sequentially applied chronotherapeutics (e.g., partial SD, sleep phase advance) are linked with extended cortical excitability and mood response remains unknown. However, the contribution of microsleeps, napping, and reduced sleep times on extending response and delaying relapse have not been carefully examined following ketamine.

Interestingly, the extension of chronotherapeutic benefits and their interaction with ECT and rTMS have also been investigated. One study found that rTMS (administered over the left DLPFC) prolonged the effects of SD (Eichhammer et al. 2002), but a second study found that active rTMS (left DLPFC) was not superior to sham rTMS in stabilizing the antidepressant effects of SD (Kreuzer et al. 2012; Eichhammer et al. 2002).

Drug interventions have also been used to extend the rapid antidepressant effects of chronotherapies. While chronotherapies, particularly bright light therapy, augment the effects of antidepressant medications (Lam and Kennedy 2015), the augmenting effects of an antidepressant and mood stabilizer (sertraline and lithium) with three chronotherapies (SD; bright light therapy; sleep phase advance) also showed rapid benefits in BD with persisting effects (Wu et al. 2009). In a study of 39 BD patients (13 of whom were treatment-resistant and 23 of whom were medicated with lithium and/or an SSRI), three interventions of SD plus bright light therapy reduced Hamilton Depression Rating Scale (HAM-D) scores by 50% in 26 of the 39 patients (Benedetti et al. 2007b). In a third study, the use of a mood stabilizer (lithium) combined with SD and bright light therapy alleviated suicidal ideation and mood symptoms (Benedetti et al. 2014). The benefits of combination therapies with ketamine have not been fully evaluated. Differences are evident in the observation that the antidepressant effects of ketamine in BD patients maintained on mood stabilizers (lithium and valproate) are not remarkably greater or more prolonged than those in drug-free MDD patients (Diazgranados et al. 2010a; Ionescu et al. 2015).

While naps blunt rapid antidepressant response to SD or partial SD (Dallaspezia and Benedetti 2011), pharmacological treatments that reduce microsleeps/naps (i.e., modafinil, caffeine, flumazenil) do not substantially enhance the rapid antidepressant response associated with SD (Beck et al. 2010; Hemmeter et al. 2007). However, one case report found that modafinil reduced napping and increased clinical benefit during SD (Even et al. 2005). On the other hand, enhanced vigilance associated with the benzodiazepine receptor antagonist flumazenil prolonged rapid antidepressant response to SD to the day *after* recovery sleep (Hemmeter et al. 2007), possibly by affecting the dynamics of process "S."

7.2 Molecular Associations with Extended Response

Extended antidepressant response has also been investigated at the molecular level. One study found that the glycogen synthase kinase (GSK) promotor variant (rs334558*C) interacts with the long/short form of the serotonin transporter (5-HTTLPR) 5HT allele to extend post-SD antidepressant response after recovery sleep (Benedetti et al. 2012), suggesting a molecular target for serotonergic interventions that might prolong response. Extended mood benefits from SD by the drug flumazenil, which affects sleep homeostasis (Hemmeter et al. 2007), and by BDNF release subsequent to ketamine infusion (Haile et al. 2014), also suggest a path for investigating extended ketamine mood effects, sleep homeostasis, and plasticity (Laje et al. 2012; Bachmann et al. 2012). Whether the active ketamine metabolite 2R,6R-HNK (Zanos et al. 2016) could also extend clinical antidepressant response is an important question.

8 Summary and Future Directions

The past decade has been a period of steady progress in developing new strategies for rapidly treating mood disorders, and understanding the antidepressant mechanisms of the glutamatergic drug ketamine has been a critical part of this progress. Ketamine's rapid effects on sleep and clock-gene related pathways have provided mechanistic antidepressant links with previously established, rapid-acting chronotherapeutic interventions. Overall, ketamine's capacity to increase neurotrophic activity and synaptic strength, normalize sleep, and reinforce the circadian system are seen as critical elements for rapid antidepressant mood effects.

Moving forward, two important areas of clinical development are needed. First, the ability of repeated infusions to extend ketamine's antidepressant effects is being evaluated. Extended effects are reminiscent of sequentially applied chronotherapeutic effects; elucidating whether repeated treatments are also associated with plasticity-associated sleep markers and molecular changes such as ketamine-associated cortical excitability will be critical for developing interventions with durable effects. Second, the potential antisuicidal effects of ketamine highlight the need for more research into the relationship between suicide risk, sleep, and circadian rhythms. One common pathway for ketamine's rapid antidepressant effects may be normalization of the circadian sleep/wake system transcriptome that, in turn, may compensate for clock-gene variant effects or for insufficient sleep caused by numerous behavioral or environmental factors. The recent identification of ketamine's active metabolite (2*R*,6*R*-HNK) (Zanos et al. 2016) will be an important element in identifying future novel treatments with fewer side effects and more durable clinical benefits.

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References

Aeschbach D, Cutler AJ, Ronda JM (2008) A role for non-rapid-eye-movement sleep homeostasis in perceptual learning. J Neurosci 28(11):2766–2772

Agargun MY, Cartwright R (2003) REM sleep, dream variables and suicidality in depressed patients. Psychiatry Res 119(1–2):33–39

Antonijevic IA (2006) Depressive disorders – is it time to endorse different pathophysiologies? Psychoneuroendocrinology 31:1–15

Archer SN, Laing EE, Moller-Levet CS, van der Veen DR, Bucca G, Lazar AS, Santhi N, Slak A, Kabiljo R, von Schantz M, Smith CP, Dijk DJ (2014) Mistimed sleep disrupts circadian regulation of the human transcriptome. Proc Natl Acad Sci U S A 111(6):E682–E691

Bachmann V, Klein C, Bodenmann S, Schafer N, Berger W, Brugger P, Landolt HP (2012) The BDNF Val66Met polymorphism modulates sleep intensity: EEG frequency- and state-specificity. Sleep 35(3):335–344

Bagley J, Moghaddam B (1997) Temporal dynamics of glutamate efflux in the prefrontal cortex and in the hippocampus following repeated stress: effects of pretreatment with saline or diazepam. Neuroscience 77(1):65–73

Ballard ED, Ionescu DF, Vande Voort JL, Niciu MJ, Richards EM, Luckenbaugh DA, Brutsche NE, Ameli R, Furey ML, Zarate CA Jr (2014) Improvement in suicidal ideation after ketamine infusion: relationship to reductions in depression and anxiety. J Psychiatr Res 58:161–166

Ballard ED, Lally N, Nugent AC, Furey ML, Luckenbaugh DA, Zarate CA Jr (2015) Neural correlates of suicidal ideation and its reduction in depression. Int J Neuropsychopharmacol 18 (1)

Ballard ED, Vande Voort JL, Bernert RA, Luckenbaugh DA, Richards EM, Niciu MJ, Furey ML, Duncan WC, Zarate CA Jr (2016) Nocturnal wakefulness is associated with next-day suicidal ideation in major depressive disorder and bipolar disorder. J Clin Psychiatry 77(7):825–831

Barbini B, Benedetti F, Colombo C, Dotoli D, Bernasconi A, Cigala-Fulgosi M, Florita M, Smeraldi E (2005) Dark therapy for mania: a pilot study. Bipolar Disord 7(1):98–101

Beck J, Hemmeter U, Brand S, Muheim F, Hatzinger M, Holsboer-Trachsler E (2010) Modafinil reduces microsleep during partial sleep deprivation in depressed patients. J Psychiatr Res 44 (13):853–864

Bellet MM, Vawter MP, Bunney BG, Bunney WE, Sassone-Corsi P (2011) Ketamine influences CLOCK:BMAL1 function leading to altered circadian gene expression. PLoS One 6(8): e23982

Benca RM, Obermeyer WH, Thisted RA, Gillin JC (1992) Sleep and psychiatric-disorders – a metaanalysis. Arch Gen Psychiatry 49(8):651–668

Benedetti F, Colombo C (2011) Sleep deprivation in mood disorders. Neuropsychobiology 64 (3):141–151

Benedetti F, Colombo C, Barbini B, Campori E, Smeraldi E (1999) Ongoing lithium treatment prevents relapse after total sleep deprivation. J Clin Psychopharmacol 19(3):240–245

Benedetti F, Barbini B, Campori E, Fulgosi MC, Pontiggia A, Colombo C (2001) Sleep phase advance and lithium to sustain the antidepressant effect of total sleep deprivation in bipolar depression: new findings supporting the internal coincidence model? J Psychiatr Res 35 (6):323–329

Benedetti F, Barbini B, Colombo C, Smeraldi E (2007a) Chronotherapeutics in a psychiatric ward. Sleep Med Rev 11(6):509–522

Benedetti F, Dallaspezia S, Fulgosi MC, Barbini B, Colombo C, Smeraldi E (2007b) Phase advance is an actimetric correlate of antidepressant response to sleep deprivation and light therapy in bipolar depression. Chronobiol Int 24(5):921–937

Benedetti F, Calabrese G, Bernasconi A, Cadioli M, Colombo C, Dallaspezia S, Falini A, Radaelli D, Scotti G, Smeraldi E (2009) Spectroscopic correlates of antidepressant response to sleep deprivation and light therapy: a 3.0 Tesla study of bipolar depression. Psychiatry Res 173(3):238–242

Benedetti F, Dallaspezia S, Lorenzi C, Pirovano A, Radaelli D, Locatelli C, Poletti S, Colombo C, Smeraldi E (2012) Gene-gene interaction of glycogen synthase kinase 3-beta and serotonin transporter on human antidepressant response to sleep deprivation. J Affect Disord 136 (3):514–519

Benedetti F, Riccaboni R, Locatelli C, Poletti S, Dallaspezia S, Colombo C (2014) Rapid treatment response of suicidal symptoms to lithium, sleep deprivation, and light therapy (chronotherapeutics) in drug-resistant bipolar depression. J Clin Psychiatry 75(2):133–140

Berger M, Vollmann J, Hohagen F, Konig A, Lohner H, Voderholzer U, Riemann D (1997) Sleep deprivation combined with consecutive sleep phase advance as a fast-acting therapy in depression: an open pilot trial in medicated and unmedicated patients. Am J Psychiatry 154 (6):870–872

Bernert RA, Turvey CL, Conwell Y, Joiner TE Jr (2014) Association of poor subjective sleep quality with risk for death by suicide during a 10-year period: a longitudinal, population-based study of late life. JAMA Psychiat 71(10):1129–1137

Borbély A (1982) Two process model of sleep regulation. Hum Neurobiol 1:195–204

Borbély AA (1987) The S-deficiency hypothesis of depression and the two-process model of sleep regulation. Pharmacopsychiatry 20(1):23–29

Borbély AA, Wirz-Justice A (1982) Sleep, sleep deprivation and depression. A hypothesis derived from a model of sleep regulation. Hum Neurobiol 1(3):205–210

Breitstein J, Penix B, Roth BJ, Baxter T, Mysliwiec V (2014) Intensive sleep deprivation and cognitive behavioral therapy for pharmacotherapy refractory insomnia in a hospitalized patient. J Clin Sleep Med 10(6):689–690

Bunney BG, Bunney WE (2012) Rapid-acting antidepressant strategies: mechanisms of action. Int J Neuropsychopharmacol 15(5):695–713

Bunney BG, Bunney WE (2013) Mechanisms of rapid antidepressant effects of sleep deprivation therapy: clock genes and circadian rhythms. Biol Psychiatry 73(12):1164–1171

Campbell IG, Feinberg I (1996) NREM delta stimulation following MK-801 is a response of sleep systems. J Neurophysiol 76(6):3714–3720

Chen ZY, Patel PD, Sant G, Meng CX, Teng KK, Hempstead BL, Lee FS (2004) Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. J Neurosci 24(18):4401–4411

Clow A, Law R, Evans P, Vallence AM, Hodyl NA, Goldsworthy MR, Rothwell JR, Ridding MC (2014) Day differences in the cortisol awakening response predict day differences in synaptic plasticity in the brain. Stress 17(3):219–223

Cohen DA, Freitas C, Tormos JM, Oberman L, Eldaief M, Pascual-Leone A (2010) Enhancing plasticity through repeated rTMS sessions: the benefits of a night of sleep. Clin Neurophysiol 121(12):2159–2164

Cohrs S, Tergau F, Riech S, Kastner S, Paulus W, Ziemann U, Ruther E, Hajak G (1998) High-frequency repetitive transcranial magnetic stimulation delays rapid eye movement sleep. Neuroreport 9(15):3439–3443

Cornwell BR, Salvadore G, Furey M, Marquardt CA, Brutsche NE, Grillon C, Zarate CA Jr (2012) Synaptic potentiation is critical for rapid antidepressant response to ketamine in treatment-resistant major depression. Biol Psychiatry 72(7):555–561

Cuesta M, Clesse D, Pevet P, Challet E (2009) New light on the serotonergic paradox in the rat circadian system. J Neurochem 110(1):231–243

Dallaspezia S, Benedetti F (2011) Chronobiological therapy for mood disorders. Expert Rev Neurother 11(7):961–970

Diazgranados N, Ibrahim L, Brutsche NE, Newberg A, Kronstein P, Khalife S, Kammerer WA, Quezado Z, Luckenbaugh DA, Salvadore G, Machado-Vieira R, Manji HK, Zarate CA Jr (2010a) A randomized add-on trial of an N-methyl-D-aspartate antagonist in treatment-resistant bipolar depression. Arch Gen Psychiatry 67(8):793–802

DiazGranados N, Ibrahim LA, Brutsche NE, Ameli R, Henter ID, Luckenbaugh DA, Machado-Vieira R, Zarate CA Jr (2010b) Rapid resolution of suicidal ideation after a single infusion of an N-methyl-D-aspartate antagonist in patients with treatment-resistant major depressive disorder. J Clin Psychiatry 71(12):1605–1611

Dijk D-J, Czeisler CA (1995) Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. J Neurosci 15:3526–3538

Duman RS, Aghajanian GK (2012) Synaptic dysfunction in depression: potential therapeutic targets. Science 338(6103):68–72

Duncan WC (2009) Non-pharmacological treatments and chronobiological aspects of bipolar disorder: implications for novel therapeutics. In: Zarate C, Manji H (ed) Bipolar depression: molecular neurobiology, clinical diagnosis and pharmacology. Birkhauser, Switzerland, pp 95–116

Duncan WC Jr, Zarate CA Jr (2013) Ketamine, sleep, and depression: current status and new questions. Curr Psychiatry Rep 15(9):394

Duncan WC Jr, Pettigrew KD, Gillin JC (1979) REM architecture changes in bipolar and unipolar depression. Am J Psychiatry 136(11):1424–1427

Duncan WC Jr, Selter J, Brutsche N, Sarasso S, Zarate CA Jr (2013a) Baseline delta sleep ratio predicts acute ketamine mood response in major depressive disorder. J Affect Disord 145 (1):115–119

Duncan WC, Sarasso S, Ferrarelli F, Selter J, Riedner BA, Hejazi NS, Yuan P, Brutsche N, Manji HK, Tononi G, Zarate CA (2013b) Concomitant BDNF and sleep slow wave changes indicate ketamine-induced plasticity in major depressive disorder. Int J Neuropsychopharmacol 16 (2):301–311

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112(2):257–269

Eichhammer P, Kharraz A, Wiegand R, Langguth B, Frick U, Aigner JM, Hajak G (2002) Sleep deprivation in depression stabilizing antidepressant effects by repetitive transcranial magnetic stimulation. Life Sci 70(15):1741–1749

Eidelman P, Talbot LS, Gruber J, Hairston I, Harvey AG (2010) Sleep architecture as correlate and predictor of symptoms and impairment in inter-episode bipolar disorder: taking on the challenge of medication effects. J Sleep Res 19(4):516–524

Esser SK, Hill SL, Tononi G (2007) Sleep homeostasis and cortical synchronization: I. Modeling the effects of synaptic strength on sleep slow waves. Sleep 30(12):1617–1630

Even C, Thuile J, Santos J, Bourgin P (2005) Modafinil as an adjunctive treatment to sleep deprivation in depression. J Psychiatry Neurosci 30(6):432–433

Faraguna U, Vyazovskiy VV, Nelson AB, Tononi G, Cirelli C (2008) A causal role for brain-derived neurotrophic factor in the homeostatic regulation of sleep. J Neurosci 28 (15):4088–4095

Feinberg I, Campbell IG (1993) Ketamine administration during waking increases delta EEG intensity in rat sleep. Neuropsychopharmacology 9:41–48

Franken P (2013) A role for clock genes in sleep homeostasis. Curr Opin Neurobiol 23(5):864–872

Franken P, Dijk DJ (2009) Circadian clock genes and sleep homeostasis. Eur J Neurosci 29 (9):1820–1829

Frey S, Birchler-Pedross A, Hofstetter M, Brunner P, Gotz T, Munch M, Blatter K, Knoblauch V, Wirz-Justice A, Cajochen C (2012) Young women with major depression live on higher homeostatic sleep pressure than healthy controls. Chronobiol Int 29:278–294

Giese M, Beck J, Brand S, Muheim F, Hemmeter U, Hatzinger M, Holsboer-Trachsler E, Eckert A (2014) Fast BDNF serum level increase and diurnal BDNF oscillations are associated with therapeutic response after partial sleep deprivation. J Psychiatr Res 59:1–7

Gillin JC, Duncan W, Pettigrew KD, Frankel BL, Snyder F (1979) Successful separation of depressed, normal, and insomniac subjects by EEG sleep data. Arch Gen Psychiatry 36 (1):85–90

Gorgulu Y, Caliyurt O (2009) Rapid antidepressant effects of sleep deprivation therapy correlates with serum BDNF changes in major depression. Brain Res Bull 80(3):158–162

Gould TD, Manji HK (2002) The Wnt signaling pathway in bipolar disorder. Neuroscientist 8 (5):497–511

Gould TD, Manji HK (2005) Glycogen synthase kinase-3: a putative molecular target for lithium mimetic drugs. Neuropsychopharmacology 30(7):1223–1237

Gunnell D, Chang SS, Tsai MK, Tsao CK, Wen CP (2013) Sleep and suicide: an analysis of a cohort of 394,000 Taiwanese adults. Soc Psychiatry Psychiatr Epidemiol 48(9):1457–1465

Haile CN, Murrough JW, Iosifescu DV, Chang LC, Al Jurdi RK, Foulkes A, Iqbal S, Mahoney JJ 3rd, De La Garza R 2nd, Charney DS, Newton TF, Mathew SJ (2014) Plasma brain derived neurotrophic factor (BDNF) and response to ketamine in treatment-resistant depression. Int J Neuropsychopharmacol 17(2):331–336

Hefti K, Holst SC, Sovago J, Bachmann V, Buck A, Ametamey SM, Scheidegger M, Berthold T, Gomez-Mancilla B, Seifritz E, Landolt HP (2013) Increased metabotropic glutamate receptor subtype 5 availability in human brain after one night without sleep. Biol Psychiatry 73 (2):161–168

Hemmeter U, Hatzinger M, Brand S, Holsboer-Trachsler E (2007) Effect of flumazenil-augmentation on microsleep and mood in depressed patients during partial sleep deprivation. J Psychiatr Res 41(10):876–884

Hemmeter UM, Hemmeter-Spernal J, Krieg JC (2010) Sleep deprivation in depression. Expert Rev Neurother 10(7):1101–1115

Hiltunen L, Suominen K, Lonnqvist J, Partonen T (2011) Relationship between daylength and suicide in Finland. J Circadian Rhythms 9:10

Homayoun H, Moghaddam B (2007) NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. J Neurosci 27(43):11496–11500

Huber R, Ghilardi MF, Massimini M, Tononi G (2004) Local sleep and learning. Nature 430 (6995):78–81

Huber R, Ghilardi MF, Massimini M, Ferrarelli F, Riedner BA, Peterson MJ, Tononi G (2006) Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. Nat Neurosci 9:1169–1176

Huber R, Tononi G, Cirelli C (2007) Exploratory behavior, cortical BDNF expression, and sleep homeostasis. Sleep 30(2):129–139

Huber R, Maki H, Rosanova M, Casarotto S, Canali P, Casali AG, Tononi G, Massimini M (2013) Human cortical excitability increases with time awake. Cereb Cortex 23(2):332–338

Ibrahim L, Duncan WC, Luckenbaugh DA, Yuan P, Machado-Vieira R, Zarate CAJ (2011) Rapid antidepressant changes with sleep deprivation in major depressive disorder are associated with changes in vascular endothelial growth factor (VEGF): a pilot study. Brain Res Bull 86:129–133

Ionescu DF, Luckenbaugh DA, Niciu MJ, Richards EM, Zarate CA Jr (2015) A single infusion of ketamine improves depression scores in patients with anxious bipolar depression. Bipolar Disord 17:438–443

JC W, Kelsoe JR, Schachat C, Bunney BG, DeModena A, Golshan S, Gillin JC, Potkin SG, Bunney WE (2009) Rapid and sustained antidepressant response with sleep deprivation and chronotherapy in bipolar disorder. Biol Psychiatry 66(3):298–301

Kasper S, Hamon M (2009) Beyond the monoaminergic hypothesis: agomelatine, a new antidepressant with an innovative mechanism of action. World J Biol Psychiatry 10(2):117–126

Kasper S, Hajak G, Wulff K, Hoogendijk WJ, Montejo AL, Smeraldi E, Rybakowski JK, Quera-Salva MA, Wirz-Justice AM, Picarel-Blanchot F, Bayle FJ (2010) Efficacy of the novel antidepressant agomelatine on the circadian rest-activity cycle and depressive and anxiety

symptoms in patients with major depressive disorder: a randomized, double-blind comparison with sertraline. J Clin Psychiatry 71(2):109–120
Kreuzer PM, Langguth B, Schecklmann M, Eichhammer P, Hajak G, Landgrebe M (2012) Can repetitive transcranial magnetic stimulation prolong the antidepressant effects of sleep deprivation? Brain Stimul 5(2):141–147
Kuhn M, Wolf E, Maier JG, Mainberger F, Feige B, Schmid H, Burklin J, Maywald S, Mall V, Jung NH, Reis J, Spiegelhalder K, Kloppel S, Sterr A, Eckert A, Riemann D, Normann C, Nissen C (2016) Sleep recalibrates homeostatic and associative synaptic plasticity in the human cortex. Nat Commun 7:12455
Kupfer DJ, Ehlers CL (1989) Two roads to rapid eye movement latency. Arch Gen Psychiatry 46 (10):945–948
Kupfer DJ, Ulrich RF, Coble PA, Jarrett DB, Grochocinski VJ, Doman J, Matthews G, Borbély AA (1985) Electroencephalographic sleep of younger depressives. Comparison with normals. Arch Gen Psychiatry 42(8):806–810
Laje G, Lally N, Mathews D, Brutsche N, Chemerinski A, Akula N, Kelmendi B, Simen A, McMahon FJ, Sanacora G, Zarate C Jr (2012) Brain-derived neurotrophic factor Val66Met polymorphism and antidepressant efficacy of ketamine in depressed patients. Biol Psychiatry 72(11):e27–e28
Lam RW, Kennedy SH (2015) STAR*D and measurement-based care for depression: don't toss out the baby. Can J Psychiatry 60(1):6–8
Lenox RH, Gould TD, Manji HK (2002) Endophenotypes in bipolar disorder. Am J Med Genet 114(4):391–406
Lewy AJ, Emens JS, Sack RL, Hasler BP, Bernert RA (2002) Low, but not high, doses of melatonin entrained a free-running blind person with a long circadian period. Chronobiol Int 19(3):649–658
Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, Li XY, Aghajanian G, Duman RS (2010) mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science 329(5994):959–964
Li SX, Liu LJ, LZ X, Gao L, Wang XF, Zhang JT, Lu L (2013) Diurnal alterations in circadian genes and peptides in major depressive disorder before and after escitalopram treatment. Psychoneuroendocrinology 38(11):2789–2799
Linkowski P, Kerkhofs M, Rielaert C, Mendlewicz J (1986) Sleep during mania in manic-depressive males. Eur Arch Psychiatry Neurol Sci 235(6):339–341
Machado-Vieira R, Baumann J, Wheeler-Castillo C, Latov D, Henter ID, Salvadore G, Zarate CA (2010) The timing of antidepressant effects: a comparison of diverse pharmacological and somatic treatments. Pharmaceuticals (Basel) 3:19–41
Maeng S, Zarate CA Jr (2007) The role of glutamate in mood disorders: results from the ketamine in major depression study and the presumed cellular mechanism underlying its antidepressant effects. Curr Psychiatry Rep 9(6):467–474
McCarthy MJ, Nievergelt CM, Kelsoe JR, Welsh DK (2012) A survey of genomic studies supports association of circadian clock genes with bipolar disorder spectrum illnesses and lithium response. PLoS One 7(2):e32091
Murck H, Schubert MI, Schmid D, Schussler P, Steiger A, Auer DP (2009) The glutamatergic system and its relation to the clinical effect of therapeutic-sleep deprivation in depression – an MR spectroscopy study. J Psychiatr Res 43(3):175–180
Murrough JW, Perez AM, Pillemer S, Stern J, Parides MK, Aan Het Rot M, Collins KA, Mathew SJ, Charney DS, Iosifescu DV (2013) Rapid and longer-term antidepressant effects of repeated ketamine infusions in treatment-resistant major depression. Biol Psychiatry 74:250–256
Murrough JW, Soleimani L, DeWilde KE, Collins KA, Lapidus KA, Iacoviello BM, Lener M, Kautz M, Kim J, Stern JB, Price RB, Perez AM, Brallier JW, Rodriguez GJ, Goodman WK, Iosifescu DV, Charney DS (2015) Ketamine for rapid reduction of suicidal ideation: a randomized controlled trial. Psychol Med 45(16):3571–3580

Perlis ML, Grandner MA, Brown GK, Basner M, Chakravorty S, Morales KH, Gehrman PR, Chaudhary NS, Thase ME, Dinges DF (2016) Nocturnal wakefulness as a previously unrecognized risk factor for suicide. J Clin Psychiatry 77(6):726–733

Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, Kilduff TS (1998) Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 18 (23):9996–10015

Pigeon WR, Pinquart M, Conner K (2012) Meta-analysis of sleep disturbance and suicidal thoughts and behaviors. J Clin Psychiatry 73(9):e1160–e1167

Pillai V, Kalmbach DA, Ciesla JA (2011) A meta-analysis of electroencephalographic sleep in depression: evidence for genetic biomarkers. Biol Psychiatry 70(10):912–919

Posternak MA, Zimmerman M (2005) Is there a delay in the antidepressant effect? A meta-analysis. J Clin Psychiatry 66(2):148–158

Price RB, Nock MK, Charney DS, Mathew SJ (2009) Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression. Biol Psychiatry 66 (5):522–526

Price RB, Iosifescu DV, Murrough JW, Chang LC, Al Jurdi RK, Iqbal SZ, Soleimani L, Charney DS, Foulkes AL, Mathew SJ (2014) Effects of ketamine on explicit and implicit suicidal cognition: a randomized controlled trial in treatment-resistant depression. Depress Anxiety 31 (4):335–343

Quitkin FM, Rabkin JG, Stewart JW, McGrath PJ, Harrison W, Davies M, Goetz R, Puig-Antich J (1985) Sleep of atypical depressives. J Affect Disord 8:61–67

Roecklein KA, Rohan KJ, Duncan WC, Rollag MD, Rosenthal NE, Lipsky RH, Provencio I (2009) A missense variant (P10L) of the melanopsin (OPN4) gene in seasonal affective disorder. J Affect Disord 114:279–285

Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, Niederehe G, Thase ME, Lavori PW, Lebowitz BD, McGrath PJ, Rosenbaum JF, Sackeim HA, Kupfer DJ, Luther J, Fava M (2006) Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. Am J Psychiatry 163(11):1905–1917

Sabo E, Reynolds CF 3rd, Kupfer DJ, Berman SR (1991) Sleep, depression, and suicide. Psychiatry Res 36(3):265–277

Saeki T, Nakamura M, Hirai N, Noda Y, Hayasaka S, Iwanari H, Hirayasu Y (2013) Localized potentiation of sleep slow-wave activity induced by prefrontal repetitive transcranial magnetic stimulation in patients with a major depressive episode. Brain Stimul 6(3):390–396

Sahlem GL, Kalivas B, Fox JB, Lamb K, Roper A, Williams EN, Williams NR, Korte JE, Zuschlag ZD, El Sabbagh S, Guille C, Barth KS, Uhde TW, George MS, Short EB (2014) Adjunctive triple chronotherapy (combined total sleep deprivation, sleep phase advance, and bright light therapy) rapidly improves mood and suicidality in suicidal depressed inpatients: an open label pilot study. J Psychiatr Res 59:101–107

Sakurai T (2007) The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness. Nat Rev Neurosci 8(3):171–181

Tononi G, Cirelli C (2006) Sleep function and synaptic homeostasis. Sleep Med Rev 10(1):49–62

Uz T (2005) Effect of fluoxetine and cocaine on the expression of clock genes in the mouse hippocampus and striatum. Neuroscience 134:1309–1316

Vande Voort JL, Ballard ED, Luckenbaugh DA, Bernert RA, Richards EM, Niciu MJ, Park L, Machado-Vieira R, Duncan WC, Zarate CA (2016) Antisuicidal response following ketamine infusion is associated with decreased nighttime wakefulness in major depressive disorder and bipolar disorder. J Clin Psychiatry. Doi: 10.4088/JCP.15m10440. [Epub ahead of print]

Verkes RJ, Kerkhof GA, Beld E, Hengeveld MW, van Kempen GM (1996) Suicidality, circadian activity rhythms and platelet serotonergic measures in patients with recurrent suicidal behaviour. Acta Psychiatr Scand 93(1):27–34

Vyazovskiy VV, Riedner BA, Cirelli C, Tononi G (2007) Sleep homeostasis and cortical synchronization: II. A local field potential study of sleep slow waves in the rat. Sleep 30(12):1631–1642

Wehr TA, Goodwin FK (eds) (1983) Circadian rhythms in psychiatry. Psychobiology and psychopathology, vol 2. Boxwood Press, New York, NY

Wehr TA, Turner EH, Shimada JM, Lowe CH, Barker C, Leibenluft E (1998) Treatment of a rapidly cycling bipolar patient by using extended bed rest and darkness to stabilize the timing and duration of sleeo. Biol Psychiatry 43:822–828

Wiegand M, Berger M, Zulley J, von Zerssen D (1986) The effect of trimipramine on sleep in patients with major depressive disorder. Pharmacopsychiatry 19:198–199

Wiegand M, Berger M, Zulley J, Lauer C, von Zerssen D (1987) The influence of daytime naps on the therapeutic effect of sleep deprivation. Biol Psychiatry 22(3):389–392

Wiegand M, Riemann D, Schreiber W, Lauer CJ, Berger M (1993) Effect of morning and afternoon naps on mood after total sleep deprivation in patients with major depression. Biol Psychiatry 33:467–476

Willie JT, Chemelli RM, Sinton CM, Yanagisawa M (2001) To eat or to sleep? Orexin in the regulation of feeding and wakefulness. Annu Rev Neurosci 24:429–458

Wirz-Justice A, Terman M (2012) Chronotherapeutics (light and wake therapy) as a class of interventions for affective disorders. Handb Clin Neurol 106:697–713

Wirz-Justice A, Van den Hoofdakker RH (1999) Sleep deprivation in depression: what do we know, where do we go? Biol Psychiatry 46(4):445–453

Wirz-Justice A, Quinto C, Cajochen C, Werth E, Hock C (1999) A rapid-cycling bipolar patient treated with long nights, bedrest and light. Biol Psychiatry 45:1075–1077

Wright RA, Johnson BG, Zhang C, Salhoff C, Kingston AE, Calligaro DO, Monn JA, Schoepp DD, Marek GJ (2013) CNS distribution of metabotropic glutamate 2 and 3 receptors: transgenic mice and [(3)H]LY459477 autoradiography. Neuropharmacology 66:89–98

Wu JC, Kelsoe JR, Schachat C, Bunney BG, DeModena A, Golshan S, Gillin JC, Potkin SG, Bunney WE (2009) Rapid and sustained antidepressant response with sleep deprivation and chronotherapy in bipolar disorder. Biol Psychiatry 66 (3):298–301

Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI, Alkondon M, Yuan P, Pribut HJ, Singh NS, Dossou KS, Fang Y, Huang XP, Mayo CL, Wainer IW, Albuquerque EX, Thompson SM, Thomas CJ, Zarate CA Jr, Gould TD (2016) NMDAR inhibition-independent antidepressant actions of ketamine metabolites. Nature 533(7604):481–486

Zarate CA Jr, Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA, Charney DS, Manji HK (2006) A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch Gen Psychiatry 63(8):856–864

Zarate CA Jr, Brutsche NE, Ibrahim L, Franco-Chaves J, Diazgranados N, Cravchik A, Selter J, Marquardt CA, Liberty V, Luckenbaugh DA (2012) Replication of ketamine's antidepressant efficacy in bipolar depression: a randomized controlled add-on trial. Biol Psychiatry 71 (11):939–946

Zhao C, Eisinger BE, Driessen TM, Gammie SC (2014) Addiction and reward-related genes show altered expression in the postpartum nucleus accumbens. Front Behav Neurosci 8:388

Adenosine and Sleep

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Contents

The authors dedicate this chapter to the late Prof. Osamu Hayaishi, whose demise saddened everyone who knew him as a great scientist and extraordinary individual.

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Abstract

The classic endogenous somnogen adenosine promotes sleep via A_1 and A_{2A} receptors. In this chapter, we present an overview of the current knowledge regarding the regulation of adenosine levels, adenosine receptors, and available pharmacologic and genetic tools to manipulate the adenosine system. This is followed by a summary of current knowledge of the role of adenosine and its receptors in the regulation of sleep and wakefulness. Despite strong data implicating numerous brain areas, including the basal forebrain, the tuberomammillary nucleus, the lateral hypothalamus, and the nucleus accumbens, in the adenosinergic control of sleep, the complete neural circuitry in the brain involved in the sleep-promoting effects of adenosine remains unclear. Moreover, the popular demand for natural sleep aids has led to a search for natural compounds that can promote sleep via adenosine receptor activation. Finally, we discuss the effects of caffeine in man and the possible use of more selective adenosine receptor drugs for the treatment of sleep disorders.

Keywords

Adeno-associated virus • Astrocytes • CGS 21680 • DREADD • Istradefylline • Modafinil • Non-rapid eye movement sleep • Optogenetics • Prostaglandin D_2 • Slow-wave sleep

1 The Concept of a Sleep Substance

The neural and cellular basis of the need for sleep or, alternatively, the "sleep drive" remains unresolved, but can be conceptualized as a homeostatic pressure that builds up during the waking period and dissipates during sleep. One potential mechanism is that the gradual accumulation of one or more endogenous somnogenic factors during wake is the underpinning of sleep homeostatic pressure. Rosenbaum presented the first formal hypothesis that sleep is regulated by humoral factors in 1892 (Rosenbaum 1892), and Ishimori (Ishimori 1909; Kubota 1989) and Pieron (Legendre and Pieron 1913) independently demonstrated the existence of sleep-promoting chemicals a few years later. Both Ishimori and Pieron proposed, and indeed established, the presence of hypnogenic substances or "hypnotoxins" in the cerebrospinal fluid of sleep-deprived dogs (Inoué et al. 1995). Over the past century, several additional putative hypnogenic substances implicated in the sleep homeostatic process have been identified [for review, see Urade and Hayaishi (2011)], including prostaglandin D_2 (Qu et al. 2006; Ueno et al. 1982) [for review,

see Urade and Lazarus (2013)], cytokines (Krueger et al. 1984) [for review, see Krueger et al. (2011)], adenosine (Porkka-Heiskanen et al. 1997), anandamide (Garcia-Garcia et al. 2009), and the urotensin II peptide (Huitron-Resendiz et al. 2005). Extensive evidence suggests that sleep regulation is interrelated with components of the host defense (immune) system, such as pro-inflammatory cytokines (Krueger and Majde 2003; Krueger et al. 2001; Mullington et al. 2000, 2001) and prostaglandins (Lazarus et al. 2007; Oishi et al. 2015; Urade and Lazarus 2013; Ushikubi et al. 1998). Several excellent reviews of the different theories of how neural switching occurs between sleep and wakefulness are available [for example, Fuller et al. (2015); Saper et al. (2005, 2010)]. In the present chapter, we focus on the possible role of adenosine as a sleep substance.

2 Adenosine in Physiology and Pathophysiology

Adenosine is a neuromodulator and not a neurotransmitter. Although it is released from nerve endings, its formation can be increased by various processes in all types of cells, and in all parts of these cells. Furthermore, the basal level of adenosine depends only on fundamental cell biology and is independent of nerve activity. Adenosine acts on four evolutionarily well-conserved receptors that are present on most if not all cells. Adenosine fulfills physiologic and pathophysiologic functions (Fredholm 2014).

2.1 Regulation of Adenosine Levels

Adenosine is formed by hydrolysis of adenosine monophosphate (AMP) or *S*-adenosylhomocysteine (Fredholm 2007; Schrader 1983). Adenosine is formed from *S*-adenosylhomocysteine by the enzyme *S*-adenosylhomocysteine hydrolase, which can also act to trap adenosine in the presence of excess L-homocysteine. This takes place intracellularly and the fact that the enzyme is bidirectional ensures the constant presence of a finite concentration of adenosine in the cell. The formation of adenosine from 5′-AMP can occur both intracellularly and extracellularly, mediated by different enzymes. The intracellular 5′nucleotidase generates adenosine, which can be used to generate AMP by adenosine kinase. This bidirectional reaction ensures the constant presence of a finite intracellular concentration of adenosine in the range of 10 to a few hundred nanomolar (nM) under physiologic conditions (Ballarin et al. 1991).

The fact that the intracellular concentration of adenosine is not zero ensures that there is also a not insubstantial extracellular concentration of adenosine, because all cells appear to possess one or more equilibrative purine transporters (Geiger and Fyda 1991). Extracellular adenosine is increased when an adenine nucleotide is released. Extracellular adenosine is formed by the conversion of ATP to adenosine by a series of ecto enzymes on the cell surface. Extracellular ATP can be released from various cell types by multiple mechanisms, including co-release from storage

vesicles together with other hormones (neurotransmitter), a "kiss-and-run" mechanism (MacDonald et al. 2006), lysosome exocytosis (Zhang et al. 2007), controlled release through pannexin hemichannels (Chekeni et al. 2010; Elliott et al. 2009), release from inflammatory cells or vascular endothelia through connexin hemichannels and channels such as P2X7 receptors (Chen et al. 2006; Faigle et al. 2008; Linden 2006), and uncontrolled leakage from necrotic cells (Eltzschig 2009). Extracellular ATP and adenosine diphosphate (ADP) are broken down to AMP via many different ecto enzymes, especially CD39 (Yegutkin 2008). In the brain, the AMP formed is then broken down to adenosine only via ecto-5′-nucleotidase, CD73 (Resta et al. 1998). Importantly, the levels of extracellular adenine nucleotides are particularly high when cell membranes are broken, which allows the high intracellular content to escape. Thus, trauma in any form is associated with elevated levels of adenine nucleotides, as is blood sampling because most methods for this lead to a breakdown of blood platelets. AMP is also released directly from apoptotic cells (Yamaguchi et al. 2014).

ADP is released by platelets (Hollopeter et al. 2001), and ATP can be released by many different cells, including endothelial cells (Bodin and Burnstock 1998), astrocytes (Guthrie et al. 1999), and neurons (Fields and Stevens 2000), via many different mechanisms (Burnstock and Verkhratsky 2012). In the brain, extracellular adenosine might originate from neurons (both nerve terminals and postsynaptic components) and from surrounding non-neuronal cells such as glial cells (Halassa et al. 2007, 2009). For example, using inducible, astrocyte-specific transgenic dominant negative SNARE mouse approaches, Haydon's group suggested that astrocytes are an important source of extracellular adenosine via gliotransmission (Halassa et al. 2009). ATP released from neurons (both nerve terminals and postsynaptic components) also contributes to extracellular adenosine production via the CD73 enzyme, the only enzyme that degrades AMP to adenosine in the brain (Lovatt et al. 2012; Wall and Dale 2013). In striatal neurons, extracellular adenosine formed via CD73 may preferentially act at the A_{2A} receptor as extracellular CD73 is selectively co-expressed (Ena et al. 2013) and is physically associated with A_{2A} receptors in striatopallidal neurons (Augusto et al. 2013). Under pathologic conditions, such as cortical seizures, adenosine-mediated synaptic depression is independent of CD73 activity and not a consequence of astrocytic (or neuronal) ATP release, but is due to the activation of postsynaptic neurons, which leads to the release of adenosine, thus constituting an autonomic feedback mechanism that suppresses excitatory transmission during prolonged activity (Lovatt et al. 2012). It may also be that under physiologic conditions, such as those involved in sleep and wakefulness, adenosine is generated in a similar CD73-independent manner.

Adenosine levels are decreased by the enzyme adenosine deaminase (ADA; this enzyme is particularly important when adenosine levels are high) and by uptake into cells other than those that produced it. In these cells, the adenosine taken up is rapidly phosphorylated to AMP by adenosine kinase. The formation and removal of extracellular adenosine determine its levels. Under basal conditions, these levels are low, usually in the order of 30–300 nM (Ballarin et al. 1991). Under more extreme conditions, such as mild hypoxia or strenuous exercise, the levels can approach

1 μM or more, and in severely traumatic situations, including local ischemia, can reach up to several tens of μM (Fredholm 2007).

To link adenosine levels to sleep, one must be able to measure adenosine levels in the brain but adenosine levels rapidly change in both the blood and tissue upon sampling and tissue samples must be frozen within a second or less to preserve in vivo levels. Such rapid inactivation is difficult to achieve, even with focused microwave techniques, and hence measuring regional adenosine brain levels by sampling tissue is highly problematic. Microdialysis could be a feasible method, but as already demonstrated in the first reports using this technique, it takes a long time to overcome the consequences of the initial traumatization required to insert the microdialysis probes (Ballarin et al. 1991; Benveniste et al. 1989; Zetterstrom et al. 1982). Furthermore, if the probe is allowed to remain in the tissue too long it will be covered with glial cells that hamper the exchange of purines (Benveniste et al. 1989). Electrochemical methods were recently used to measure local adenosine levels (Dale and Frenguelli 2012), but this method is also associated with caveats. Thus, reported adenosine levels in sleep and wakefulness should not be uncritically accepted.

2.2 Adenosine Receptors

Extracellular adenosine reacts with one of the four adenosine receptors, A_1, A_{2A}, A_{2B}, and A_3 (Fredholm et al. 2011). If these receptors are expressed at the same level (~200,000 receptors/cell), adenosine appears to be equally potent at A_1, A_{2A}, and A_3 receptors (Fig. 1), and the levels of adenosine occurring under basal physiologic conditions are sufficient to activate these receptors. The data suggest that higher concentrations of adenosine are needed to activate A_{2B} receptors. Nevertheless, it is important to remember that the potency of an agonist such as adenosine on its receptor depends on the number of receptors available, i.e., in the presence of only a few receptors higher adenosine concentrations are required to see an effect. Local expression of the A_1 and A_{2A} receptors appears to be higher than that of the other two receptors (Fredholm et al. 2005a). Thus, these two receptor types may be primarily involved in sleep regulation.

First, we briefly discuss how the role(s) of adenosine in physiology and pathophysiology can be determined.

3 How Do We Learn About the Roles of Adenosine?

3.1 Receptor Antagonists Including Caffeine

To assess the in vivo actions of adenosine receptors, selective pharmacologic tools are crucial. Over the last 20 years, medicinal chemistry has generated agonists and antagonists with high affinity (Kd values in the low nM range) and selectivity (>100–200-fold over other adenosine receptor subtypes) for the human variants of

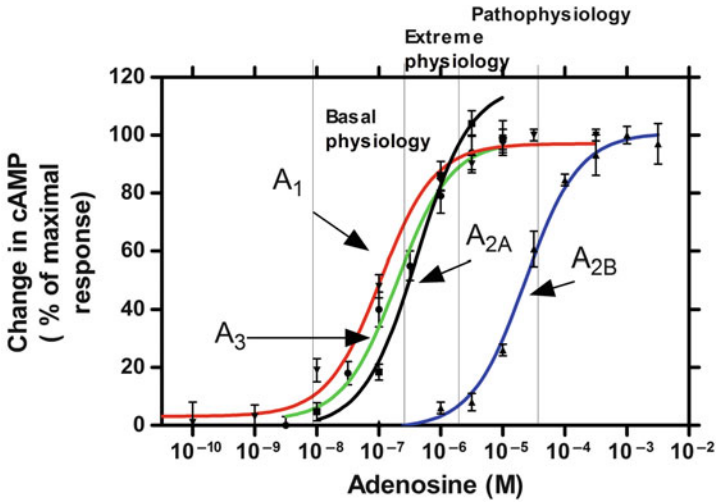

Fig. 1 Schematic illustration of the ability of adenosine to activate the four adenosine receptors. Note that A_1, A_{2A}, and A_3 receptors are activated by basal levels of adenosine at sites where the receptor number is high. In contrast, A_{2B} receptors are mostly activated in pathologic conditions

each of the four receptors (Fredholm et al. 2011). Most of the known adenosine receptor agonists are derivatives of purine nucleosides, either adenosine or xanthosine, while adenosine receptor antagonists have diverse structures (Müller and Jacobson 2011). Many A_{2A}-selective antagonists from different structural classes have been developed, including 8-(3-chlorostyryl)caffeine, MSX-2, and its water-soluble prodrugs MSX-3, ZM 241385, SCH-58261, and KW6002. In addition, radioactive, and more recently, fluorescent, ligands of adenosine receptors were also developed and introduced for drug screening and monitoring in vivo receptor occupancy in humans.

Caffeine, the most widely consumed arousal-promoting psychostimulant, is a classic nonselective adenosine receptor antagonist, although it is rather weakly potent at adenosine receptors ($K_i \approx 10$ μM). At doses commonly consumed by humans, caffeine produces its profound arousal effect by partial (estimated to be 25–50%) and nonselective (similar affinity for both A_1 and A_{2A} receptors) blockade of adenosine receptors (Fredholm et al. 1999). Caffeine is metabolized to paraxanthine and theophylline (Arnaud 2011). These metabolites are more potent than caffeine as inhibitors of A_1 and A_{2A} receptors. Therefore, elimination of caffeine does not predict the elimination of adenosine receptor blockade and hence the effects of caffeine administration.

Importantly, several A_{2A} receptor antagonists are in clinical trials for Parkinson's disease (PD), including istradefylline (KW6002), SYN-115, and preladenant (SCH-442416). Phase IIB and III clinical trials with A_{2A} receptor antagonists showed a very consistent and excellent safety profile in more than 3,000 patients with advanced PD (Hauser et al. 2011; Jenner et al. 2009). The safety profile of these

A_{2A} receptor antagonists is entirely consistent with the widespread use of the nonselective adenosine receptor antagonist caffeine in 70% of the human population. Importantly, this provides an opportunity to rapidly translate A_{2A} receptor antagonists to achieve pharmacologic control of the sleep–wake cycle.

3.2 Receptor Knockouts and Other Genetic Targeting Techniques

Over the past two decades, genetic knockout (KO) models for all four G-protein-coupled adenosine receptors were generated by targeted deletion of critical exons (Fredholm et al. 2005b; Wei et al. 2011). These adenosine receptor KO models have provided insights into the physiologic function of modulation of the sleep–wake cycle by overcoming the limitations of pharmacologic agents with partial specificity and by targeting the adenosine receptor in defined cellular populations. For example, the use of A_{2A} receptor KO models can overcome concerns about the partial specificity of A_{2A} receptor antagonists (particularly after focal injection at relatively high concentrations), and convincingly demonstrated that the sleep-promoting effect of A_{2A} receptor agonists and caffeine-induced arousal effect are mediated by A_{2A} receptors (not A_1 receptors). Global A_1 and A_{2A} receptor KO approaches, however, have intrinsic limitations of the confounding developmental effect and lack of cell-type specificity (Fredholm et al. 2005b). To overcome these limitations, conditional KO of some adenosine receptor genes in defined brain regions (e.g., forebrain versus striatum) and cell-type (e.g., neurons versus astrocytes) has been achieved using the Cre-*loxP* system [for review see Fuller et al. (2015); Wei et al. (2011)]. Brain-regional deletion of A_{2A} receptors has been achieved in the forebrain (i.e., striatum, cortex, hippocampus) (Bastia et al. 2005; Yu et al. 2008) and striatum (Shen et al. 2008). Local deletion of A_1 receptors in hippocampal CA1 or CA3 neurons and A_{2A} receptors in the nucleus accumbens (NAc) has also been achieved by local injection of adeno-associated virus (AAV) vectors containing the *cre* transgene into the brains of mice carrying *loxP*-flanked A_1 receptor (Scammell et al. 2003) or A_{2A} receptor (Lazarus et al. 2011) genes. The conditional KO strategy permits a temporal and regional specificity that has uncovered previously under-appreciated functions of adenosine receptors in the basal ganglia for controlling the sleep–wake cycle (see detailed discussion below). In addition, the development of AAV carrying short-hairpin RNA targeted to produce site-specific silencing of the A_{2A} receptor gene allowed for the clear demonstration in rats that the arousal effect of caffeine is mediated by A_{2A} receptors in the NAc shell (Lazarus et al. 2011). Lastly, the recent development of optogenetics based on specific local modulation of neuronal activity using genetically engineered optical switches (e.g., channelrhodopsin) (Boyden et al. 2005; Deisseroth 2014; Yizhar et al. 2011) or chemogenetics to study G-protein signaling in freely behaving animals by the directed molecular evolution of designer receptors exclusively activated by designer dugs (DREADD) (Farrell et al. 2013; Giguere et al. 2014) has refined our understanding of novel brain circuits underlying the sleep–wake

cycle (Fuller et al. 2015). Recently, a probe for selective optogenetic control of A_{2A} receptor signaling (optoA_{2A} receptor) was developed (Li et al. 2015).

4 Adenosine and Sleep

4.1 Adenosine Levels During Sleep and Wakefulness

Adenosine has long been known to represent a state of relative energy deficiency: ATP depletion and the elevation of extracellular adenosine levels are positively correlated (Kalinchuk et al. 2003) and positively associated with sleep (Porkka-Heiskanen et al. 1997). Adenosine levels in samples collected from several brain areas of cats during spontaneous sleep–wake cycles by in vivo microdialysis were higher during non-rapid eye movement (non-REM, NREM) sleep than wakefulness for all probed brain areas (Porkka-Heiskanen et al. 1997, 2000). Moreover, in vivo microdialysis experiments in the brain of cats also revealed that adenosine concentrations specifically increase twofold in the basal forebrain (BF) during a prolonged 6-hour period of wakefulness compared with that at the beginning of sleep deprivation (Porkka-Heiskanen et al. 1997, 2000).

Sixty years have passed since the discovery of the hypnotic effect of adenosine in the mammalian brain (Feldberg and Sherwood 1954); however, the brain cell types involved in the sleep-promoting effects of adenosine remain unclear. In principle, adenosine (and ATP, which is rapidly degraded to adenosine) can be released from neurons or glia cells. Genetically engineered mice that selectively express a dominant negative SNARE domain in astrocytes to nonspecifically block the release of ATP exhibit decreased concentrations of extracellular adenosine (Pascual et al. 2005). Although the amount of wakefulness, NREM, and REM sleep in these mice is indistinguishable from that in wild-type mice, these mice exhibit reduced slow-wave activity and recovery sleep after sleep deprivation (Halassa et al. 2009), suggesting that adenosine released from astrocytes is involved in the accumulation of sleep pressure. Direct proof is still lacking, however, and thus the exact sources of adenosine remain unknown.

The late Miodrag Radulovacki and colleagues extensively investigated the effects of adenosine on wakefulness. They used the ADA inhibitor deoxycoformycin to increase the levels of adenosine in the central nervous system of rats and found that REM and NREM sleep were increased (Radulovacki et al. 1983), further supporting a hypnotic role for adenosine.

Adenosine is reported to promote sleep by acting through A_1 or A_{2A} receptors, but the relative contribution of these receptors to sleep induction remains controversial (Basheer et al. 2004; Huang et al. 2007). Indirect evidence by comparison of the effects of caffeine, the A_1 receptor antagonist 8-cyclopentyltheophylline, and the nonselective A_1/A_{2A} receptor antagonist alloxazine on sleep in rats (Virus et al. 1990) might partially account for the prevailing opinion that the A_1 receptor is more important in sleep–wake regulation than the A_{2A} receptor. The aforementioned pharmacologic approach and related studies, however, have non-trivial limitations,

particularly with respect to data interpretation. For example, receptor antagonists are difficult to compare due to differences in solubility, blood–brain-barrier permeability, and neuropharmacodynamics, and most importantly, have "off-target" effects, especially at higher concentrations. Moreover, the diffuse expression of inhibitory A_1 receptors in the brain may have differential effects on sleep and wakefulness in a region-specific manner (Ochiishi et al. 1999a, b; Reppert et al. 1991; Rivkees et al. 1995). The advent of genetically engineered systems, including transgenic animals and recombinant viral vectors, and findings in humans have convincingly established over the last decade a pivotal role of A_{2A} receptors in the regulation of sleep and wakefulness (Holst and Landolt 2015; Lazarus et al. 2012, 2013).

4.2 Effects of A_1 Receptors

The A_1 receptor agonist N^6-cyclopentyladenosine produces dose-dependent increases in slow-wave activity in electroencephalography during NREM sleep when administered systemically or intracerebroventricularly in rats (Benington et al. 1995), but lateral ventricle infusions of N^6-cyclopentyladenosine in mice do not change the amounts of observed NREM and REM sleep (Urade et al. 2003), which may indicate opposing effects on sleep and wakefulness in different areas of the brain. For example, adenosine acting via A_1 receptors induces sleep by inhibiting arousal-related cell groups in the BF, such as the horizontal limb of the diagonal band of Broca and the substantia innominata (Fig. 2a) (Alam et al. 1999; Strecker et al. 2000). Moreover, adenosine may promote sleep by A_1 receptor-mediated inhibition of glutamatergic inputs to cortically projecting cholinergic and γ-aminobutyric acid (GABA) neurons of the BF (Yang et al. 2013). Adenosine could also promote sleep by suppressing hypocretin/orexin neurons in the lateral hypothalamus, because an A_1 receptor agonist produced NREM and REM sleep and the receptor's antagonist induced wakefulness (Fig. 2b) (Thakkar et al. 2008). ADA is predominantly localized in the tuberomammillary nucleus (TMN) of the brain and the TMN is enriched in histamine neurons containing A_1 receptors, thereby suggesting that the histaminergic arousal system is actively regulated by adenosine in the TMN. In fact, bilateral injections of the A_1 receptor agonist N^6-cyclopentyladenosine into the rat TMN significantly increase the amount of NREM sleep (Fig. 2c) (Oishi et al. 2008). Moreover, bilateral injections of adenosine or the ADA inhibitor coformycin into the rat TMN also increase NREM sleep, which is completely abolished by co-administration of the selective A_1 receptor antagonist 1,3-dimethyl-8-cyclopenthylxanthine. These results indicate that endogenous adenosine in the TMN suppresses the histaminergic system via A_1 receptors to promote NREM sleep. Interestingly, single-nucleotide polymorphism analyses have identified a human genetic ADA variant with low enzymatic activity that is linked to the enhancement of deep sleep and slow-wave activity during sleep (Bachmann et al. 2012; Mazzotti et al. 2012; Rétey et al. 2005). By contrast, activation of A_1 receptors in the lateral preoptic area of the hypothalamus by

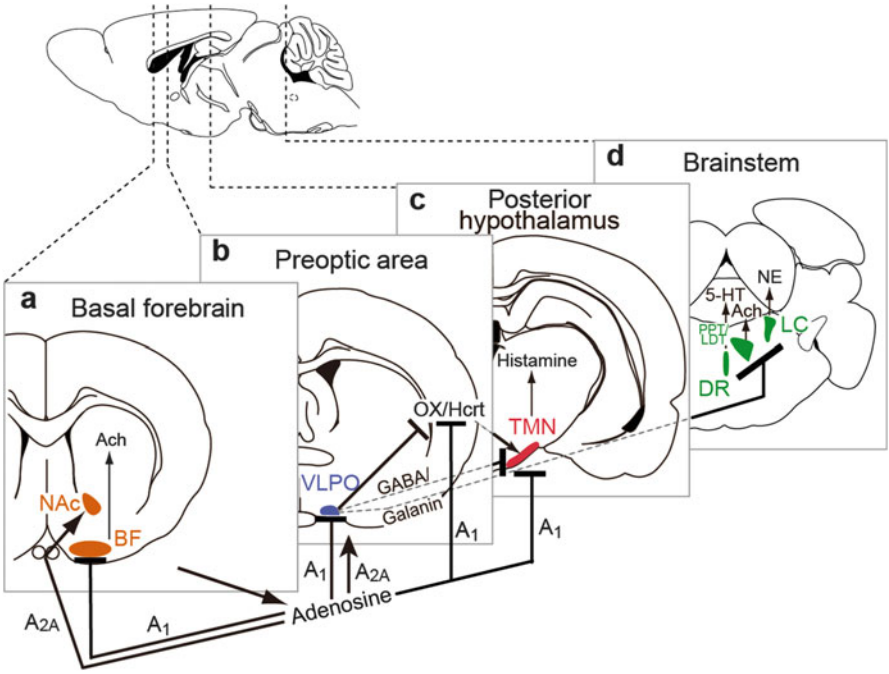

Fig. 2 Circuit basis of sleep–wake regulation. Model 1 (shown in panel **a**): adenosine inhibits the release of acetylcholine from the basal forebrain (BF) cholinergic neurons to produce slow-wave sleep. Model 2 (shown in panels **b**–**d**): a flip–flop switching mechanism involving mutually inhibitory interactions between sleep-promoting neurons in the ventrolateral preoptic area (VLPO) and wake-promoting neurons in the hypothalamus [i.e., histaminergic tuberomammillary nucleus (TMN)], and brainstem [i.e., noradrenergic locus coeruleus (LC), serotonergic dorsal raphe nucleus (DR), and cholinergic laterodorsal tegmental nucleus (LDT)]. The flip-flop switch between the VLPO and hypothalamus and brainstem is stabilized by orexin/hypocretin (OX/Hcrt) inputs from the lateral hypothalamus (LHA). Adenosine acts as an endogenous somnogen and promotes sleep via inhibitory A_1 receptors (A_1) in the basal forebrain, VLPO, LHA, and TMN and excitatory A_{2A} receptors (A_{2A}) in the nucleus accumbens (NAc) and VLPO (Huang et al. 2007, 2011; Lazarus et al. 2012, 2013). Other abbreviations: *Ach* acetylcholine, *5-HT* serotonin, *NE* norepinephrine

local infusion of an A_1 receptor agonist promotes wakefulness (Methippara et al. 2005).

4.3 Effects of A_{2A} Receptors

CGS 21680, a highly selective A_{2A} receptor agonist, produces profound increases in NREM and REM sleep after infusion into the subarachnoid space underlying the ventral surface region of the rostral BF in rats or into the lateral ventricle of mice (Satoh et al. 1996; Urade et al. 2003). In vivo microdialysis experiments demonstrated that infusions of CGS 21680 into the BF inhibit the release of

histamine in both the frontal cortex and medial preoptic area in a dose-dependent manner, and increase the release of GABA in the TMN of the hypothalamus, but not in the frontal cortex (Hong et al. 2005). CGS 21680-induced blocking of histamine release is antagonized when the TMN is perfused with the GABA antagonist picrotoxin, suggesting that the A_{2A} receptor agonist induces sleep by inhibiting the histaminergic system through an increase in GABA release in the TMN. It was previously proposed that sleep is promoted by activating sleep neurons in the ventrolateral preoptic area (VLPO) and reciprocal suppression of histaminergic wake neurons in the TMN through GABAergic and galaninergic inhibitory projections (Sherin et al. 1996, 1998). The existence of two distinct types of VLPO neurons in terms of their responses to serotonin and adenosine was demonstrated by intracellular recordings of VLPO neurons in rat brain slices (Fig. 2b). VLPO neurons are uniformly inhibited by the arousing neurotransmitters noradrenaline and acetylcholine, and primarily inhibited by an A_1 receptor agonist. Serotonin inhibits type-1 neurons but excites type-2 neurons, whereas an A_{2A} receptor agonist postsynaptically excites type-2, but not type-1 neurons. These results implicate the involvement of type-2 neurons in the initiation of sleep, whereas type-1 neurons contribute to sleep consolidation as they are only activated in the absence of inhibitory effects from the arousal systems (Gallopin et al. 2005).

The administration of CGS 21680 into the rostral BF, however, produces c-fos expression not only in the VLPO, but also within the NAc shell and the medial portion of the olfactory tubercle (Satoh et al. 1999; Scammell et al. 2001). Interestingly, direct perfusion of the A_{2A} receptor agonist into the NAc induces NREM and REM sleep that corresponds to about three-quarters of the amount of sleep measured when the A_{2A} receptor agonist is infused into the subarachnoid space (Satoh et al. 1999). These results may indicate that A_{2A} receptors within or close to the NAc predominantly promote sleep (Fig. 2a). Acting opposite to adenosine, caffeine enhances wakefulness because it acts as an antagonist of both A_1 and A_{2A} receptor subtypes (Fredholm et al. 1999). Experiments using global genetic KOs of the A_1 and A_{2A} receptors revealed that A_{2A} receptors, but not A_1 receptors, mediate the arousal-inducing effect of caffeine (Huang et al. 2005). The specific role of A_{2A} receptors in the basal ganglia (BG) was investigated using powerful tools for site-specific gene manipulations, such as conditional KO mice of the A_{2A} receptor based on the Cre/lox technology or local infection with AAV carrying short-hairpin RNA of A_{2A} receptors to silence the expression of the receptor subtype (Lazarus et al. 2011). Deletion of A_{2A} receptors selectively in the NAc shell blocked caffeine-induced wakefulness (Fig. 3). Excitatory A_{2A} receptors within the NAc shell must be tonically activated by adenosine for caffeine to be effective as an A_{2A} receptor antagonist. This tonic activation probably occurs in the NAc shell because sufficient levels of adenosine are available under basal conditions and A_{2A} receptors are abundantly expressed throughout the striatum, including the NAc shell (Rosin et al. 1998; Svenningsson et al. 1999). Thus, activation of A_{2A} receptors in the NAc shell contributes to the restraint of the arousal system, whereby caffeine overrides the "adenosine brake" to promote wakefulness. Interestingly, deletion of the dopamine transporter (DAT), which is responsible for the re-uptake of dopamine (Giros and

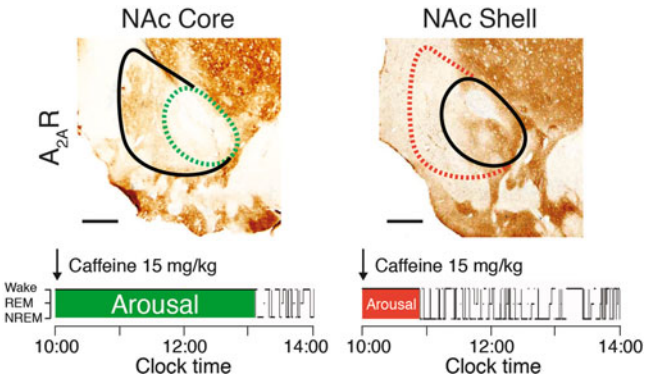

Fig. 3 The arousal effects of caffeine are abolished in rats with site-specific deletion of A_{2A} receptors ($A_{2A}R$) in the shell of the nucleus accumbens (NAc). To identify the neurons on which caffeine acts to produce arousal, A_{2A} receptors were focally depleted by bilateral injections of adeno-associated virus carrying short-hairpin RNA for the A_{2A} receptor into the core (*dashed green line* in the *left panel*) or shell (*dashed red line* in the *right panel*) of the nucleus accumbens of rats (Lazarus et al. 2011). Typical hypnograms that show changes in wakefulness and in rapid eye movement (REM) and non-REM (NREM) sleep after administration of caffeine at a dose of 15 mg/kg indicate that rats with shell, but not core, knockdown of the A_{2A} receptors showed a strongly attenuated caffeine arousal. *Green* and *red* areas in the hypnograms represent wakefulness after caffeine administration that correspond to the depletion of A_{2A} receptors in the core and shell of the nucleus accumbens, respectively

Caron 1993), in mice reduces NREM sleep, increases wakefulness, and unmasks hypersensitivity to the wake-promoting effects of caffeine (Wisor et al. 2001). The last observation may indicate that the expression of NAc D_2 receptors working opposite to A_{2A} receptors is involved in the arousal effect of modafinil, a wakefulness-promoting compound. Despite the fact that stimulating A_{2A} receptors leads to decreased affinity for dopamine at D_2 receptors via intramembrane interactions and to a reduction in Gi-protein coupling of the D_2 receptor for the inhibition of cAMP production (Fuxe et al. 2003), adenosine and its antagonists, such as caffeine, can modulate the activity of medium spiny projection neurons in the striatum via A_{2A} receptors independently of D_2 receptors (Aoyama et al. 2000; Chen et al. 2001). Interestingly, humans with a genetic reduction of striatal DAT show an elevated sensitivity to caffeine stimulation and increased homeostatic response when they are sleep deprived (Holst and Landolt 2015).

The study of NAc adenosine-mediated modulation of the sleep–wake cycle led to a new proposal that the BG represents a key structural element for the control of sleep and wakefulness (Lazarus et al. 2011, 2012). Dysfunction in the BG, such as PD and Huntington's disease and lesions in the BG, results in a wide range of disorders of movement, cognition, and sleep–wake function (Adler and Thorpy 2005; Dale et al. 2004; Goodman and Barker 2010; Obeso et al. 2000; Wetter et al. 2000). In fact, bilateral lesions using ibotenic acid to kill intrinsic neurons in the striatum (caudoputamen), internal and external globus pallidus (GPe), subthalamic nucleus, substantia nigra (pars reticulata or compacta), or thalamus revealed that

bilateral lesions made in the striatum or specifically in the NAc result in a significant reduction in time spent in wakefulness and fragmentation of both sleep and wakefulness (Qiu et al. 2010). Only lesions in the caudate–putamen and globus pallidus increased sleep by 10% and wake by 50%, respectively (Qiu et al. 2010). Moreover, the complete deletion of D_2 receptors, which are prominently, albeit not exclusively, expressed in the BG, significantly decreases wakefulness with a concomitant increase in NREM and REM sleep and a drastic decrease in the NREM sleep delta power (Qu et al. 2010). Excessive sleepiness in PD and other sleep disorders are commonly treated with modafinil (Hogl et al. 2002; Minzenberg and Carter 2007) and interestingly, the arousal effect of modafinil is exclusively mediated by D_1 and D_2 receptors, with D_2 receptors being of primary importance (Qu et al. 2008). Based on these findings, it was proposed that activation of A_{2A} receptors leads to enhanced activity of GABAergic output neurons in the striatopallidal pathway and subsequently arousal systems in the thalamus, hypothalamus, brainstem, and ultimately the cerebral cortex are maintained under a tight inhibitory control. In fact, stereotaxic-based brain microinjections of Cre-recombinase-dependent AAV vectors carrying channelrhodopsin or DREADD into the NAc of transgenic mice in which Cre-recombinase is expressed under the A_{2A}-receptor promoter robustly induced NREM sleep during selective activation of striatopallidal neurons by light or the small molecule clozapine-N-oxide (Oishi Y, Xu Q, et al., unpublished).

Moreover, a recent study found that blocking A_{2A} receptors or A_{2A} receptor-expressing neurons in the olfactory bulb of rodents increases REM sleep, suggesting the possibility that the olfactory bulb is a key site for regulating REM sleep by the adenosine/A_{2A} receptor system (Wang et al. 2016). Because olfactory dysfunction can be ameliorated with an A_{2A} receptor antagonist, for example, caffeine or ZM 241385 (Prediger et al. 2005), it is possible that REM sleep and the perception of odors are linked in the olfactory bulb. Interestingly, the ability to smell is reduced in patients with REM sleep behavior disorder (Stiasny-Kolster et al. 2007).

4.4 Effects of Natural Compounds on Sleep and Wakefulness via Adenosine Receptors

Several studies recently demonstrated that a variety of natural compounds promote sleep via adenosine receptor activation. In strong support of the role of A_{2A} receptors in the regulation of sleep, Japanese sake yeast supplementation improves the quality of sleep in humans (Monoi et al. 2016) and sake yeast-induced NREM sleep was abolished in mice by pretreatment with the A_{2A} receptor antagonist ZM 241385 (Nakamura et al. 2016). Because sake yeast, but not other *Saccharomyces cerevisiae* yeasts (e.g., baker's and brewer's yeast), contains a large amount of *S*-adenosyl-L-methionine and the *S*-adenosyl-L-methionine metabolite methylthioadenosine, Urade and colleagues suggested that the sleep-inducing effect of sake yeast is likely due to the activation of A_{2A} receptors by *S*-adenosyl-L-methionine or methylthioadenosine.

In contrast, paeoniflorin, one of the principal active ingredients of *Paeonia Radix*, shortens sleep latency and increases the amount of NREM sleep exclusively via the activation of A_1 receptors, a conclusion based on the finding that paeoniflorin effects can be blocked by treatment with an A_1 receptor antagonist and are absent in A_1 receptor-KO mice (Chen et al. 2015). In addition, paeoniflorin significantly increases the mechanical pain threshold, prolongs the thermal latency, and increases NREM sleep in partial sciatic nerve ligation mice, a mouse neuropathic pain model characterized by persistent pain and insomnia (Yin et al. 2015). Therefore, the A_1 receptor-mediated analgesic and hypnotic effects of paeoniflorin may be of potential use for the treatment of neuropathic pain and associated insomnia.

Moreover, N^6-(4-hydroxybenzyl) adenine riboside isolated from *Gastrodia elata* has hypnotic effects in mice (Zhang et al. 2012) and may dose-dependently increase NREM sleep via mechanisms that involve A_1 and A_{2A} receptors. Finally, cordycepin (3-deoxyadenosine), an adenosine analogue isolated from *Cordyceps* fungi, promotes NREM sleep in rats, but it remains unclear if the sleep-inducing effect is, in fact, mediated by adenosine receptors (Hu et al. 2013).

5 Effects of Caffeine in Man and the Possible Role of More Selective Adenosine Receptor Drugs

Whereas the above data linking adenosine to sleep were obtained from animal experiments, the evidence suggests that this link also holds true in humans. This is largely due to the well-known effects of caffeine on sleep. Caffeine is the most consumed psychoactive compound in the world. It is readily available through dietary products, such as coffee, tea, soft drinks, and chocolate, but it is also added to non-prescription medications, such as pain-relievers and cold remedies. Regardless of the source, worldwide average caffeine consumption is estimated to be just under 80 mg/d, although the levels of intake in countries such as Sweden and Finland are in the range of 400 mg caffeine per day (Fredholm et al. 1999). Caffeine is widely used to promote wakefulness and to counteract fatigue in doses that are well in the range where adenosine antagonism is the dominant effect. Some individuals, however, experience anxiety and panic attacks (Chait 1992; Evans and Griffiths 1991) at normal consumption levels, and this is more common at higher doses. One study found that people with polymorphisms at the A_{2A}-receptor-gene are at risk of experiencing increased anxiety when consuming coffee, tea, energy drinks, or other caffeine-containing products (Alsene et al. 2003). A_{2A} receptor polymorphisms also consistently modulate the objective and subjective effects of caffeine on sleep quality and electroencephalogram (Bodenmann et al. 2012; Byrne et al. 2012; Rétey et al. 2007).

Whether caffeine affects circadian rhythm and thereby alters the timing of sleep is widely unknown; however, recent developments revealed caffeine effects on the mammalian circadian clock. For example, caffeine delays the human circadian melatonin rhythm by blocking A_1 receptors (Burke et al. 2015). Chronic treatment

with caffeine lengthens the circadian period of molecular oscillations in human osteosarcoma U2OS cells expressing clock gene luciferase reporters. Further, application of pharmacologic tools and small-interfering RNA knockdown revealed that the effect of caffeine on molecular oscillation is attenuated by perturbation of A_1 receptor signaling but not ryanodine receptor or phosphodiesterase activity. This finding establishes a possible molecular mechanism for the clinical observation in a double-blind, placebo-controlled, ~49-day long, within-subject study that bedtime caffeine consumption induces a ~40-min phase delay of the circadian melatonin rhythm in humans. Another study revealed that caffeine increases the light sensitivity of the mouse circadian clock (van Diepen et al. 2014).

Society demands the means to bend sleep to the needs of modern lifestyle instead of the other way around and thus sleep-avoidance has become a popular research topic. Scientists and clinicians worldwide are searching for new methods of keeping people alert on limited sleep. Much more about the effects of coffee and other beverages containing caffeine on sleep in man will be covered elsewhere in this book [for review, see Clark and Landolt (2017)]. Moreover, consistent with the fact that the arousal effect of caffeine in mice is exclusively mediated by A_{2A} receptors, emerging evidence supports the modulation of the sleep–wake cycle by A_{2A} receptor antagonists. For example, the newly developed dual adenosine A_{2A}/A_1 receptor antagonist JNJ-40255293 enhances wakefulness (Atack et al. 2014). Moreover, since the clinical approval of the A_{2A} receptor antagonist istradefylline (KW-6002) for motor improvement in PD in Japan in 2013, a report of four cases indicated that evening treatment with this antagonist reduces sleep duration in the evening and increases daytime sleepiness in patients (Matsuura and Tomimoto 2015). Thus, A_{2A} receptor antagonists may have considerable potential as eugeroics (wakefulness enhancing drugs) while avoiding some of the aforementioned A_{2A}-independent side effects of caffeine (such as anxiety and disturbance of the circadian rhythm) or negative effects of other psychostimulants, including dependence.

The possibility that stimulation of adenosine receptors could be used to promote sleep should also be considered. Currently, there are 60 million prescriptions for sleeping pills in the USA each year, 43 million of which are for the nonbenzodiazepine zolpidem, also known as Ambien. Benzodiazepines and nonbenzodiazepines, both of which enhance the effect of the neurotransmitter GABA at the $GABA_A$ receptor, are used for the treatment of insomnia and poor sleep quality despite their wide range of disadvantages and safety issues, ranging from low sleep quality by increasing light sleep at the expense of physiological deep sleep to side effects (e.g., next-day sedation, cognitive impairment, and amnesic effects) and the development of tolerance and dependence by long-term administration. Because an A_{2A} receptor agonist strongly increases sleep in wild-type mice (Satoh et al. 1996; Urade et al. 2003), pharmacologic A_{2A} receptor activation may be an alternative strategy for the treatment of insomnia. Due to the lack of brain-permeability, however, not all currently existing A_{2A} receptor agonists are suitable for treating the nervous system. If the observation in animals that adenosine levels are elevated during prolonged wakefulness holds also true for humans, a freely penetrating allosteric modulator may effectively enhance the

sleep-inducing effect of endogenous adenosine and help people with insomnia to fall asleep.

6 Conclusion

Here we demonstrated that adenosine is indeed one of the several somnogenic substances that act in concert to ensure normal sleep–wake patterns. Adenosine tends to increase sleep, but the source of the adenosine is still poorly understood. Many cells and processes could play a role. Similarly, adenosine promotes sleep by several mechanisms, in several locations, via A_1 or A_{2A} receptors. We emphasized A_1 receptor-mediated effects on histamine neurons and the A_{2A} receptor-mediated effects in the NAc. Both of these receptors are antagonized by caffeine – to a large extent explaining the awakening effect of this common drug.

References

Adler CH, Thorpy MJ (2005) Sleep issues in Parkinson's disease. Neurology 64:S12–S20

Alam MN, Szymusiak R, Gong H, King J, McGinty D (1999) Adenosinergic modulation of rat basal forebrain neurons during sleep and waking: neuronal recording with microdialysis. J Physiol 521:679–690. doi:10.1111/j.1469-7793.1999.00679.x

Alsene K, Deckert J, Sand P, de Wit H (2003) Association between A2a receptor gene polymorphisms and caffeine-induced anxiety. Neuropsychopharmacology 28:1694–1702. doi:10.1038/sj.npp.1300232

Aoyama S, Kase H, Borrelli E (2000) Rescue of locomotor impairment in dopamine D2 receptor-deficient mice by an adenosine A2A receptor antagonist. J Neurosci 20:5848–5852

Arnaud MJ (2011) Pharmacokinetics and metabolism of natural methylxanthines in animal and man. Handb Exp Pharmacol 200:33–91. doi:10.1007/978-3-642-13443-2_3

Atack JR et al (2014) JNJ-40255293, a novel adenosine A_{2A}/A_1 antagonist with efficacy in preclinical models of Parkinson's disease. ACS Chem Neurosci 5:1005–1019. doi:10.1021/cn5001606

Augusto E et al (2013) Ecto-5′-nucleotidase (CD73)-mediated formation of adenosine is critical for the striatal adenosine A_{2A} receptor functions. J Neurosci 33:11390–11399. doi:10.1523/jneurosci.5817-12.2013

Bachmann V et al (2012) Functional ADA polymorphism increases sleep depth and reduces vigilant attention in humans. Cereb Cortex 22:962–970. doi:10.1093/cercor/bhr173

Ballarin M, Fredholm BB, Ambrosio S, Mahy N (1991) Extracellular levels of adenosine and its metabolites in the striatum of awake rats: inhibition of uptake and metabolism. Acta Physiol Scand 142:97–103. doi:10.1111/j.1748-1716.1991.tb09133.x

Basheer R, Strecker RE, Thakkar MM, McCarley RW (2004) Adenosine and sleep–wake regulation. Prog Neurobiol 73:379–396. doi:10.1016/j.pneurobio.2004.06.004

Bastia E, Xu YH, Scibelli AC, Day YJ, Linden J, Chen JF, Schwarzschild MA (2005) A crucial role for forebrain adenosine A(2A) receptors in amphetamine sensitization. Neuropsychopharmacology 30:891–900. doi:10.1038/sj.npp.1300630

Benington JH, Kodali SK, Heller HC (1995) Stimulation of A1 adenosine receptors mimics the electroencephalographic effects of sleep deprivation. Brain Res 692:79–85. doi:10.1016/0006-8993(95)00590-m

Benveniste H, Hansen AJ, Ottosen NS (1989) Determination of brain interstitial concentrations by microdialysis. J Neurochem 52:1741–1750. doi:10.1111/j.1471-4159.1989.tb07252.x

Bodenmann S, Hohoff C, Freitag C, Deckert J, Rétey JV, Bachmann V, Landolt HP (2012) Polymorphisms of ADORA2A modulate psychomotor vigilance and the effects of caffeine on neurobehavioural performance and sleep EEG after sleep deprivation. Br J Pharmacol 165:1904–1913. doi:10.1111/j.1476-5381.2011.01689.x

Bodin P, Burnstock G (1998) Increased release of ATP from endothelial cells during acute inflammation. Inflamm Res 47:351–354

Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K (2005) Millisecond-timescale, genetically targeted optical control of neural activity. Nat Neurosci 8:1263–1268. doi:10.1038/nn1525

Burke TM et al (2015) Effects of caffeine on the human circadian clock in vivo and in vitro. Sci Transl Med 7:146–305. doi:10.1126/scitranslmed.aac5125

Burnstock G, Verkhratsky A (2012) Mechanisms of ATP release and inactivation. In: Purinergic signalling and the nervous system. Springer, Berlin Heidelberg, pp 79–118. doi:10.1007/978-3-642-28863-0_4

Byrne EM, Johnson J, McRae AF, Nyholt DR, Medland SE, Gehrman PR, Heath AC, Madden PA, Montgomery GW, Chenevix-Trench G, Martin NG (2012) A genome-wide association study of caffeine-related sleep disturbance: confirmation of a role for a common variant in the adenosine receptor. Sleep 35:967–975. doi:10.5665/sleep.1962

Chait LD (1992) Factors influencing the subjective response to caffeine. Behav Pharmacol 3:219–228

Chekeni FB et al (2010) Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis. Nature 467:863–867. doi:10.1038/nature09413

Chen J-F et al (2001) The role of the D2 dopamine receptor (D2R) in A2A adenosine receptor (A2AR)-mediated behavioral and cellular responses as revealed by A2A and D2 receptor knockout mice. Proc Natl Acad Sci U S A 98:1970–1975. doi:10.1073/pnas.98.4.1970

Chen Y et al (2006) ATP release guides neutrophil chemotaxis via P2Y2 and A3 receptors. Science 314:1792–1795. doi:10.1126/science.1132559

Chen C et al (2015) Paeoniflorin promotes non-rapid eye movement sleep via adenosine A1 receptors. J Pharmacol Exp Ther 356:64–73. doi:10.1124/jpet.115.227819

Clark I, Landolt HP (2017) Coffee, caffeine, and sleep: a systematic review of epidemiological studies and randomized controlled trials. Sleep Med Rev 31:70–78. doi:10.1016/j.smrv.2016.01.006

Dale N, Frenguelli BG (2012) Measurement of purine release with microelectrode biosensors. Purinergic Signal 8:27–40. doi:10.1007/s11302-011-9273-4

Dale RC et al (2004) Encephalitis lethargica syndrome: 20 new cases and evidence of basal ganglia autoimmunity. Brain 127:21–33. doi:10.1093/brain/awh008

Deisseroth K (2014) Circuit dynamics of adaptive and maladaptive behaviour. Nature 505:309–317. doi:10.1038/nature12982

Elliott MR et al (2009) Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. Nature 461:282–286. doi:10.1038/nature08296

Eltzschig HK (2009) Adenosine: an old drug newly discovered. Anesthesiology 111:904–915. doi:10.1097/ALN.0b013e3181b060f2

Ena SL, De Backer J-F, Schiffmann SN, de Kerchove d'Exaerde A (2013) FACS array profiling identifies Ecto-5′ nucleotidase as a striatopallidal neuron-specific Gene involved in striatal-dependent learning. J Neurosci 33:8794–8809. doi:10.1523/jneurosci.2989-12.2013

Evans SM, Griffiths RR (1991) Dose-related caffeine discrimination in normal volunteers: individual differences in subjective effects and self-reported cues. Behav Pharmacol 2:345–356

Faigle M, Seessle J, Zug S, El Kasmi KC, Eltzschig HK (2008) ATP release from vascular endothelia occurs across Cx43 hemichannels and is attenuated during hypoxia. PLoS One 3:e2801. doi:10.1371/journal.pone.0002801

Farrell MS et al (2013) A Galphas DREADD mouse for selective modulation of cAMP production in striatopallidal neurons. Neuropsychopharmacology 38:854–862. doi:10.1038/npp.2012.251

Feldberg W, Sherwood SL (1954) Injections of drugs into the lateral ventricle of the cat. J Physiol 123:148–167
Fields RD, Stevens B (2000) ATP: an extracellular signaling molecule between neurons and glia. Trends Neurosci 23:625–633. doi:10.1016/S0166-2236(00)01674-X
Fredholm BB (2007) Adenosine, an endogenous distress signal, modulates tissue damage and repair. Cell Death Differ 14:1315–1323
Fredholm BB (2014) Adenosine – a physiological or pathophysiological agent? J Mol Med 92:201–206. doi:10.1007/s00109-013-1101-6
Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE (1999) Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev 51:83–133
Fredholm BB, Chen J-F, Cunha RA, Svenningsson P, Vaugeois J-M (2005a) Adenosine and brain function. Int Rev Neurobiol 63:191–270. doi:10.1016/S0074-7742(05)63007-3
Fredholm BB, Chen JF, Masino SA, Vaugeois JM (2005b) Actions of adenosine at its receptors in the CNS: insights from knockouts and drugs. Annu Rev Pharmacol Toxicol 45:385–412. doi:10.1146/annurev.pharmtox.45.120403.095731
Fredholm BB, IJzerman AP, Jacobson KA, Linden J, Muller CE (2011) International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors – an update. Pharmacol Rev 63:1–34. doi:10.1124/pr.110.003285
Fuller PM, Yamanaka A, Lazarus M (2015) How genetically engineered systems are helping to define, and in some cases redefine, the neurobiological basis of sleep and wake. Temperature 2:406–417. doi:10.1080/23328940.2015.1075095
Fuxe K et al (2003) Receptor heteromerization in adenosine A2A receptor signaling: relevance for striatal function and Parkinson's disease. Neurology 61:S19–S23
Gallopin T et al (2005) The endogenous somnogen adenosine excites a subset of sleep-promoting neurons via A2A receptors in the ventrolateral preoptic nucleus. Neuroscience 134:1377–1390
Garcia-Garcia F, Acosta-Pena E, Venebra-Munoz A, Murillo-Rodriguez E (2009) Sleep-inducing factors. CNS Neurol Disord Drug Targets 8:235–244
Geiger JD, Fyda DM (1991) Adenosine transport in nervous system tissues. In: Stone TW (ed) Adenosine in the nervous system. Academic Press, London, pp 1–23. doi:10.1016/B978-0-12-672640-4.50007-8
Giguere PM, Kroeze WK, Roth BL (2014) Tuning up the right signal: chemical and genetic approaches to study GPCR functions. Curr Opin Cell Biol 27:51–55. doi:10.1016/j.ceb.2013.11.006
Giros B, Caron MG (1993) Molecular characterization of the dopamine transporter. Trends Pharmacol Sci 14:43–49. doi:10.1016/0165-6147(93)90029-J
Goodman A, Barker R (2010) How vital is sleep in Huntington's disease? J Neurol 257:882–897. doi:10.1007/s00415-010-5517-4
Guthrie PB, Knappenberger J, Segal M, Bennett MVL, Charles AC, Kater SB (1999) ATP released from astrocytes mediates glial calcium waves. J Neurosci 19:520–528
Halassa MM, Fellin T, Haydon PG (2007) The tripartite synapse: roles for gliotransmission in health and disease. Trends Mol Med 13:54–63. doi:10.1016/j.molmed.2006.12.005
Halassa MM et al (2009) Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. Neuron 61:213–219
Hauser RA et al (2011) Preladenant in patients with Parkinson's disease and motor fluctuations: a phase 2, double-blind, randomised trial. Lancet Neurol 10:221–229. doi:10.1016/s1474-4422(11)70012-6
Hogl B et al (2002) Modafinil for the treatment of daytime sleepiness in Parkinson's disease: a double-blind, randomized, crossover, placebo-controlled polygraphic trial. Sleep 25:905–909
Hollopeter G et al (2001) Identification of the platelet ADP receptor targeted by antithrombotic drugs. Nature 409:202–207. doi:10.1038/35051599
Holst SC, Landolt H-P (2015) Sleep homeostasis, metabolism, and adenosine. Curr Sleep Med Rep 1:27–37. doi:10.1007/s40675-014-0007-3

Hong Z-Y, Huang Z-L, Qu W-M, Eguchi N, Urade Y, Hayaishi O (2005) An adenosine A2A receptor agonist induces sleep by increasing GABA release in the tuberomammillary nucleus to inhibit histaminergic systems in rats. J Neurochem 92:1542–1549. doi:10.1111/j.1471-4159.2004.02991.x

Hu Z et al (2013) Cordycepin increases nonrapid eye movement sleep via adenosine receptors in rats. Evid Based Complement Alternat Med 2013:8. doi:10.1155/2013/840134

Huang Z-L et al (2005) Adenosine A2A, but not A1, receptors mediate the arousal effect of caffeine. Nat Neurosci 8:858–859. http://www.nature.com/neuro/journal/v8/n7/suppinfo/nn1491_S1.html

Huang Z-L, Urade Y, Hayaishi O (2007) Prostaglandins and adenosine in the regulation of sleep and wakefulness. Curr Opin Pharmacol 7:33–38. doi:10.1016/j.coph.2006.09.004

Huang ZL, Urade Y, Hayaishi O (2011) The role of adenosine in the regulation of sleep. Curr Top Med Chem 11:1047–1057

Huitron-Resendiz S et al (2005) Urotensin II modulates rapid eye movement sleep through activation of brainstem cholinergic neurons. J Neurosci 25:5465–5474. doi:10.1523/jneurosci.4501-04.2005

Inoué S, Honda K, Komoda Y (1995) Sleep as neuronal detoxification and restitution. Behav Brain Res 69:91–96. doi:10.1016/0166-4328(95)00014-k

Ishimori K (1909) True cause of sleep: a hypnogenic substance as evidenced in the brain of sleep-deprived animals. Tokyo Igakkai Zasshi 23:429–457

Jenner P, Mori A, Hauser R, Morelli M, Fredholm BB, Chen JF (2009) Adenosine, adenosine A2A antagonists, and Parkinson's disease. Parkinsonism Relat Disord 15:406–413. doi:10.1016/j.parkreldis.2008.12.006

Kalinchuk AV et al (2003) Local energy depletion in the basal forebrain increases sleep. Eur J Neurosci 17:863–869. doi:10.1046/j.1460-9568.2003.02532.x

Krueger JM, Majde JA (2003) Humoral links between sleep and the immune system: research issues. Ann N Y Acad Sci 992:9–20

Krueger JM, Walter J, Dinarello CA, Wolff SM, Chedid L (1984) Sleep-promoting effects of endogenous pyrogen (interleukin-1). Am J Phys 246:R994–R999

Krueger JM, Obal F Jr, Fang J, Kubota T, Taishi P (2001) The role of cytokines in physiological sleep regulation. Ann N Y Acad Sci 933:211–221

Krueger JM, Clinton JM, Winters BD, Zielinski MR, Taishi P, Jewett KA, Davis CJ (2011) Involvement of cytokines in slow wave sleep. Prog Brain Res 193:39–47. doi:10.1016/b978-0-444-53839-0.00003-x

Kubota K (1989) Kuniomi Ishimori and the first discovery of sleep-inducing substances in the brain. Neurosci Res 6:497–518

Lazarus M, Yoshida K, Coppari R, Bass CE, Mochizuki T, Lowell BB, Saper CB (2007) EP3 prostaglandin receptors in the median preoptic nucleus are critical for fever responses. Nat Neurosci 10:1131–1133. http://www.nature.com/neuro/journal/v10/n9/suppinfo/nn1949_S1.html

Lazarus M et al (2011) Arousal effect of caffeine depends on adenosine A2A receptors in the shell of the nucleus accumbens. J Neurosci 31:10067–10075. doi:10.1523/jneurosci.6730-10.2011

Lazarus M, Huang Z-L, Lu J, Urade Y, Chen J-F (2012) How do the basal ganglia regulate sleep–wake behavior? Trends Neurosci 35:723–732. doi:10.1016/j.tins.2012.07.001

Lazarus M, Chen J-F, Urade Y, Huang Z-L (2013) Role of the basal ganglia in the control of sleep and wakefulness. Curr Opin Neurobiol 23:780–785. doi:10.1016/j.conb.2013.02.001

Legendre R, Pieron H (1913) Recherches sur le besoin de sommeil consécutif à une veille prolongée. Z Allegem Physiol 14:235–262

Li Y et al (2015) Optogenetic activation of adenosine a receptor signaling in the dorsomedial striatopallidal neurons suppresses goal-directed behavior. Neuropsychopharmacology 41:1003–1013. doi:10.1038/npp.2015.227

Linden J (2006) Purinergic chemotaxis. Science 314:1689–1690. doi:10.1126/science.1137190

Lovatt D et al (2012) Neuronal adenosine release, and not astrocytic ATP release, mediates feedback inhibition of excitatory activity. Proc Natl Acad Sci U S A 109:6265–6270. doi:10.1073/pnas.1120997109

MacDonald PE, Braun M, Galvanovskis J, Rorsman P (2006) Release of small transmitters through kiss-and-run fusion pores in rat pancreatic beta cells. Cell Metab 4:283–290. doi:10.1016/j.cmet.2006.08.011

Matsuura K, Tomimoto H (2015) Istradefylline is recommended for morning use: a report of 4 cases. Intern Med 54:509–511. doi:10.2169/internalmedicine.54.3522

Mazzotti DR, Guindalini C, de Souza AAL, Sato JR, Santos-Silva R, Bittencourt LRA, Tufik S (2012) Adenosine deaminase polymorphism affects sleep EEG spectral power in a large epidemiological sample. PLoS One 7:e44154. doi:10.1371/journal.pone.0044154

Methippara MM, Kumar S, Alam MN, Szymusiak R, McGinty D (2005) Effects on sleep of microdialysis of adenosine A1 and A2a receptor analogs into the lateral preoptic area of rats. Am J Physiol Regul Integr Comp Physiol 289:R1715–R1723. doi:10.1152/ajpregu.00247.2005

Minzenberg MJ, Carter CS (2007) Modafinil: a review of neurochemical actions and effects on cognition. Neuropsychopharmacology 33:1477–1502

Monoi N et al (2016) Japanese sake yeast supplementation improves the quality of sleep: a double-blind randomised controlled clinical trial. J Sleep Res 25:116–123. doi:10.1111/jsr.12336

Müller CE, Jacobson KA (2011) Recent developments in adenosine receptor ligands and their potential as novel drugs. Biochim Biophys Acta 1808:1290–1308. doi:10.1016/j.bbamem.2010.12.017

Mullington J, Korth C, Hermann DM, Orth A, Galanos C, Holsboer F, Pollmächer T (2000) Dose-dependent effects of endotoxin on human sleep. Am J Physiol Regul Integr Comp Physiol 278:R947–R955

Mullington JM, Hinze-Selch D, Pollmacher T (2001) Mediators of inflammation and their interaction with sleep: relevance for chronic fatigue syndrome and related conditions. Ann N Y Acad Sci 933:201–210

Nakamura Y, Midorikawa T, Monoi N, Kimura E, Murata-Matsuno A, Sano T, Oka K, Sugafuji T, Uchiyama A, Murakoshi M, Sugiyama K, Nishino H, Urade Y (2016) Oral administration of Japanese sake yeast (Saccharomyces cerevisiae sake) promotes non-rapid eye movement sleep in mice via adenosine A2A receptors. J Sleep Res 25:746–753. doi:10.1111/jsr.12434

Obeso JA et al (2000) Pathophysiologic basis of surgery for Parkinson's disease. Neurology 55:S7–12

Ochiishi T et al (1999a) Cellular localization of adenosine A1 receptors in rat forebrain: immunohistochemical analysis using adenosine A1 receptor-specific monoclonal antibody. J Comp Neurol 411:301–316

Ochiishi T et al (1999b) High level of adenosine A1 receptor-like immunoreactivity in the CA2/CA3a region of the adult rat hippocampus. Neuroscience 93:955–967

Oishi Y, Huang Z-L, Fredholm BB, Urade Y, Hayaishi O (2008) Adenosine in the tuberomammillary nucleus inhibits the histaminergic system via A1 receptors and promotes non-rapid eye movement sleep. Proc Natl Acad Sci U S A 105:19992–19997. doi:10.1073/pnas.0810926105

Oishi Y, Yoshida K, Scammell TE, Urade Y, Lazarus M, Saper CB (2015) The roles of prostaglandin E2 and D2 in lipopolysaccharide-mediated changes in sleep. Brain Behav Immun 47:172–177. doi:10.1016/j.bbi.2014.11.019

Pascual O et al (2005) Astrocytic purinergic signaling coordinates synaptic networks. Science 310:113–116. doi:10.1126/science.1116916

Porkka-Heiskanen T, Strecker RE, Thakkar M, Bjorkum AA, Greene RW, McCarley RW (1997) Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness. Science 276:1265–1268

Porkka-Heiskanen T, Strecker RE, McCarley RW (2000) Brain site-specificity of extracellular adenosine concentration changes during sleep deprivation and spontaneous sleep: an in vivo microdialysis study. Neuroscience 99:507–517. doi:10.1016/S0306-4522(00)00220-7

Prediger RDS, Batista LC, Takahashi RN (2005) Caffeine reverses age-related deficits in olfactory discrimination and social recognition memory in rats: involvement of adenosine A_1 and A_{2A} receptors. Neurobiol Aging 26:957–964. doi:10.1016/j.neurobiolaging.2004.08.012

Qiu M-H, Vetrivelan R, Fuller PM, Lu J (2010) Basal ganglia control of sleep–wake behavior and cortical activation. Eur J Neurosci 31:499–507. doi:10.1111/j.1460-9568.2009.07062.x

Qu W-M et al (2006) Lipocalin-type prostaglandin D synthase produces prostaglandin D2 involved in regulation of physiological sleep. Proc Natl Acad Sci U S A 103:17949–17954

Qu W-M, Huang Z-L, Xu X-H, Matsumoto N, Urade Y (2008) Dopaminergic D1 and D2 receptors are essential for the arousal effect of modafinil. J Neurosci 28:8462–8469. doi:10.1523/jneurosci.1819-08.2008

Qu W-M, Xu X-H, Yan M-M, Wang Y-Q, Urade Y, Huang Z-L (2010) Essential role of dopamine D2 receptor in the maintenance of wakefulness, but not in homeostatic regulation of sleep, in mice. J Neurosci 30:4382–4389. doi:10.1523/jneurosci.4936-09.2010

Radulovacki M, Virus RM, Djuricic-Nedelson M, Green RD (1983) Hypnotic effects of deoxycorformycin in rats. Brain Res 271:392–395

Reppert SM, Weaver DR, Stehle JH, Rivkees SA (1991) Molecular cloning and characterization of a rat A1-adenosine receptor that is widely expressed in brain and spinal cord. Mol Endocrinol 5:1037–1048. doi:10.1210/mend-5-8-1037

Resta R, Yamashita Y, Thompson LF (1998) Ecto-enzyme and signaling functions of lymphocyte CD73. Immunol Rev 161:95–109

Rétey JV et al (2005) A functional genetic variation of adenosine deaminase affects the duration and intensity of deep sleep in humans. Proc Natl Acad Sci U S A 102:15676–15681. doi:10.1073/pnas.0505414102

Rétey JV, Adam M, Khatami R, Luhmann UF, Jung HH, Berger W, Landolt HP (2007) A genetic variation in the adenosine A2A receptor gene (ADORA2A) contributes to individual sensitivity to caffeine effects on sleep. Clin Pharmacol Ther 81:692–698

Rivkees SA, Price SL, Zhou FC (1995) Immunohistochemical detection of A1 adenosine receptors in rat brain with emphasis on localization in the hippocampal formation, cerebral cortex, cerebellum, and basal ganglia. Brain Res 677:193–203

Rosenbaum E (1892) Warum müssen wir schlafen?: eine neue Theorie des Schlafes. August Hirschwald, Berlin

Rosin DL, Robeva A, Woodard RL, Guyenet PG, Linden J (1998) Immunohistochemical localization of adenosine A2A receptors in the rat central nervous system. J Comp Neurol 401:163–186

Saper CB, Scammell TE, Lu J (2005) Hypothalamic regulation of sleep and circadian rhythms. Nature 437:1257–1263

Saper CB, Fuller PM, Pedersen NP, Lu J, Scammell TE (2010) Sleep state switching. Neuron 68:1023–1042

Satoh S, Matsumura H, Suzuki F, Hayaishi O (1996) Promotion of sleep mediated by the A2a-adenosine receptor and possible involvement of this receptor in the sleep induced by prostaglandin D2 in rats. Proc Natl Acad Sci U S A 93:5980–5984

Satoh S, Matsumura H, Koike N, Tokunaga Y, Maeda T, Hayaishi O (1999) Region-dependent difference in the sleep-promoting potency of an adenosine A2A receptor agonist. Eur J Neurosci 11:1587–1597. doi:10.1046/j.1460-9568.1999.00569.x

Scammell TE et al (2001) An adenosine A2a agonist increases sleep and induces Fos in ventrolateral preoptic neurons. Neuroscience 107:653–663. doi:10.1016/s0306-4522(01)00383-9

Scammell TE, Arrigoni E, Thompson MA, Ronan PJ, Saper CB, Greene RW (2003) Focal deletion of the adenosine A1 receptor in adult mice using an adeno-associated viral vector. J Neurosci 23:5762–5770

Schrader J (1983) Metabolism of adenosine and sites of production in the heart. In: Berne R, Rall T, Rubio R (eds) Regulatory function of adenosine, Developments in pharmacology, vol 2. Springer US, New York, pp 133–156. doi:10.1007/978-1-4613-3909-0_9

Shen HY et al (2008) A critical role of the adenosine A2A receptor in extrastriatal neurons in modulating psychomotor activity as revealed by opposite phenotypes of striatum and forebrain A2A receptor knock-outs. J Neurosci 28:2970–2975

Sherin JE, Shiromani PJ, McCarley RW, Saper CB (1996) Activation of ventrolateral preoptic neurons during sleep. Science 271:216–219

Sherin JE, Elmquist JK, Torrealba F, Saper CB (1998) Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. J Neurosci 18:4705–4721

Stiasny-Kolster K, Clever SC, Moller JC, Oertel WH, Mayer G (2007) Olfactory dysfunction in patients with narcolepsy with and without REM sleep behaviour disorder. Brain 130:442–449. doi:10.1093/brain/awl343

Strecker RE et al (2000) Adenosinergic modulation of basal forebrain and preoptic/anterior hypothalamic neuronal activity in the control of behavioral state. Behav Brain Res 115:183–204. doi:10.1016/s0166-4328(00)00258-8

Svenningsson P, Fourreau L, Bloch B, Fredholm BB, Gonon F, Le Moine C (1999) Opposite tonic modulation of dopamine and adenosine on c-fos gene expression in striatopallidal neurons. Neuroscience 89:827–837

Thakkar MM, Engemann SC, Walsh KM, Sahota PK (2008) Adenosine and the homeostatic control of sleep: effects of A1 receptor blockade in the perifornical lateral hypothalamus on sleep–wakefulness. Neuroscience 153:875–880. doi:10.1016/j.neuroscience.2008.01.017

Ueno R, Ishikawa Y, Nakayama T, Hayaishi O (1982) Prostaglandin D2 induces sleep when microinjected into the preoptic area of conscious rats. Biochem Biophys Res Commun 109:576–582. doi:10.1016/0006-291x(82)91760-0

Urade Y, Hayaishi O (2011) Prostaglandin D2 and sleep/wake regulation. Sleep Med Rev 15:411–418. doi:10.1016/j.smrv.2011.08.003

Urade Y, Lazarus M (2013) Prostaglandin D2 in the regulation of sleep. In: Shaw PJ, Tafti M, Thorpy MJ (eds) The genetic basis of sleep and sleep disorders. Cambridge University Press, New York, pp 73–83

Urade Y et al (2003) Sleep regulation in adenosine A2A receptor-deficient mice. Neurology 61: S94–S96

Ushikubi F et al (1998) Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. Nature 395:281–284

van Diepen HC, Lucassen EA, Yasenkov R, Groenen I, Ijzerman AP, Meijer JH, Deboer T (2014) Caffeine increases light responsiveness of the mouse circadian pacemaker. Eur J Neurosci 40:3504–3511. doi:10.1111/ejn.12715

Virus RM, Ticho S, Pilditch M, Radulovacki M (1990) A comparison of the effects of caffeine, 8-cyclopentyltheophylline, and alloxazine on sleep in rats. Possible roles of central nervous system adenosine receptors. Neuropsychopharmacology 3:243–249

Wall MJ, Dale N (2013) Neuronal transporter and astrocytic ATP exocytosis underlie activity-dependent adenosine release in the hippocampus. J Physiol 591:3853–3871. doi:10.1113/jphysiol.2013.253450

Wang Y-Q et al (2016) Adenosine A_{2A} receptors in the olfactory bulb suppress rapid eye movement sleep in rodents. Brain Struct Funct. doi:10.1007/s00429-016-1281-2

Wei CJ, Li W, Chen JF (2011) Normal and abnormal functions of adenosine receptors in the central nervous system revealed by genetic knockout studies. Biochim Biophys Acta 1808:1358–1379. doi:10.1016/j.bbamem.2010.12.018

Wetter TC, Collado-Seidel V, Pollmacher T, Yassouridis A, Trenkwalder C (2000) Sleep and periodic leg movement patterns in drug-free patients with Parkinson's disease and multiple system atrophy. Sleep 23:361–367

Wisor JP, Nishino S, Sora I, Uhl GH, Mignot E, Edgar DM (2001) Dopaminergic role in stimulant-induced wakefulness. J Neurosci 21:1787–1794

Yamaguchi H, Maruyama T, Urade Y, Nagata S (2014) Immunosuppression via adenosine receptor activation by adenosine monophosphate released from apoptotic cells. Elife 3: e02172. doi:10.7554/eLife.02172

Yang C, Franciosi S, Brown RE (2013) Adenosine inhibits the excitatory synaptic inputs to basal forebrain cholinergic, GABAergic and parvalbumin neurons in mice. Front Neurol 4:77. doi:10.3389/fneur.2013.00077

Yegutkin GG (2008) Nucleotide- and nucleoside-converting ectoenzymes: important modulators of purinergic signalling cascade. Biochim Biophys Acta 1783:673–694. doi:10.1016/j.bbamcr.2008.01.024

Yin D et al (2015) Paeoniflorin exerts analgesic and hypnotic effects via adenosine A_1 receptors in a mouse neuropathic pain model. Psychopharmacology 233:281–293. doi:10.1007/s00213-015-4108-6

Yizhar O, Fenno LE, Davidson TJ, Mogri M, Deisseroth K (2011) Optogenetics in neural systems. Neuron 71:9–34. doi:10.1016/j.neuron.2011.06.004

Yu L et al (2008) Adenosine A2A receptor antagonists exert motor and neuroprotective effects by distinct cellular mechanisms. Ann Neurol 63:338–346. doi:10.1002/ana.21313

Zetterstrom T, Vernet L, Ungerstedt U, Tossman U, Jonzon B, Fredholm BB (1982) Purine levels in the intact rat brain. Studies with an implanted perfused hollow fibre. Neurosci Lett 29:111–115

Zhang Z et al (2007) Regulated ATP release from astrocytes through lysosome exocytosis. Nat Cell Biol 9:945–953

Zhang Y, Li M, Kang R-X, Shi J-G, Liu G-T, Zhang J-J (2012) NHBA isolated from Gastrodia elata exerts sedative and hypnotic effects in sodium pentobarbital-treated mice. Pharmacol Biochem Behav 102:450–457. doi:10.1016/j.pbb.2012.06.002

Metabolite Clearance During Wakefulness and Sleep

Stephen B. Hladky and Margery A. Barrand

Contents

Abstract

Mechanisms for elimination of metabolites from ISF include metabolism, blood–brain barrier transport and non-selective, perivascular efflux, this last being assessed by measuring the clearance of markers like inulin. Clearance describes elimination. Clearance of a metabolite generated within the brain is determined as its elimination rate divided by its concentration in interstitial fluid (ISF). However, the more frequently measured parameter is the rate constant for elimination determined as elimination rate divided by amount present, which thus depends on both the elimination processes and the distribution of the

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metabolite in the brain. The relative importance of the various elimination mechanisms depends on the particular metabolite. Little is known about the effects of sleep on clearance via metabolism or blood–brain barrier transport, but studies with inulin in mice comparing perivascular effluxes during sleep and wakefulness reveal a 4.2-fold increase in clearance. Amongst the important brain metabolites considered, CO_2 is eliminated so rapidly across the blood–brain barrier that clearance is blood flow limited and elimination quickly balances production. Glutamate is removed from ISF primarily by uptake into astrocytes and conversion to glutamine, but also by transport across the blood–brain barrier. Both lactate and amyloid-β are eliminated by metabolism, blood–brain barrier transport and perivascular efflux and both show decreased production, decreased ISF concentration and increased perivascular clearance during sleep. Taken altogether available data indicate that sleep increases perivascular and non-perivascular clearances for amyloid-β which reduces its concentration and may have long-term consequences for the formation of plaques and cerebral arterial deposits.

Keywords

Amyloid-beta • Blood—brain barrier transport • Brain interstitial fluid volume • Carbon dioxide • Cerebrospinal fluid • Clearance • Glutamate • Glymphatic circulation • Inulin • Lactate • Metabolite elimination mechanisms • Perivascular efflux • Perivascular spaces • Rate constant for elimination • Volume of distribution

1 Introduction

All substances required for brain cell activity have ultimately to derive from outside the brain. Likewise all metabolites created within the brain have to be eliminated in one way or another. The principal routes of entry and exit are indicated in Fig. 1. This review deals with the processes affecting elimination and how they may differ between wakefulness and sleep.

1.1 How Is Clearance Defined?

The clearance of a substance from the brain is a statement of how easily that substance can be eliminated. It is defined as

$$\text{clearance} = (\text{rate of elimination})/\text{concentration}. \quad (1)$$

If rate of elimination is expressed as amount per unit time, e.g. with units mol min^{-1}, and the concentration as amount per unit volume, e.g. with units mol mL^{-1}, the clearance has the units of a volume flow, e.g. mL min^{-1}. Both elimination rate and substance concentration can be measured and both are required

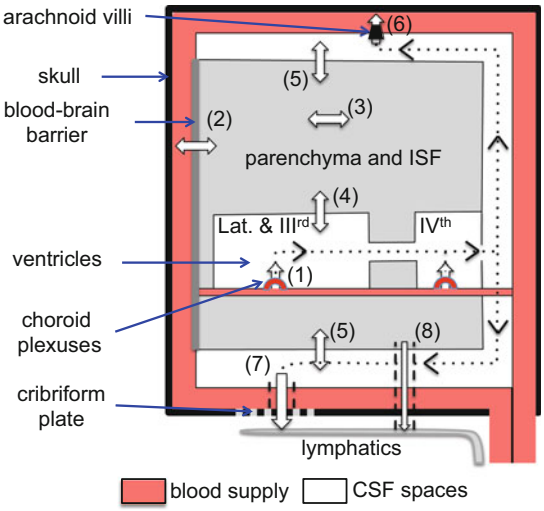


Fig. 1 Routes by which substances enter and leave cerebrospinal fluid (CSF), interstitial fluid (ISF) and the brain. The extracellular fluid of the brain consists of CSF filling the larger cavities including the ventricles, cisterns and subarachnoid spaces and ISF filling the small spaces between the cells of the brain parenchyma. CSF is secreted into the ventricles across the choroid plexuses (1). There is rapid movement of O_2, CO_2, H_2O and lipid soluble substances between blood and ISF across the blood–brain barrier (2). Movements within the ISF (3) are primarily by diffusion and there is also primarily diffusional exchange between ISF and CSF in the ventricles. Movements between ISF and the CSF in the subarachnoid spaces and basal cisterns surrounding the brain (5) are faster involving influx and efflux along the perivascular spaces (see Sect. 2.3) of the blood vessels that penetrate the parenchyma. There is outflow of CSF across the arachnoid villi (6) leading to the dural venous sinuses (including but not restricted to the superior sagittal sinus) and along cranial nerves, most notably the olfactory nerve leading to the cribriform plate (7) and thence to the nasal mucosa. There is also outflow of fluid associated with arteries or veins (8) leading to lymph nodes in the neck. The traditional view of the directions of net CSF flow from the choroid plexuses to the sites of outflow is indicated by the *dotted lines* with *arrowheads*. Figure modified from Hladky and Barrand (2014)

to describe the processes of elimination. The rate of elimination itself is not a good description because even if the elimination mechanism is unchanged, the rate can and usually does vary with substance concentration. The concentration alone is not a good description because it is affected by both production and elimination of the substance of interest. During many studies of sleep it is only the changes in concentrations of metabolites in interstitial fluid (ISF) or cerebrospinal fluid (CSF) that have been reported. Whether these can be interpreted as changes in rate of production of these metabolites or changes in their clearance can be difficult to establish.

As an additional complication, it is not immediately obvious which concentration should be used. The metabolite may be both inside and outside of cells and some of it may have precipitated. Probably the best choice is to use the exchangeable concentration in ISF because that concentration can be measured, e.g. by

microdialysis (Cirrito et al. 2003), and the different elimination processes can be described as depending upon it.

Though there are many different metabolites generated within the brain, this review will consider in specific terms only a few important examples (CO_2, glutamate/glutamine, lactate and amyloid-β) and how their clearances differ or may differ during periods of wakefulness and sleep. Comparing periods of wakefulness to periods of sleep, there is known to be increased nervous activity, increased glucose and O_2 consumption, increased lactate production and ISF lactate concentration, progressive increases in glutamate concentrations in ISF and increased production and ISF concentration of amyloid-β (Aβ) (Kennedy et al. 1981; Netchiporouk et al. 2001; Vyazovskiy et al. 2008b, 2009; Ball et al. 2010; Porkka-Heiskanen and Kalinchuk 2011; Dash et al. 2012; Naylor et al. 2012; Tononi and Cirelli 2014; Cedernaes et al. 2017). During non-rapid eye movement sleep (nREM sleep or slow-wave sleep) all of these changes are reversed at least in part. Thus, other things being equal, rates of elimination of these metabolites are expected to decrease during sleep as their ISF concentrations decline.

1.2 Pharmacokinetic Considerations

Providing the rate of elimination of a substance is proportional to its concentration then its clearance is a constant, independent of concentration. With only a few exceptions, drugs in clinical use show constant clearance over their therapeutic range. This makes dosing much simpler and safer. However, this may not be the case with brain metabolites for which elimination processes may saturate. In these situations clearance will decrease as concentration increases. Nevertheless, clearance is still a much better description of the elimination processes than either the rate of elimination or the rate constant for elimination described in the next section.

Averaged over a sufficient length of time, the concentration of any metabolite in the extracellular fluid must take on the value at which its rate of production (and release into the extracellular fluid) is balanced by its elimination. In terms of the clearance, CL, and concentration, c, on average

$$\text{CL} \times c = \text{rate of elimination} = \text{rate of production}. \tag{2}$$

Hence if the metabolite concentration, c, is increased after awakening then either the rate of production has increased or the clearance, CL, has decreased. Similarly if c decreases on falling asleep either the rate of production has decreased or CL has increased. Equation (2) is central to understanding control of the rate of elimination. If at any time the rate of elimination doesn't balance the rate of production, the concentration and thus the rate of elimination will change until it does. Thus in a very real sense so long as concentration can change the rate of elimination, that rate is determined by the rate of production. Changes in clearance alter the concentration at which this balance of rates occurs.

1.2.1 The Relationship between Clearance, Rate Constant of Elimination, Volume of Distribution and Half-Life

Calculation of the clearance requires measurement of the concentration in ISF, e.g. by microdialysis (Cirrito et al. 2003), which can be difficult. Thus, in many studies either the rate constant, k, or half-life, $t_{1/2}$, is reported instead. The rate constant is defined by

$$k = (\text{rate of elimination})/N \tag{3}$$

where N is the amount present. The units of the rate constant are thus reciprocal time, e.g. min^{-1}. From Eqs. (1) and (3) it can be seen that the rate constant and clearance are related, i.e. that $k \times N = \text{CL} \times c$ or $\text{CL}/k = N/c$. Thus when comparing clearances and rate constants what needs to be considered is the apparent volume in which the substance of interest is distributed, defined as the volume of distribution,

$$V_D = N/c. \tag{4}$$

If all of a metabolite is freely dissolved in ISF then the volume of distribution is just the same as the volume of the solution, but if some is reversibly bound to cells or in large aggregates or if it can rapidly enter and leave the cells, then the volume of distribution can be much larger than the solution volume (compare the discussion of soluble and exchangeable amyloid-β in Cirrito et al. 2003). *The rate constant for elimination of a substance depends not only on the processes of elimination described by the clearance but also on the volume over which the substance is distributed,*

$$k = \text{CL}/V_D. \tag{5}$$

It is this dependence on distribution of the substance that makes the rate constant less suitable than the clearance as a means for describing elimination.

Another parameter describing elimination is the half-life. This is the time taken for the amount present to decrease by half if production is abruptly stopped. If the decrease in amount remaining follows an exponential curve, the half-life and the rate constant for elimination are simply related by

$$t_{1/2} = 0.69/k. \tag{6}$$

Evaluation of half-lives and clearances using marker substances added to ISF can provide important indications of the nature and routes of elimination of particular metabolites. Marker substances such as albumin, inulin, mannitol and sucrose are stable, water soluble, do not enter cells, do not aggregate, do not bind to cell membranes and are very slow to cross the blood–brain barrier. Because these exit the brain primarily by non-selective transport together with many other components of ISF, they are used to describe elimination by perivascular pathways (see Sect. 2.3). The volumes of distribution for these substances are all just equal to

the volume of ISF, about 0.2 mL g^{-1}, measured values of the half-lives for these substances are in the range of 2–4 h (Shibata et al. 2000; Groothuis et al. 2007; Ball et al. 2010; Iliff et al. 2012) and the corresponding clearances are in the range of 0.6–1.2 μL g^{-1} min^{-1}.

Metabolites with half-lives shorter than those for markers like albumin, inulin, sucrose and mannitol must either be distributed over a smaller volume or have a higher clearance. The volume cannot be smaller because within the parenchyma these markers are already restricted to ISF. Thus any metabolite with a shorter half-life must have a higher clearance, i.e. it is eliminated by other routes in addition to the perivascular.

Because non-selective efflux via the perivascular route will contribute to the elimination of every water soluble substance, half-lives longer than a few hours for elimination from ISF imply either that clearance by this route is reduced, e.g. by obstruction of the perivascular spaces, or there is more of the substance present than expected from its concentration, i.e. the volume of distribution is larger.

2 Mechanisms of Elimination from the Brain Parenchyma

A metabolite can be eliminated, i.e. removed, from the extracellular fluid of the brain parenchyma by three types of mechanism, indicated in Fig. 2 as (a) further metabolism, (b) transport across the blood–brain barrier to blood, and (c) perivascular efflux along blood vessels. Elimination via the choroid plexuses of metabolites originating in the parenchyma is likely to be small, because under normal circumstances, i.e. in the absence of oedema, efflux from the parenchyma into the ventricles has been

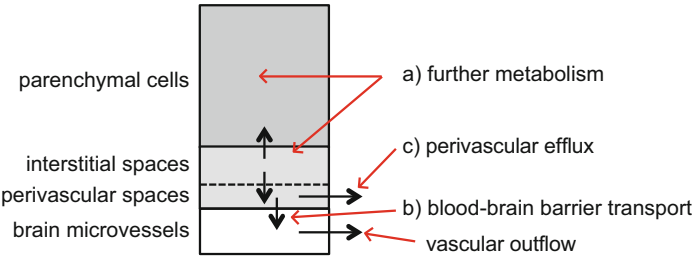


Fig. 2 Principal routes of metabolite clearance from ISF. A metabolite can: (a) enter cells where it can undergo further metabolism, (b) be transported across the blood–brain barrier that separates the interstitial fluid of the parenchyma from blood in the vasculature or (c) leave along with other components of ISF as part of perivascular efflux. The perivascular "space" around the capillaries may be (see text) the basement membrane between endothelial cells and astrocyte endfeet. The location of the perivascular spaces around larger blood vessels is controversial: candidates include a space between the vessel wall and the astrocyte endfeet and for arteries the basement membranes of the smooth muscle layer. Some perivascular efflux enters the CSF in the sub-arachnoid spaces, the remainder may follow vessel walls out of the brain to peripheral lymphatics (Yamada et al. 1991; Carare et al. 2008; Ball et al. 2010; Iliff et al. 2012) and for further discussion (Hladky and Barrand 2014)

found to be slow (Patlak and Fenstermacher 1975; Iliff et al. 2012; and discussion in Hladky and Barrand 2014). However, when fluid is infused into the parenchyma such efflux can be observed (Bedussi et al. 2015; McIntee et al. 2016).

2.1 Elimination by Further Metabolism

Further metabolism is often an important route of elimination for metabolic intermediates such as lactate produced by partial metabolism of glucose (see Sect. 3.3). It is also important in removing peptides including amyloid-β (Aβ) (see Sect. 3.4). Further metabolism eliminates one metabolite but, obviously, produces another. Metabolism of substances in the extracellular fluid often follows uptake into cells either via specific transporters or endocytosis. Transfer by specific transporters can be extensive and rapid, with persistence in the extracellular fluid for only seconds or minutes rather than hours.

2.2 Elimination across the Blood–Brain Barrier

Elimination from the parenchyma across the interfaces between brain and blood is primarily across the blood–brain barrier provided by the endothelial layer lining the brain microvasculature. Transfer across this interface may occur by simple diffusion across the membranes and cytoplasm of the endothelial cells. For instance diffusion may account for most of the transport of CO_2 between brain and blood and is so rapid that it is blood-flow limited (discussed in Hladky and Barrand 2016) (see Sect. 3.1). For larger or less lipid soluble metabolites interaction with specific transporters is required and endothelial cells have transporters for a large variety of substances (see the classic summaries Bradbury 1979; Davson and Segal 1996; and more recent reviews Abbott et al. 2010; Strazielle and Ghersi-Egea 2013; Pan and Kastin 2016). The presence of these transporters suggests that metabolites for which there are transporters will be cleared more rapidly across the blood–brain barrier than via perivascular routes but this may not always be the case, as will be discussed below for lactate (see Sect. 3.3).

To be cleared across the blood–brain barrier a metabolite in ISF must by some means reach the surface of the brain endothelial cells. Since the brain parenchyma is very well vascularised the distances for transfer are short, usually less than 20 μm. Diffusion over such short distances is rapid and rarely rate limiting. The metabolite must also be able to cross the layer of glial endfeet surrounding the microvessels. However, there are gaps between the endfeet (Mathiisen et al. 2010) and the gaps are not sealed by tight junctions. Because there are enough gaps, the endfoot layer is usually not rate limiting in comparison with transfer across the endothelial cells. If there are specific transporters for a metabolite at the blood–brain barrier and the concentration of the metabolite is low enough that the transporters are not saturated, this route of clearance may correspond to half-lives within the extracellular solution of less than 10 or 15 min.

2.3 Elimination via Perivascular Pathways

Elimination by efflux via perivascular routes is of great importance because it provides a method for removing anything that is water soluble and not too large including occasional unwanted waste products that must not be allowed to accumulate over a lifetime. Perivascular elimination provides a mechanism that can deal with the unexpected on a time scale of hours.

The clearance by perivascular efflux is the same or at least similar for substances with a wide range of sizes as has been measured for markers such as mannitol, inulin, sucrose, serum albumin, polyethylene glycols and various dextrans (Cserr et al. 1977; Carare et al. 2008; Iliff et al. 2012). Every metabolite in ISF will be cleared to some extent by this mechanism but its importance for any particular metabolite will depend on whether or not there are other faster routes of clearance. The markers listed here that have been used to determine the perivascular clearance are those for which all other routes of elimination are negligible.

2.3.1 Routes of Perivascular Efflux

"Perivascular" is taken here to describe various possible routes available along the walls of blood vessels but separated from the blood flowing through the vascular lumen (see "Nomenclature", p. 59 in Hladky and Barrand 2016).

There are two proposals for where substances move parallel to microvessels: either primarily via the basement membranes separating the endothelial cells from the astrocyte endfeet (Rennels et al. 1990; Carare et al. 2008) or distributed throughout the extracellular space between the brain cells (see e.g. Iliff et al. 2012). Which is more important may depend on solute size. In either case the distance to the nearest perivascular space adjacent to a larger vessel is likely to be relatively small, e.g. 100–200 μm. At least over distances less than ~100 μm diffusion is the dominant mechanism of transfer of solutes through the extracellular spaces of gray matter (Nicholson and Sykova 1998; Nicholson 2001; Sykova and Nicholson 2008; Asgari et al. 2016; Jin et al. 2016).

The route of solute efflux adjacent to larger vessels has been controversial (Hladky and Barrand 2014; Tarasoff-Conway et al. 2015; Simon and Iliff 2016). Efflux of markers along arteries has been seen in many studies (e.g. Szentistvanyi et al. 1984; Carare et al. 2008; Arbel-Ornath et al. 2013), but others have found convincing evidence for influx along arteries and suggestions of efflux along veins (Rennels et al. 1990; Iliff et al. 2012). There is also disagreement over the principal routes along arteries with some favouring an actual extramural perivascular space between the vessel walls and the endfeet, see e.g. (Szentistvanyi et al. 1984; Ichimura et al. 1991; Iliff et al. 2012; Arbel-Ornath et al. 2013; Bedussi et al. 2017) and others advocating instead an intramural perivascular pathway along the basement membranes of the smooth muscle layer (Carare et al. 2008, 2013b; Tarasoff-Conway et al. 2015) (see Appendix 1).

Some of the perivascular solute efflux from the parenchyma enters CSF from which it is delivered partly to venous blood and partly to lymph. The remainder appears to pass to lymph without first mixing with CSF (Bradbury et al. 1981;

Szentistvanyi et al. 1984; Weller et al. 2009; Pollay 2010; Aspelund et al. 2015; Louveau et al. 2015; Tarasoff-Conway et al. 2015; Bakker et al. 2016; Engelhardt et al. 2016; Simon and Iliff 2016).

2.3.2 Mechanisms Driving Perivascular Solute Efflux

There have been three proposals for how substances are propelled along perivascular pathways.

Proposal 1: there is secretion of fluid by the blood–brain barrier that provides a small pressure gradient for outflow of ISF along perivascular spaces (see Cserr and Ostrach 1974; Cserr et al. 1981; Szentistvanyi et al. 1984; Cserr and Patlak 1992). This was a plausible suggestion when it was believed that the half-life for clearance of solutes by outflow was of the order of 12 h. It now seems, however, that such half-lives are 2–4 h (Shibata et al. 2000; Groothuis et al. 2007; Ball et al. 2010; Iliff et al. 2012). The flow required in order that the clearance can be via an outflow of fluid with the same concentration of the solute as in the interstitial fluid is >0.6 μL g^{-1} min^{-1} (calculated as 0.69 $V/t_{1/2} = 0.69 \times 200$ μL g^{-1}/240 min). This is much larger than 0.1 μL g^{-1} min^{-1} which is at the high end of estimates for the blood–brain barrier secretion rate (see Sect. 4.1 in Hladky and Barrand 2016). Hence outflow of the fluid secreted across the blood–brain barrier is insufficient to account for perivascular clearance of solutes.

Proposal 2: there is convection in the perivascular spaces, arterial or venular, that allows relatively rapid movements of solutes both inwards and outwards. In this proposal movement in the interstitial spaces of the parenchyma is primarily by diffusion but movements along the larger vessels cannot be just diffusion because solutes added to CSF can appear in the parenchyma much too quickly for diffusion over the distance involved, a millimeter or more (Rennels et al. 1990; Ichimura et al. 1991; Iliff et al. 2012). Increased rate of solute movement along the perivascular spaces down a concentration gradient in either direction may result from convective mixing (Bradbury et al. 1981; Hladky and Barrand 2014; Bakker et al. 2016), which is perhaps better called dispersion (Asgari et al. 2016). Diffusion may well be adequate for movement from the interstitial spaces in the parenchyma to the perivascular spaces around the vessels because the distances involved are sufficiently short (Jin et al. 2016).

Proposal 3: there is a pressure driven flow of CSF inwards along periarterial spaces and into the parenchyma and outwards from the parenchyma along the perivenous spaces. This has been called the glymphatic circulation (Iliff et al. 2012; Nedergaard 2013). However, it is unclear how the flow required, at least 0.6 μL g^{-1} min (see proposal 1), can be driven through the parenchyma (Asgari et al. 2016; Jin et al. 2016). Jin et al. have calculated solute concentrations and fluid flows within the parenchyma assuming a realistic geometry; a range of membrane permeabilities spanning the expected physiological values; and the assumption that the ISF between the cells behaves as a free fluid with the viscosity of water. They concluded that “little or no advective solute transport is predicted to occur with physiological paravascular pressure differences” taken

to be less than 5 mmHg. The minimum pressures required for advection (the solute transport coupled to flow) to be important may be larger, perhaps much larger, than Jin et al. have calculated if the contents of the interstitial spaces in the brain have gel-like properties as for the extracellular spaces in the periphery. These properties conferred by the presence of macromolecules include much higher resistance to bulk flow than in simple solution (Levick 1987) with only slight restriction of diffusion (Nicholson 1980; Nicholson and Sykova 1998; Sykova and Nicholson 2008). Closely analogous effects are produced by adding agar to solutions to reduce convection.

Further considerations arguing against the glymphatic hypothesis are that net inward flow along periarterial spaces explains neither outward movements of solutes along arteries (Bradbury et al. 1981; Szentistvanyi et al. 1984; Cserr and Patlak 1992; Carare et al. 2008; Arbel-Ornath et al. 2013; Bedussi et al. 2017) nor the continuation of rapid inward movement of large solutes when the proposed glymphatic flow across the glial endfoot layer is hindered by deleting the major water channel AQP4 (Iliff et al. 2012) as discussed in Hladky and Barrand (2014).

An important part of the driving force for either net flow or mixing in the perivascular spaces is thought to be the periodic compression of the perivascular pathways resulting from changes in blood pressure during the cardiac cycle (Bradbury et al. 1981; Carare et al. 2008; Iliff et al. 2012, 2013; Bakker et al. 2016). It should be noted that the length of perivascular space around a cortical vessel that is compressed at any one time is as long as the vessel (Bradbury et al. 1981; Asgari et al. 2016). Bradbury et al. (1981) were of the opinion that periodic compression of this space "would cause to-and-fro movement of fluid in and out of the brain" such that "A basis would be provided for substances in solution or suspension to be moved either out of or into the brain depending on the relative concentration in subarachnoid CSF." The back-and-forth convective movements would only be apparent using techniques with both a time resolution better than a fraction of a second and good spatial resolution. With the techniques now available all that would be seen would be accelerated movement down the concentration gradient regardless of its direction.

2.4 How Do the Mechanisms Differ between Sleep and Wakefulness?

There is surprisingly little known about how the elimination mechanisms described above may differ between sleep and wakefulness and how these differences may impact on the clearance of various metabolites. While rates of further metabolism of metabolites are very likely to change, simply because their concentrations change, very little is known about whether clearance due to further metabolism differs between sleep and wakefulness. The little that is known about variations in blood–brain barrier transporters between sleep and wakefulness has been discussed by Pan and Kastin (2016) while diurnal changes in the activity of P-glycoprotein

have been documented by Kervezee et al. (2014). Variations in the blood–brain barrier clearance for amyloid-β are considered in Sections "Clearance across the Blood–Brain Barrier" and 3.4.3.

In order to compare perivascular influx and efflux in awake, asleep or anaesthetised mice, Xie et al. (2013) analysed the movements of three different tracers: Texas red dextran (3kD) which enters the brain from CSF by perivascular influx; inulin, which is eliminated from the brain almost entirely by perivascular efflux; and tetramethylammonium (TMA) ions, whose diffusion within the parenchyma can be used to measure ISF volume. Their results demonstrated that for awake mice compared to those that are either asleep or anaesthetised:

- influx of Texas red dextran (3kD) into the parenchyma following 30 min infusion into the cisterna magna was ~95% less, a 20-fold reduction
- the rate constant for ^{14}C-inulin elimination ($=0.69/t_{1/2}$) was ~63% smaller, a 2.7-fold reduction (calculated assuming a single exponential decay)
- astrocytes were swollen while the extracellular space volume was about 38% less, a 1.6-fold reduction (assessed from space available for diffusion of tetramethylammonium (TMA) ions).

From their results one can calculate the change in clearance for inulin:

- using CL = (rate constant) × V, the clearance is lower in awake mice than in mice that are asleep by a factor of $1.6 \times 2.7 = 4.3$, i.e. a 77% decrease.

Xie et al. proposed that the effects of awakening on extracellular volume and clearance are mediated by noradrenergic activity because they noted that in the presence of antagonists of noradrenergic transmission values of ISF volume and influx of fluorescent markers were similar to those seen with mice that were asleep. The importance of β-adrenoceptors in similar volume changes has been shown by Sherpa et al. (2016).

Xie et al. (see also Kress et al. 2014) interpreted both the change in rate constant for inulin efflux and the dramatic change in influx seen for Texas red dextran as being consequences of a large change in the rate of glymphatic circulation (see proposal 3, Sect. 2.3.2). They further concluded that it was the reduction in the volume of the extracellular space during wakefulness inferred from the TMA results that decreased the rate of glymphatic circulation. It is, however, difficult to understand how a change in flow rate would have had a much smaller effect on inulin clearance (<5-fold) than on dextran influx (~20-fold). From inspection of Xie et al.'s published images, it appears likely that the dramatic effect on dextran influx results not from changes within the parenchyma, such as alterations in the volume of ISF, the properties of astrocyte endfeet or the tightness of the blood–brain barrier, but rather from changes in delivery along the arteries in the subarachnoid space produced in a manner that has yet to be explained. Altered delivery might result from subtle variations in either the pressure differences between these spaces and the cisterna magna or the dimensions of the subarachnoid spaces.

There may or may not be changes in interstitial and perivascular fluid flows association with the changes in ISF volume reported in sleep-wake transitions. Furthermore such changes in flows may or may not account for the difference in inulin clearance. For the case against the importance of fluid flow and thus per force against the importance of changes in the flow see Asgari et al. (2016) and Jin et al. (2016) (see also Sect. 2.3.2).

In summary, perivascular clearance as measured using inulin is ~4-fold lower in mice that are awake compared to those that are asleep. The reasons for this are not completely clear (see also Sect. 3.4.3).

3 Clearance of Important Brain Metabolites

This section discusses the extent to which each of the elimination mechanisms described above are involved in clearance of particular brain metabolites and how differences between sleep and wakefulness affect these clearances.

3.1 Carbon Dioxide

In terms of molar amounts, by far the most important waste products of metabolism in the brain are carbon dioxide and water. These are disposed of rapidly by diffusion across the blood–brain barrier, the only sufficiently accessible interface between brain and blood. In fact these transfers are so rapid that they are to a large extent cerebral blood-flow limited.

3.1.1 Differences in CO_2 Clearances between Sleep and Wakefulness

During slow-wave sleep rates of elimination are reduced simply because the rates of production are less. However, pCO_2 in the brain should fall rather less than the rate of CO_2 production because cerebral blood flow and hence CO_2 clearance decreases (for more detailed consideration see Sect. 6 in Hladky and Barrand 2016).

The obvious sleep related disorder of CO_2 elimination is sleep apnea. In this condition CO_2 can still be transferred from brain to blood but, because of the absence of adequate ventilation, CO_2 accumulates in the whole body and pCO_2 increases in the blood to the extent that net diffusion out of the brain can no longer match production.

3.2 Glutamate and Glutamine

Handling of glutamate, glutamine and ammonium within the brain are interlinked and are in addition linked to the handling of many other amino acids by transamination reactions. Glutamine and ammonium are transported in both directions across the blood–brain barrier. Glutamate in the brain arises primarily by deamination of glutamine in neurons and by transfer of amino groups from other amino

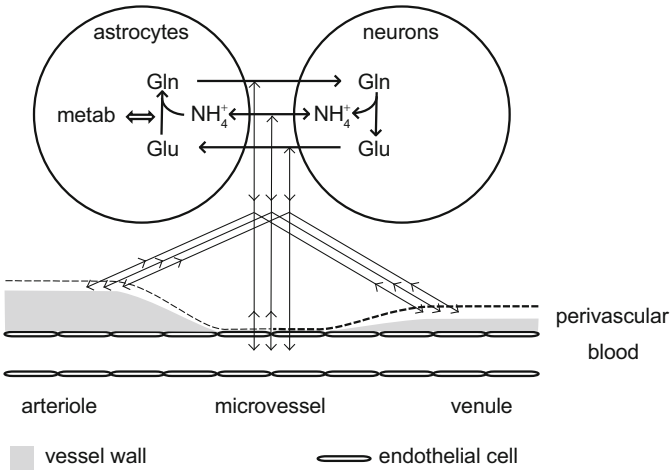


Fig. 3 Simplified view of the clearance of glutamate, glutamine and NH_4^+. Glutamate (Glu) enters the ISF primarily via release from glutaminergic neurons. It is removed primarily by uptake into astrocytes where it is converted into glutamine (Gln), most of which returns to the neurons where it is converted back to glutamate and stored ready for release. This cycle is shown with *bold arrows*. The overall levels of both glutamate and glutamine appear to be determined primarily by metabolism within astrocytes (metab in the figure) and elimination of glutamine across the blood–brain barrier. Glutamate can enter the endothelial cells of the blood–brain barrier from the brain but flux in the opposite direction is thought to be negligible because the only transporters for glutamate in the abluminal membrane (facing the brain) are Na^+-linked and glutamate is thus driven into the cells by the gradient of Na^+ (Hawkins et al. 2006, 2013). Most of the glutamate transported from the endothelial cells to the blood is produced within the cells from glutamine (Lee et al. 1998; Hawkins et al. 2006). The rates of elimination of glutamine and glutamate via perivascular routes are unknown

acids to α-ketoglutarate. It enters ISF by neurotransmitter release from neurons (see Fig. 3). Glutamate entry to the brain from the blood is negligible (Hawkins 2009).

Most glutamate is removed from brain ISF by uptake into astrocytes. This keeps the extracellular glutamate concentration in the brain parenchyma well below that in plasma. Within astrocytes the glutamate is converted to glutamine for recycling to neurons (see Fig. 3). Glutamate also leaves the brain by being transported into blood across the blood–brain barrier. Much of this glutamate leaving to blood is produced from glutamine within the endothelial cells (Lee et al. 1998; Hawkins et al. 2006). A role for the perivascular route in elimination of glutamine and glutamate has apparently never been investigated, presumably because the rates of elimination via this route would be expected to be small compared to reuptake into cells and elimination across the blood–brain barrier.

3.2.1 Differences in Glutamate Levels between Sleep and Wakefulness

Glutamate ISF concentrations are thought to increase progressively at night when rats are often awake and to decrease to a basal level during the day when they are

often asleep. There is a marked increase to a plateau when they are sleep-deprived (Dash et al. 2009). The normal progressive changes may be a direct consequence of increased release of glutamate from neurons associated with an upregulation of synaptic transmission during wakefulness and its reversal during sleep (Vyazovskiy et al. 2008a, 2009; Liu et al. 2010; Hulse et al. 2011; Lante et al. 2011). If there is a decrease in ISF volume on awakening (see Sect. 2.4) this may contribute to the increase in ISF glutamate concentration observed in the first half hour or so of wakefulness. Increased concentration during wakefulness would be expected to increase the rates of elimination across the blood–brain barrier.

3.3 Lactic Acid/Lactate

Lactic acid is the product of glycolysis and conversion of pyruvate to lactate and can also be produced from metabolism of other substrates, including glutamate (Sonnewald 2014). In solution almost all of it dissociates to lactate$^-$ and H^+. Lactic acid production by metabolism in the brain cells and the possibility that the lactate$^-$ can be removed from the brain leaving the H^+ behind have important consequences for pH regulation (discussed in Sect. 6 of Hladky and Barrand 2016). The extent to which lactic acid released from astrocytes is taken up by neurons and used as fuel for their activity remains controversial (see e.g. Dienel 2012; Dienel and Cruz 2016; Petit and Magistretti 2016).

One route for lactate removal from the brain is via efflux across the blood–brain barrier mediated by MCT1 transporters that are present in both luminal and abluminal membranes of the endothelial cells lining brain microvessels (see Fig. 4). At this barrier there is also uptake of lactate from plasma because MCT1 facilitates transport in both directions (for references see p. 47 and footnote 26 of Hladky and Barrand 2016). Under conditions where there is little lactate production, i.e. in unstimulated anaesthetised rats, the amount of lactate released into ISF was thought to be balanced by the amount removed via a barely detectable *net* efflux across the blood–brain barrier (Hawkins et al. 1973; Siesjö 1978; Dienel and Cruz 2003).

Another possible pathway for lactate removal from the brain is via perivascular efflux. However, in unstimulated anaesthetised rats this route is not expected to be important. This is because it has been shown that the clearance for lactate (usually referred to as the permeability-surface-area product, *PS*) via MCT1 at the blood–brain barrier is about 60 μL g^{-1} min^{-1} in adult rats (Daniel et al. 1972; Drewes and Gilboe 1973) and 100 μL g^{-1} min^{-1} in humans (Knudsen et al. 1991). These values far exceed estimates of clearance via perivascular efflux as assessed using markers like inulin, ~1 μL g^{-1} min^{-1} (see Sect. 1.2.1). However, this comparison may be misleading for two reasons. Firstly, locally in regions of nervous activity ISF concentration can be high and can saturate the MCT1 transporters. Secondly, lactate can be distributed through the parenchyma by transfers within and between astrocytes (see Fig. 4), a mechanism not available to the markers of perivascular efflux. Lactate unlike the markers can be released from astrocyte endfeet adjacent to

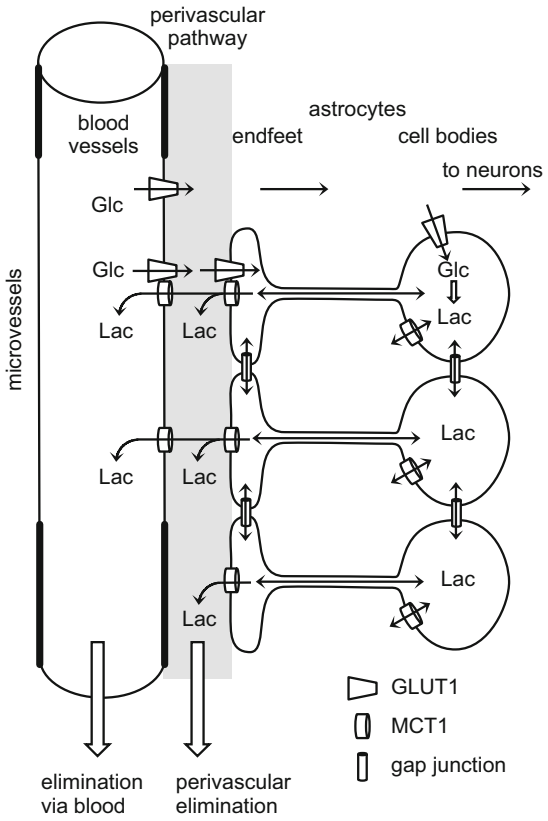


Fig. 4 Production of lactate and its removal from the brain. Glucose (Glc) is shown entering the brain in a region of activity. Lactate (Lac) generated in astrocytes within that region can be transferred via gap junctions to other astrocytes and be released from endfeet either near microvessels or larger vessels. MCT1 monocarboxylate transporter 1 and GLUT1 glucose transporter 1 mediate passive transport down the concentration gradient for lactic acid or glucose respectively. The *arrows* between blood and the perivascular spaces indicate the direction of net transport. Transport in the perivascular pathway may be occurring along arteries or veins. In the diagram *heavy lines* indicate the walls of arterioles and venules that are presumed to prevent exchange of lactate. Diagram modified from Fig. 7c in Gandhi et al. (2009a)

perivascular spaces of larger vessels from which efflux may be rapid (Ball et al. 2007, 2010; Gandhi et al. 2009a, b).

Indication that there are at least two routes of lactate elimination was obtained in investigations of spreading depression in rats (Cruz et al. 1999). This is an extreme condition in which there is extensive release of lactate, obvious net transport of lactate out of the brain across the blood–brain barrier and obvious loss of lactate by another, presumably perivascular route (Cruz et al. 1999; Ball et al. 2010).

Two routes of lactate elimination are also evident with the more physiological scenario of general sensory stimulation (observed in rats with removal of a shelter

and gentle stroking over the body with a paint brush). Under these conditions more lactate was produced within the brain and in the rest of the body and lactate concentrations were increased in both ISF and plasma. Although there was increased lactate exit across the blood–brain barrier this was nearly balanced by increased entry and there was little change in the net movement. Thus the extra lactate released within the brain must either have been distributed to regions outside those assayed or removed via other, presumably perivascular routes (Madsen et al. 1999; Dienel and Cruz 2003, 2004).

At least some of the lactate that leaves via perivascular routes reaches the meninges (Ball et al. 2010) and lymph nodes (Ball et al. 2010; Lundgaard et al. 2016). The rest reaches CSF from which a fraction will travel with CSF to venous blood via the arachnoid villi and much of the remainder will be carried by the CSF via the cribrifrom plate to the nasal mucosa. From there most of the lactate along with other small solutes is expected to cross into venous blood in the peripheral capillaries of the mucosa (Bradbury and Westrop 1983).

3.3.1 Differences in Lactate Clearances between Sleep and Wakefulness

The concentration of lactate in ISF provides a useful indicator of whether an experimental animal is awake or asleep with clear increases (by about 0.15 mM) during wakefulness to a maintained level and subsequent decrease during sleep. Naylor et al. (2012) used microbiosensors to measure the time course of lactate concentration changes in the ISF of mice during the onset of individual episodes of sleep and wakefulness. The half-lives both for increases on awakening or decreases on falling asleep were short, 4–5 min. The obvious first suggestion to explain such rapid changes is that the rates of production and release of lactate were rapidly altered by brain activity with the concentration adjusting to the new rate with a half-life governed by the clearance. However, the rapid changes could also be explained by constant rate of release and rapid small changes in clearance (total by all routes). If the clearance does change, the half-lives for the increases and decreases should also differ (shorter when asleep), but for the small changes in concentration observed (perhaps 15%), the difference in half-lives would be too small to be determined.

Lundgaard et al. (2016) used microdialysis probes (that are considerably larger than the microbiosensors (Naylor et al. 2012)) to measure lactate concentrations in the brains of unrestrained mice exposed to a daily cycle of 12 h darkness and 12 h light. Average concentrations measured in the light when the mice were asleep for about 140 min of the 12 h were about 28% lower than those measured in the dark when the mice were asleep for about 20 min of the 12 h. It is difficult to calculate clearances in awake and sleeping mice from these concentrations partly because the rates of release of lactate from the cells and rates of influx of lactate from blood are likely to have differed (compare Madsen et al. 1999; Dienel and Cruz 2003) but also because the animals were awake and asleep during parts of both periods.

In experiments designed to circumvent such difficulties, Lundgaard et al. looked at three different treatments that had been shown in previous studies to reduce

perivascular clearance as assessed by clearance of inulin (Plog et al. 2015): administration of acetazolamide, which reduces CSF production by the choroid plexuses; knock-out of AQP4, which reduces the water permeability of astrocyte endfeet and slows access of CSF-bourn-markers to ISF (Iliff et al. 2012); and puncture of the cisterna magna, which allows drainage of CSF. *None of these procedures produced a significant change in the lactate concentration measured in the dark phase when the mice were almost always awake suggesting that perivascular clearance was then relatively unimportant*. However all three interventions made 20–30% smaller the decrease in lactate concentration observed between mice in the light (rarely asleep) and the same mice in the dark (more often asleep). *This is strong, though circumstantial, evidence that perivascular clearance of lactate is more important when the mice are asleep than when they are awake* (Lundgaard et al. 2016). It is unclear whether a reduction in lactate concentration during sleep has any beneficial or harmful effect.

3.4 Amyloid-β

The Aβ polypeptides are a group of metabolites of some considerable interest because of their association with Alzheimer's disease. These polypeptides are produced in the brain by cleavage of the membrane bound amyloid precursor protein (APP) (see Fig. 5). Various functions have been ascribed to these and other APP cleavage products for normal brain cell activity but it is the disproportionate generation in excess of elimination of the Aβ products that leads to their aggregation, plaque formation and deposition around blood vessels.

Of the Aβ peptides formed in the brain, most work has focussed on $A\beta_{1–40}$ and $A\beta_{1–42}$, these being present at the highest concentrations. They exist as monomers in solution at low nanomolar concentrations and, particularly for $A\beta_{1–42}$, also as oligomers (Cirrito et al. 2003; Bell et al. 2007). Monomers and oligomers together are sometimes referred to as soluble Aβ. In young animals only soluble Aβ is detectable. However in older animals there are often deposits along cerebral arteries (cerebral arterial angiopathy or CAA). These are mainly aggregates of $A\beta_{1–40}$. Large aggregates or plaques are also observed in the brain parenchyma; these are mainly composed of $A\beta_{1–42}$. Small changes in soluble Aβ concentrations may over time produce important changes in the rate of formation of Aβ aggregates (Lomakin et al. 1996; Harper and Lansbury 1997; Hortschansky et al. 2005; Iwata et al. 2005; Yan et al. 2009; Ye et al. 2015). While it is not known which form of Aβ is responsible for the toxic effects ascribed to it, current evidence appears to suggest that within the parenchyma the main culprits are the oligomers (Lambert et al. 1998; Zerbinatti et al. 2004; Haass and Selkoe 2007; Nisbet et al. 2015; McIntee et al. 2016).

3.4.1 Clearance of Aβ from ISF

The following sections consider the pathways by which elimination of Aβ formed in the brain can occur. In the young, Aβ is produced and present in soluble form and

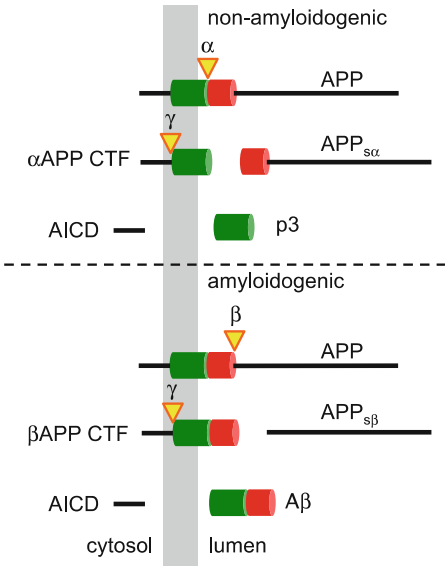


Fig. 5 Production of amyloid-β and other products by cleavage of membrane-bound amyloid precursor protein, APP. Cleavage begins at either of two locations mediated by α-secretase or by β-secretase, also called BACE1. These are mutually exclusive. One of the two products is released on the luminal/non-cytoplasmic side of the membrane. The other remains in the membrane and is then cleaved by a γ-secretase, which can cut at a number of closely spaced locations. The resultant products are a short peptide, AICD, released into the cytosol and either p3 peptides (on the α, non-amyloidogenic metabolic pathway) or Aβ peptides (on the β, amyloidogenic pathway). APP cleavage may occur in transGolgi, plasma and endosomal membranes. Based on Haass et al. (2012)

is eliminated as rapidly as it is produced with about 7–8% of the total soluble Aβ being replaced each hour (Bateman et al. 2006; Mawuenyega et al. 2010). Monomeric and small oligomeric forms of Aβ are cleared from ISF by at least four routes: incorporation into plaques, metabolism either extracellular or after uptake into cells (Iwata et al. 2000; Van Uden et al. 2002; Saido and Iwata 2006; Bu 2009; Saido and Leissring 2012; Kanekiyo et al. 2013; Ries and Sastre 2016), efflux across the blood–brain barrier (Shibata et al. 2000; Bell et al. 2007; Zhao et al. 2015; Nelson et al. 2016; Storck et al. 2016) and efflux via perivascular routes (Ball et al. 2010; Iliff et al. 2012; Xie et al. 2013; Peng et al. 2016). The relative importance of these mechanisms remains controversial (Tarasoff-Conway et al. 2015). Clearance of Aβ from blood plasma is rapid with a half-life of a few minutes, primarily by metabolism in the liver and spleen (Ghiso et al. 2004; Kandimalla et al. 2005).

Clearance by Aggregation?

In vitro studies have shown that aggregates can continue to grow at lower monomer concentrations than are required for initial aggregation (see e.g. Harper and Lansbury 1997). In humans, CSF concentrations of $A\beta_{1–42}$ are lower when plaques

are present in the parenchyma than when they are absent (Fagan et al. 2006; Potter et al. 2013) implying that plaques can take up soluble $A\beta_{1–42}$ fast enough to decrease the concentration of soluble forms. In addition, once plaques are formed there are no longer diurnal variations in the concentration of soluble $A\beta_{1–42}$ (Roh et al. 2012). In effect incorporation into aggregates reduces, at least temporarily, the amount of soluble forms.

Some suggest that formation of both oligomers and aggregates is reversible (see e.g. Harper and Lansbury 1997; Cirrito et al. 2003) though removal of "seeds" for aggregates may be very slow (Ye et al. 2015); however others believe that aggregation is effectively irreversible (Lomakin et al. 1996). In favour of the former view, it was found that cutting the nerves fibres leading to terminals releasing Aβ in a particular brain region led to plaques already formed within that region disappearing over time (Lazarov et al. 2002). Similarly if Aβ production was abruptly stopped using a γ-secretase inhibitor, the loss of Aβ exceeded the amount of soluble Aβ initially present (Cirrito et al. 2003). Aggregates that can be dissociated serve as a form of buffer and, if their dissociation is sufficiently rapid, will increase the volume of distribution (see Sect. 1.2.1). Aggregates that cannot be dissociated provide a route of elimination of the soluble forms.

Clearance by Uptake and Further Metabolism

It is clear from a number of studies that Aβ peptides can be metabolised further within the brain with some enzymes being active against Aβ in the extracellular spaces and some requiring that the peptides are first taken up into cells. Metabolites have been detected in CSF early after injection of $A\beta_{1–40}$ at high concentrations into the parenchyma (Shiiki et al. 2004; McIntee et al. 2016). Important enzymes for metabolism of Aβ include neprilysin, insulin degrading enzyme (IDE) and matrix metalloproteases (MMPs) (Iwata et al. 2000, 2001; Van Uden et al. 2002; Tanzi et al. 2004; Saido and Iwata 2006; Farris et al. 2007; Bu 2009; Miners et al. 2011; Saido and Leissring 2012; Kanekiyo et al. 2013; McIntee et al. 2016; Ries and Sastre 2016). Uptake of Aβ by LRP1 receptor-mediated endocytosis has been shown to be important for intracellular metabolism in neurons, astrocytes and vascular smooth muscle cells (Bu 2009; Kanekiyo et al. 2012; Kanekiyo and Bu 2014).

The rate of metabolism increases with Aβ concentrations well into the micromolar range (compare Shirotani et al. 2001) implying lack of saturation at physiological concentrations. At low micromolar Aβ concentrations when the principal blood–brain barrier transport process is saturated (see Section "Clearance Across the Blood–Brain Barrier") metabolism clearly plays the dominant role in elimination of Aβ peptides. However, the contribution of metabolism to elimination when concentrations of the Aβ soluble forms are in the physiological, low nanomolar range has been controversial. In favour of a substantial role Shiiki et al. (2004) found in rats that blocking metabolism using the neprilysin inhibitor thiorphan did produce an effect reducing the rate constant for elimination of $^{125}I\text{-}A\beta_{1–40}$ by 34% and this was when the Aβ concentration was in the low nanomolar range (see also Ito et al. 2013). Perhaps the strongest evidence that metabolism is important as a

normal clearance route comes from the observation that the amount of Aβ in the parenchyma was increased, in some cases by 50–100%, in neprilysin null mice (Iwata et al. 2001; Farris et al. 2007; see also Selkoe 2001) and by a similar amount when Aβ degrading enzymes were chronically inhibited (Iwata et al. 2001; Yin et al. 2006; Miners et al. 2011). Additional evidence that metabolism can in principle be important for elimination was obtained in studies in transgenic mice overexpressing neprilysin or IDE (Leissring et al. 2003).

Clearance across the Blood–Brain Barrier

The ways in which Aβ can be transported across the blood–brain barrier have been explored by several different groups. Shibata et al. (2000) were the first to propose that Aβ could cross the blood–brain barrier by LRP1-mediated transcytosis and that this could account for the loss of ^{125}I-Aβ_{1-40} from the brain that they observed by following the fate of ^{125}I. They showed that the loss of total ^{125}I from the brain was reduced by antibodies against LRP1, by RAP which binds to LRP1 and inhibits binding of substrates and by apoE knockout (apoE may influence the interaction of Aβ with LRP1). Furthermore the elimination process appeared to be saturable with K_m of 15 nM. These properties are consistent with elimination of ^{125}I-Aβ_{1-40} being primarily across the blood–brain barrier via an LRP1-dependent process. However it should be kept in mind that demonstrating the importance of LRP1 is not the same as demonstrating elimination via the blood–brain barrier because LRP1 is also present on neurons, astrocytes and vascular smooth muscle cells where it can mediate endocytosis of Aβ leading to its metabolism (Bu 2009; Kanekiyo et al. 2012; Kanekiyo and Bu 2014).

Because in Shibata et al.'s experiments there was loss of the radiotracer (^{125}I) from the brain, further metabolism of ^{25}I-Aβ_{1-40} alone cannot explain the results. The alternative explanation to transfer across the blood–brain barrier is that the administered ^{125}I-Aβ_{1-40} was degraded in the brain producing an ^{125}I-containing product that was itself effluxed (see e.g. Ito et al. 2013). Shibata et al. argued against this possibility based on their finding that the total ^{125}I remaining within the brain could still be precipitated using TCA, i.e. that it was still attached to some form of peptide, and that, in the instances where they checked, the peptide still migrated as ^{125}I-Aβ_{1-40} in HPLC. Thus, if metabolism was an important part of the elimination, the ^{125}I-containing metabolites must have been eliminated so rapidly as to be undetectable within the brain. This would appear to be unlikely (but see McIntee et al. (2016) for rapid appearance of metabolites in CSF).

Results favouring some production of metabolites were presented by Shiiki et al. (2004) who found from HPLC analysis of jugular venous blood that radioactivity was present as both intact ^{125}I-Aβ_{1-40} and metabolites. However these assays required injection of ca. 30× higher concentrations of ^{125}I-Aβ_{1-40} than would normally be seen. At these high concentrations efflux mechanisms at the blood–brain barrier would have been saturated and metabolism, which is easily demonstrated at high concentrations, would be relatively more important. Furthermore the metabolites may have been produced after efflux or possibly as part of

the efflux process. In Shiiki et al.'s hands using rats, the elimination rate constant for ^{125}I-A$\beta_{1–40}$ was not reduced by RAP.

Subsequently, Bell et al. (2007) using mice reported that iodinated and unlabelled human Aβ, detected by ELISA, were eliminated from the parenchyma of mice at the same rate, that intact Aβ was present in plasma after 30 min, and that this appearance in plasma was completely blocked by centrally administered anti-LRP1 antibody. These findings reinforce the conclusions reached by Shibata et al. (2000). Bell et al. also found that the rate constant for elimination of A$\beta_{1–42}$ was about half that for A$\beta_{1–40}$. This could reflect either a doubling of the volume of distribution or a halving of the clearance. A difference in the volume of distribution is likely because in the parenchyma the concentration of exchangeable A$\beta_{1–42}$ is less than that for A$\beta_{1–40}$ (Cirrito et al. 2003) but the amount of A$\beta_{1–42}$ in aggregates is greater. A difference in clearance is at least as plausible because A$\beta_{1–40}$ has been shown to have higher affinities than A$\beta_{1–42}$ for LRP1-mediated binding and uptake into the brain endothelial cells (Deane et al. 2004).

In further experiments not described here the properties of efflux across the blood–brain barrier have been elucidated in considerable detail including the importance of PICALM (Deane et al. 2004; Zhao et al. 2015; Nelson et al. 2016). These studies leave little room for doubting that transcytosis of intact Aβ across this barrier is important for Aβ elimination.

Results from several other studies support the idea that efflux of Aβ occurs at the blood–brain barrier and that LRP1 is important in this elimination. Jaeger et al. (2009) showed that a cocktail of phosphorothioate antisense oligonucleotides against LRP-1 substantially decreased the loss of A$\beta_{1–42}$ after intraparenchymal injection. Using an in vitro system, Pflanzner et al. (2011) demonstrated LRP1-dependent A$\beta_{1–40}$ transport across monolayers of primary mouse brain capillary endothelial cells, a transport not observed in monolayers of cells with genetically modified LRP1. Roberts et al. (2014) confirmed that efflux of Aβ from brain to blood occurs in vivo by finding that the concentration in venous blood leaving the brain was 7.5% higher than that in arterial blood. Storck et al. (2016) developed a mouse model in which LRP1 could be knocked out selectively in endothelial cells and were able to show that the knockout reduced the initial rate of loss of ^{125}I-A$\beta_{1–42}$ by 48% and increased the amount eventually retained. The increased retention is explained if the slower loss allows more time for the ^{125}I-A$\beta_{1–42}$ to be converted to a form, possibly an aggregate, that was unable to be removed from the brain (see Appendix 2).

Taken together the evidence indicates that a major proportion of the Aβ *elimination from the brain is dependent on* LRP1 *in the endothelial cells lining brain microvessels and that the interaction with* LRP1 *brings about* Aβ *efflux across the blood–brain barrier.*

While LRP1-mediated transcytosis is the most widely considered means for effluxing Aβ across the blood–brain barrier, P-glycoprotein may also have a role. Aβ has been shown to be a substrate for P-glycoprotein, a multi-substrate efflux pump located in the luminal membranes of brain endothelial cells. However how this pump can mediate transport across the entire barrier has not been established.

Its role appears to be in some way complementary to that of transcytosis (Cirrito et al. 2005; Hartz et al. 2010; Ohtsuki et al. 2010; Erickson and Banks 2013; Pan and Kastin 2014).

Clearance via Perivascular Routes

The perivascular route for elimination of Aβ peptides was investigated even before other routes of elimination were implicated. In the initial studies, deposits of Aβ were found along the external boundaries of arterial walls in the early stages of deposition (Weller et al. 1998, 2000; see also Yamaguchi et al. 1992; Hawkes et al. 2012) but also throughout the smooth muscle layer of the arteries in the later stages (Weller et al. 1998; Keable et al. 2016). These initial studies are consistent with growth of the deposits adjacent to an efflux route along the outside of the arteries, i.e. an extramural periarterial route.

Subsequently studies were undertaken using fluorescent dextran as a non-metabolizable marker for substances of the size of Aβ. This was injected into the parenchyma and within minutes fluorescence could be visualised throughout the smooth muscle layer of the arterial walls (Carare et al. 2008). From this observation it was proposed that both the fluorescent dextran and Aβ observed within the smooth muscle layer were following the same intramural periarterial route of efflux. There is at present no compelling evidence to decide between the intramural and extramural routes. Whenever either dextran or Aβ was observed within the smooth muscle layer it was at much higher density than in the adjacent regions of parenchyma (Carare et al. 2013a) suggesting that something must have concentrated or trapped it in the smooth muscle layer. For Aβ there is in fact good evidence for interactions of the peptides with components of the basement membrane matrix (Hawkes et al. 2011; Zekonyte et al. 2016). However, the fact that Aβ or dextran reaches the basement membranes within the smooth muscle layer does not prove that these are the actual route of efflux. Rapid movement between a transport pathway outside the muscle layer and the muscle layer itself is suggested by the observation of Zervas et al. (1982) that both horseradish peroxidase and 3H-leucine can rapidly enter the muscle layer of cerebral arteries from CSF.

Iliff et al. (2012) found that 1 h after injection of fluorescent tagged HyLyte-555-amyloid β_{1-40} into mouse striatum it was seen adjacent to capillaries and the walls of certain large veins. After injection of high concentrations of ^{125}I-Aβ_{1-40}, McIntee et al. (2016) found that metabolites reached CSF within 5 min followed over tens of minutes by intact ^{125}I-Aβ_{1-40}.

3.4.2 Relative Importance of the Different Routes for Elimination of Aβ

Attempts have been made to estimate the proportions of Aβ eliminated by the various routes, i.e. by metabolism, by efflux across the blood–brain barrier and by perivascular efflux. Shibata et al. (2000) and subsequently Xie et al. (2013) observed that the half-time for the elimination of ^{125}I-Aβ_{1-40} was much shorter than could be explained by elimination via the perivascular route as indicated by the half-life for elimination of inulin.

To obtain an estimate for transfer across the blood–brain barrier Shibata et al. assumed that the rate constant for perivascular elimination of ^{125}I-A$\beta_{1–40}$ was equal to the rate constant for elimination of inulin, and subtracted this from the observed rate constant. As they had concluded that metabolism played little part, this non-perivascular elimination was held to be transfer across the blood–brain barrier. However, the assumption that perivascular clearance is the same for ^{125}I-A$\beta_{1–40}$ and inulin does not imply that the rate constants are the same. With equal clearances, the rate constant for elimination of ^{125}I-A$\beta_{1–40}$ by the perivascular route should be calculated as $k_{\mathrm{Ab,ISF}} = k_{\mathrm{inulin}}(V_{\mathrm{inulin}}/V_{\mathrm{Ab}})$ (see Sect. 1.2.1). Because the volume of distribution for ^{125}I-A$\beta_{1–40}$ is greater, possibly much greater, than that for inulin, the estimate of the rate constant for the perivascular route for ^{125}I-A$\beta_{1–40}$ to be subtracted from the total should be smaller than that for inulin. Thus their estimate of the rate constant for the blood–brain barrier process, calculated as the difference, should have been larger. This strengthens the conclusion that at low concentrations of Aβ the non-perivascular process accounts for much more efflux of Aβ than the perivascular process.

The data reported by Iliff et al. (2012) and Xie et al. (2013) have also shown substantially faster elimination for Aβ than for inulin. In their studies on the influence of AQP4 water channels on the movement of substances into and out of the brain, they found that knockout of AQP4 reduced to a similar extent the rate constants for elimination of inulin and Aβ. They argued from the result for inulin that AQP4 reduces perivascular clearance and from the similar result for Aβ that a large proportion of Aβ elimination was by the perivascular route. The second contention is difficult to reconcile with the argument given in the preceding paragraph that most Aβ elimination is by a non-perivascular mechanism. The obvious alternative interpretation of the effects of AQP4 knock-out, consistent with all the results, is that AQP4 knock-out reduces elimination by both perivascular and non-perivascular routes (compare the discussion of the effects of sleep in Sect. 3.4.3).

Ito et al. (2013) measured the rate constant for loss of intact human A$\beta_{1–40}$ in anaesthetised mice at micromolar concentrations high enough to saturate the blood–brain barrier elimination mechanisms. Elimination should therefore have been primarily by other routes. They found a value of the rate constant, ~0.032 min^{-1}, which is too large to be attributed to perivascular efflux and thus represents elimination by metabolism. If as described in Section "Clearance by Uptake and Further Metabolism" the rate of metabolism of Aβ is proportional to its concentration, the rate constant measured by Ito et al. at high concentrations is also the rate constant for elimination by metabolism at low concentrations. Bell et al. (2007) using mice that had recovered from anaesthesia reported data from which the total rate constant for elimination at low concentrations can be calculated as 0.016 min^{-1} (see Section "Clearance Across the Blood–Brain Barrier" and Appendix 1). This value is twofold smaller than the rate constant for metabolism calculated by Ito et al. Bell et al.'s value may well be smaller because their mice were awake, but nevertheless the comparison does suggest that metabolism is important for Aβ elimination.

To make comparisons between the elimination mechanisms, Roberts et al. (2014) used measurements of: the turnover rate for Aβ (Potter et al. 2013); the pool size for Aβ; the difference between Aβ concentrations in arterial blood and in venous blood leaving the brain; and cerebral blood flow. They compared the rate at which Aβ is effluxed to the blood (A-V difference times blood flow) with the total rate of elimination (pool size × turnover rate × concentration). From this they concluded that about 50% of Aβ elimination was by efflux from brain to blood which they took to include both efflux across the blood–brain barrier and efflux to CSF and thence to blood. They presumed that the rest of the elimination was via metabolism. To separate efflux across the blood–brain barrier from perivascular efflux, they estimated the latter as the product of the CSF concentration times an assumed rate of outflow of CSF. On this basis they calculated that 25% of the elimination was efflux across the blood–brain barrier, 25% was via CSF and the remaining 50% was via metabolism.

Although their results do suggest that all of these mechanisms are involved, the proportions Roberts et al. estimated may need revision for two reasons. Firstly, a substantial, but unknown, fraction of Aβ leaving via the perivascular route and/or CSF exits the brain to lymph and so does not appear in the venous blood samples (see e.g. Dienel and Cruz 2008; Pappolla et al. 2014; and for further discussion Hladky and Barrand 2014; Tarasoff-Conway et al. 2015; Hladky and Barrand 2016). Hence it does not contribute to the A-V difference and a larger fraction of that difference will be a result of transport across the blood–brain barrier. The fraction of Aβ leaving the brain via lymph may be substantial. The second reason is that not all of the Aβ in CSF may have originated in the brain parenchyma which means that their calculation of perivascular elimination as the rate of outflow via CSF may be an overestimate. In summary the results of Roberts et al. suggest that the fraction of Aβ leaving the brain across the blood–brain barrier may have been underestimated and could be as high as 50%, that the fraction leaving by perivascular efflux is indeterminate (but based on their evidence could be large) and that the fraction left to be accounted for by metabolism may have been substantially overestimated.

Further difficulties encountered in quantitative interpretation of efflux kinetics are discussed in Appendix 2 but these do not compromise the conclusions of the papers concerned and are unlikely to account for the discrepancies.

On balance the available data suggests a significant involvement in elimination of Aβ *from the brain for all three routes of elimination: further metabolism, efflux across the blood–brain barrier and perivascular efflux* (see also the views expressed in Kanekiyo and Bu (2014) and in Ramanathan et al. (2015)).

3.4.3 Differences in Aβ Clearances between Sleep and Wakefulness

How the routes of Aβ elimination differ during sleep and during wakefulness has been investigated by Xie et al. (2013). They determined rate constants for elimination of added ^{125}I-Aβ_{1-40} in awake and in sleeping mice and compared them to those for inulin (see Table 1). They found in the awake and in sleeping mice that the rate constant for elimination of Aβ was 4-fold and 3.3-fold greater respectively than

Table 1 Rate constants for elimination of ^{125}I-A$\beta_{1–40}$ and of inulin in mice when awake and during sleep (Xie et al. 2013)

	Rate constant/min^{-1}			
	Inulin	Aβ	Aβ perivascular	Aβ non-perivascular
Awake	0.006[a]	0.024[a]	<0.006	0.018–0.024
Asleep	0.016[a]	0.053[a]	<0.016	0.037–0.053
	Ratios			
Asleep/awake	2.7	2.2	–	2.1–2.2

[a]Values from Fig. 3b,d in Xie et al. (2013)

that for inulin. Since the volume of distribution for Aβ is larger, perhaps much larger, than that for inulin this implies a higher clearance ($=k \times V_D$) for Aβ implying in agreement with earlier results that most of the elimination is via pathways other than perivascular. As discussed in Sections "Clearance Across the Blood–Brain Barrier" and 3.4.2 it is likely that transcytosis across the blood–brain barrier accounts for most of this elimination though further metabolism is likely to contribute.

As evident from the values in Table 1, there is faster elimination during sleep for both inulin and Aβ. Xie et al. argue that for each the change in the rate constant of elimination results from a change in the glymphatic circulation occurring as a consequence of a change in ISF volume (see Proposal 3 in Sect. 2.3.2 and Sect. 2.4). However, inspection of their data suggests that the explanation is more complicated. From the non-selective nature of perivascular clearance it is a reasonable approximation to assume that the perivascular clearances for Aβ and inulin are the same. Because the volume of distribution for Aβ is at least as large as for inulin (and possibly much greater, see Sect. 1.2.1), this means that the rate constant for Aβ elimination via the perivascular route will be less than or equal to that for inulin. Thus in awake mice the rate constant for Aβ elimination via the perivascular route is $\leq$0.006 min^{-1} and that for the non-perivascular route is between 0.018 and 0.024 min^{-1}. Similarly for asleep mice, the rate constant for the non-perivascular route will be between 0.037 and 0.053 min^{-1}. The actual values for the two conditions are both expected to be near the same end of these ranges. Thus it appears that during sleep the rate constant for non-perivascular Aβ elimination may be increased 2.1- to 2.2-fold. The volume of distribution of inulin is increased 1.6-fold and its clearance ~4.3-fold (see Section "Clearance Across the Blood–Brain Barrier"). The volume of distribution for Aβ should also increase but perhaps not by so large a factor, so the non-perivascular clearance for ^{125}I-A$\beta_{1–40}$ appears to increase by something between 2.1 and 3.5-fold.

In summary the effect of sleep on clearance of Aβ *is not restricted to an effect on clearance* via *the perivascular route but includes changes in* Aβ *clearances by other routes. Whether or not the change between sleep and wakefulness in clearance* via *the perivascular route is a consequence of an ISF volume alteration* (see Sect. 2.4), *it is unlikely that* ISF *volume alterations can explain changes in the total clearance of* Aβ.

4 Summary

Clearance describes elimination. Clearance of a metabolite generated within the brain is determined as its elimination rate divided by its concentration in ISF. However, the more frequently measured parameter is the rate constant for elimination determined as elimination rate divided by amount present. The rate constant depends on both the elimination processes and the distribution of the metabolite in the brain, specified as the volume of distribution (see Sect. 1.2.1).

Three main types of process determine the clearance of any metabolite from ISF: further metabolism (which eliminates the metabolite of interest, but of course produces another), transport to blood across the blood–brain barrier, and non-selective efflux via perivascular routes (see Sect. 2). Further metabolism, often preceded by uptake into cells, and transfer across the blood–brain barrier are important because they can be rapid. Perivascular clearance is important because it can remove any water soluble substance that is not too large. Marker substances used to characterise the perivascular route of elimination such as inulin, mannitol, sucrose and albumin are highly water soluble, do not aggregate, do not bind to or easily cross cell membranes, are not metabolized before being eliminated and are not eliminated at a significant rate by any other mechanism. These all have volumes of distribution near to the volume of ISF. If metabolites have volumes of distribution larger than those of the markers, e.g. amyloid-β, this will mean that their rate constant for perivascular elimination will be less than that for the markers. If metabolites have half-lives shorter than those for the markers, this means they must be eliminated by routes in addition to the perivascular route leading to higher total clearances and hence the shorter half-lives (see Sect. 1.2.1).

The mechanisms underlying perivascular clearance have proved controversial. There have been three proposals put forward (see Sects. 2.3.1 and 2.3.2). The first proposal, now largely discounted as a major factor, is that fluid is secreted from the blood into the parenchyma by the blood–brain barrier thence flowing out of the parenchyma carrying water soluble metabolites with it. The second proposal is that there is convective mixing or dispersion occurring in perivascular spaces that greatly increases the rate of transfer of solutes in both directions. The third is that there is a glymphatic circulation entailing flow of CSF into the parenchyma via periarterial spaces, then flow through the parenchyma and finally flow outwards via perivenous spaces. At present the available evidence favours the second proposal over the third. The many references in the literature (now in the hundreds) to a role for the glymphatic circulation in the elimination of substances from the brain can almost all be read as references to a role for perivascular transport by whatever mechanism this occurs. Further work to establish this mechanism is required.

Little is known about the effects of sleep on clearance via metabolism or blood–brain barrier transport, but studies with inulin in mice comparing perivascular effluxes during sleep and wakefulness have found increases of 2.6-fold in the rate constant of elimination, 1.6-fold in the volume of distribution and 4.2-fold in the clearance of inulin.

Table 2 summarises the principal routes/mechanisms by which certain important metabolites formed in the brain are eliminated and what is known of how their elimination is modified by sleep.

CO_2 (see Sect. 3.1) is eliminated by diffusion across the blood–brain barrier. During sleep production decreases but the clearance of CO_2 is also reduced because cerebral blood flow is reduced and hence there is little change in CO_2 concentration.

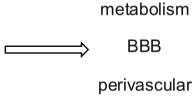
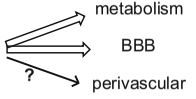
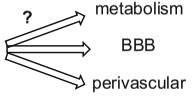
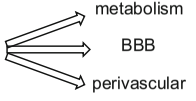
Glutamate (see Sect. 3.2) is synthesised in the brain from other amino acids and added to the extensive pool of glutamate and glutamine recycling between astrocytes and neurons. Elimination from the brain, which balances synthesis, involves fluxes much smaller than those entailed in recycling. There are transporters at the blood–brain barrier that allow efflux to blood without appreciable transfer in the opposite direction. Some perivascular elimination will occur but is expected to be minor in comparison with efflux across the blood–brain barrier.

Lactic acid/lactate is eliminated from ISF partly by uptake into cells and metabolism to CO_2 but where and to what extent this occurs have been highly controversial. Efflux of lactic acid/lactate across the blood–brain barrier via specific transporters is rapid for low lactic acid concentrations but saturates within the range of concentrations that can be reached in ISF during nervous activity. Often increased lactate concentrations in ISF occur under conditions that will also produce increased lactate concentrations in plasma. These increased plasma concentrations will lead to increased influx into the brain across the blood–brain barrier offsetting the increased efflux. There is also an augmented form of perivascular elimination of lactate that may entail transfer or shuttling of lactic acid/lactate between astrocytes and release into periarterial and/or perivenous spaces allowing rapid efflux from the parenchyma. At low lactate concentrations the blood–brain barrier route is expected to be more important than perivascular efflux, but at higher concentrations the perivascular route may account for much of the net efflux.

Amyloid-β (Aβ) is also eliminated by all three of the major routes, i.e. metabolism, transfer across the blood–brain barrier and perivascular efflux, the relative proportions remaining controversial. Most but not all of the available evidence suggests that, at the low concentrations of soluble Aβ encountered in vivo, the perivascular route is the least important. Sleep increases the rate constants for elimination both by blood–brain barrier transfer and perivascular efflux.

For all of the metabolites considered, the rates of production and elimination are decreased during sleep. Changes in sleep may be particularly important for metabolites for which small changes in the average concentration may, over a prolonged period of time, have cumulative effects. The example considered here is Aβ. Aβ is eliminated by multiple mechanisms and changes in any one of these produce only modest changes in Aβ concentrations. However, even these modest changes may be important over an extended time period. For Aβ, lack of slow-wave (nREM) sleep may increase the average concentration of soluble Aβ over the day which may lead to increased cumulative formation of vascular aggregates, cerebral arterial angiopathy and parenchymal plaques. Each of these may have a role in the development of Alzheimer's disease. There are correlations between disruption of

Table 2 Principal routes of elimination from ISF of important brain metabolites and changes observed during sleep

			Difference awake ⇒ asleep			
Metabolite	Principal routes of clearance from ISF	Major factors effecting clearance	Major factors affecting difference	Rates of production and elimination	Clearance	ISF concentration
CO_2		Rapid diffusion, blood flow limited	Production and blood flow	⇓	⇓	Little change
Glutamate/ glutamine		Rapid uptake and conversion? Specific transporters Unknown	Decreased release from cells	⇓	?	⇓
Lactate		Uptake transporters on cells Rapid but saturable transport by MCT1 Lactate shuttling between astrocytes	Production and perivascular clearance	⇓	⇓ ⇑? ⇑	⇓
Amyloid-β		Uptake and multiple routes Transcytosis via LRP1 Aggregation along pathway	Production, BBB and perivascular clearances	⇓	? ⇑ ⇑	⇓

BBB blood–brain barrier

sleep, deposition of Aβ and the incidence of Alzheimer's but as yet there is no consensus as to any causal connections (Ju et al. 2014; Lucey and Bateman 2014; Roh et al. 2014; Spira et al. 2014; Yaffe et al. 2014; Benedict et al. 2015; Sharma et al. 2015; Sprecher et al. 2015; Cedernaes et al. 2017).

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Appendices

Appendix 1

The evidence that extramural perivascular spaces can exist is convincing as large particles can be introduced into them (see e.g. Carare et al. 2008) and during influx of fluorescent tracers the spaces protrude beyond the dimensions of the vessel walls (see e.g. Figs. 2 and 3 in Iliff et al. 2012). The case against their normal existence along arterioles and venules is largely that they are rarely seen in fixed, sectioned tissue. However, spaces, particularly labile spaces, are likely to be difficult to fix, so this type of evidence is not in itself compelling (see e.g. Fig. 2 in Bakker et al. 2016). Arbel-Ornath et al. (2013) used two-photon imaging to investigate the position of a 3 kDa dextran during efflux following injection into the parenchyma. Shortly after injection they saw fluorescence within the parenchyma, in perivascular spaces surrounding small arteries and, at lower concentration, between the smooth muscle cells.

There is extensive and convincing evidence that solutes can rapidly reach the basement membranes between the smooth muscle cells (see e.g. Carare et al. 2013a), but it has not been established whether the solutes reach these locations by movement along the basement membranes as favoured by Weller, Carare, Hawkes and colleagues (see e.g. Morris et al. 2016) or via movement along the vessels via extramural pathways and penetration from these into the basement membranes within the vessel wall. This is discussed in Section "Clearance via Perivascular Routes". While it has been possible to observe solutes moving inwards via extramural periarterial spaces (Iliff et al. 2012) and outwards via some periarterial route (Dienel and Cruz 2008; Arbel-Ornath et al. 2013), so far it has not been possible to observe solutes progressing via either perivenular or specifically intramural periarterial routes.

Appendix 2

In several papers the time courses of the amount of Aβ remaining in the brain have been analysed using a scheme introduced by Shibata et al. (2000). In this an amount

$A\beta_0$ of the Aβ is assumed to be introduced initially into the parenchyma in a soluble form. This soluble Aβ can either be effluxed with rate constant $k1$ or irreversibly converted to a retained form with rate constant $k2$. The prediction of this scheme for the time course can be derived as follows. If at any time t the amount of the soluble form in the brain is $A\beta s_t$ and that of the retained form is $A\beta r_t$, then the total amount remaining is $A\beta_t = A\beta s_t + A\beta r_t$ and the changes with time of the amounts are governed by

$$\frac{dA\beta s_t}{dt} = -(k1 + k2)A\beta s_t \tag{7}$$

and

$$\frac{dA\beta r_t}{dt} = k2A\beta s_t \tag{8}$$

The first of these differential equations has as its solution

$$A\beta s_t = A\beta_0 e^{-(k1+k2)t}, \tag{9}$$

which then allows the second to be solved,

$$A\beta r_t = A\beta_0 a1\left(1 - e^{-(k1+k2)t}\right) \tag{10}$$

and thus

$$A\beta_t = A\beta_0\left[a1 + a2e^{-(k1+k2)t}\right] \tag{11}$$

where $a1 = k2/(k1 + k2)$ is the fraction of the Aβ that is eventually converted to the retained form and $a2 = 1 - a1$ is the fraction eventually effluxed. This is to be compared with the versions of the solutions for $A\beta_t$ that have been used in various studies. The version presented by Shibata et al. (2000)

$$A\beta_t = A\beta_0[a1 + a2]e^{-k1t} \tag{12}$$

was also used by Deane et al. (2004), Bell et al. (2007) and Deane et al. (2008) (Abhay Sagare, personal communication). Storck et al. (2016) used a partially corrected version

$$A\beta_t = A\beta_0[a1 + a2]e^{-(k1+k2)t}. \tag{13}$$

Use of Eqs. (12) or (13) may have affected the estimates of the fractions of Aβ effluxed and retained. In Shibata et al., it may have led to a small overestimate of the rate constant for efflux. In Bell et al., the fraction retained was small, i.e. $k2 \ll k1$, and Eq. (12) is then almost the same as Eq. (11). However, the units

stated in the methods section of that paper for $k1$ and $k2$ were pmol min^{-1} g^{-1} while those actually used in the calculations were min^{-1} as in Shibata et al. The values reported in Tables 1 and 2 are calculated rates of efflux with the units stated, pmol min^{-1} g^{-1}. These rates were calculated as the initial amount present, ~13 pmol/g ISF (assuming the mass of ISF is 1/10th that of the tissue), times the corresponding rate constant obtained using Eq. (12) (Abhay Sagare, personal communication).

References

Abbott NJ, Patabendige AAK, Dolman DEM, Yusof SR, Begley DJ (2010) Structure and function of the blood-brain barrier. Neurobiol Dis 37:13–25

Arbel-Ornath M, Hudry E, Eikermann-Haerter K et al (2013) Interstitial fluid drainage is impaired in ischemic stroke and Alzheimer's disease mouse models. Acta Neuropathol 126:353–364

Asgari M, de Zélicourt DA, Kurtcuoglu V (2016) Glymphatic solute transport does not require bulk flow. Sci Rep 6:38635. doi:10.1038/srep38635

Aspelund A, Antila S, Proulx ST et al (2015) A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. J Exp Med 212:991–999. doi:10.1084/jem.20142290

Bakker ENTP, Bacskai BJ, Arbel-Ornath M et al (2016) Lymphatic clearance of the brain: perivascular, paravascular and significance for neurodegenerative diseases. Cell Mol Neurobiol 36:181–194. doi:10.1007/s10571-015-0273-8

Ball KK, Gandhi GK, Thrash J, Cruz NF, Dienel GA (2007) Astrocytic connexin distributions and rapid, extensive dye transfer via gap junctions in the inferior colliculus: implications for [(14)C]glucose metabolite trafficking. J Neurosci Res 85:3267–3283. doi:10.1002/jnr.21376

Ball KK, Cruz NF, Mrak RE, Dienel GA (2010) Trafficking of glucose, lactate, and amyloid-beta from the inferior colliculus through perivascular routes. J Cereb Blood Flow Metab 30:162–176

Bateman RJ, Munsell LY, Morris JC, Swarm R, Yarasheski KE, Holtzman DM (2006) Human amyloid-β synthesis and clearance rates as measured in cerebrospinal fluid in vivo. Nat Med 12:856–861. doi:10.1038/nm1438

Bedussi B, van Lier MGJTB, Bartstra JW, de Vos J, Siebes M, VanBavel E, Bakker ENTP (2015) Clearance from the mouse brain by convection of interstitial fluid towards the ventricular system. Fluids Barriers CNS 12:23. doi:10.1186/s12987-015-0019-5

Bedussi B, van der Wel NN, de Vos J, van Veen H, Siebes M, VanBavel E, Bakker ENTP (2017) Paravascular channels, cisterns, and the subarachnoid space in the rat brain: a single compartment with preferential pathways. J Cereb Blood Flow Metab 37:1374–1385. doi:10.1177/0271678x16655550

Bell RD, Sagare AP, Friedman AE, Bedi GS, Holtzman DM, Deane R, Zlokovic BV (2007) Transport pathways for clearance of human Alzheimer's amyloid beta-peptide and apolipoproteins E and J in the mouse central nervous system. J Cereb Blood Flow Metab 27:909–918

Benedict C, Byberg L, Cedernaes J et al (2015) Self-reported sleep disturbance is associated with Alzheimer's disease risk in men. Alzheimers Dement 11:1090–1097. doi:10.1016/j.jalz.2014.08.104

Bradbury MWB (1979) The concept of a blood-brain barrier. Wiley, Chichester

Bradbury MWB, Westrop RJ (1983) Factors influencing exit of substances from cerebrospinal-fluid into deep cervical lymph of the rabbit. J Physiol Lond 339:519–534

Bradbury MW, Cserr HF, Westrop RJ (1981) Drainage of cerebral interstitial fluid into deep cervical lymph of the rabbit. Am J Physiol 240:F329–F336

Bu G (2009) Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. Nat Rev Neurosci 10:333–344. doi:10.1038/nrn2620

Carare RO, Bernardes-Silva M, Newman TA, Page AM, Nicoll JAR, Perry VH, Weller RO (2008) Solutes, but not cells, drain from the brain parenchyma along basement membranes of capillaries and arteries: significance for cerebral amyloid angiopathy and neuroimmunology. Neuropathol Appl Neurobiol 34:131–144

Carare RO, Hawkes CA, Jeffrey M, Kalaria RN, Weller RO (2013a) Review: cerebral amyloid angiopathy, prion angiopathy, CADASIL and the spectrum of protein elimination failure angiopathies (PEFA) in neurodegenerative disease with a focus on therapy. Neuropathol Appl Neurobiol 39:593–611

Carare RO, Teeling JL, Hawkes CA, Puntener U, Weller RO, Nicoll JAR, Perry VH (2013b) Immune complex formation impairs the elimination of solutes from the brain: implications for immunotherapy in Alzheimer's disease. Acta Neuropathol Commun 1:48. doi:10.1186/2051-5960-1-48

Cedernaes J, Osorio RS, Varga AW, Kam K, Schioth HB, Benedict C (2017) Candidate mechanisms underlying the association between sleep-wake disruptions and Alzheimer's disease. Sleep Med Rev 31:102–111. doi:10.1016/j.smrv.2016.02.002. (in press)

Cirrito JR, May PC, O'Dell MA et al (2003) In vivo assessment of brain interstitial fluid with microdialysis reveals plaque-associated changes in amyloid-β metabolism and half-life. J Neurosci 23:8844–8853

Cirrito JR, Deane R, Fagan AM et al (2005) P-glycoprotein deficiency at the blood-brain barrier increases amyloid-beta deposition in an Alzheimer disease mouse model. J Clin Investig 115:3285–3290

Cruz NF, Adachi K, Dienel GA (1999) Rapid efflux of lactate from cerebral cortex during K+-induced spreading cortical depression. J Cereb Blood Flow Metab 19:380–392

Cserr HF, Ostrach LH (1974) Bulk flow of interstitial fluid after intracranial injection of blue dextran 2000. Exp Neurol 45:50–60

Cserr HF, Patlak CS (1992) Secretion and bulk flow of interstitial fluid. In: Bradbury MWB (ed) Physiology and pharmacology of the blood-brain barrier. Springer-Verlag, Berlin, pp 245–261

Cserr HF, Cooper DN, Milhorat TH (1977) Flow of cerebral interstitial fluid as indicated by removal of extracellular markers from rat caudate-nucleus. Exp Eye Res 25:461–473

Cserr HF, Cooper DN, Suri PK, Patlak CS (1981) Efflux of radiolabeled polyethylene glycols and albumin from rat brain. Am J Physiol 240:F319–F328

Daniel PM, Love ER, Moorhouse SR, Pratt OE (1972) The movement of ketone bodies, glucose, pyruvate and lactate between blood and brain of rats. J Physiol Lond 221:P22–P23

Dash MB, Douglas CL, Vyazovskiy VV, Cirelli C, Tononi G (2009) Long-term homeostasis of extracellular glutamate in the rat cerebral cortex across sleep and waking states. J Neurosci 29:620–629. doi:10.1523/jneurosci.5486-08.2009

Dash MB, Tononi G, Cirelli C (2012) Extracellular levels of lactate, but not oxygen, reflect sleep homeostasis in the rat cerebral cortex. Sleep 35:909–919. doi:10.5665/sleep.1950

Davson H, Segal MB (1996) Physiology of the CSF and blood-brain barriers. CRC Press, Boca Raton

Deane R, Wu ZH, Sagare A et al (2004) LRP/amyloid β-peptide interaction mediates differential brain efflux of Aβ isoforms. Neuron 43:333–344

Deane R, Sagare A, Hamm K et al (2008) apoE isoform-specific disruption of amyloid beta peptide clearance from mouse brain. J Clin Investig 118:4002–4013. doi:10.1172/jci36663

Dienel GA (2012) Brain lactate metabolism: the discoveries and the controversies. J Cereb Blood Flow Metab 32:1107–1138. doi:10.1038/jcbfm.2011.175

Dienel GA, Cruz NF (2003) Neighborly interactions of metabolically-activated astrocytes in vivo. Neurochem Int 43:339–354

Dienel GA, Cruz NF (2004) Nutrition during brain activation: does cell-to-cell lactate shuttling contribute significantly to sweet and sour food for thought? Neurochem Int 45:321–351. doi:10.1016/j.neuint.2003.10.011

Dienel GA, Cruz NF (2008) Imaging brain activation: simple pictures of complex biology. Ann N Y Acad Sci 1147:139–170. doi:10.1196/annals.1427.011

Dienel GA, Cruz NF (2016) Aerobic glycolysis during brain activation: adrenergic regulation and influence of norepinephrine on astrocytic metabolism. J Neurochem 138:14–52. doi:10.1111/jnc.13630

Drewes LR, Gilboe DD (1973) Glycolysis and the permeation of glucose and lactate in the isolated, perfused dog brain during anoxia and postanoxic recovery. J Biol Chem 248:2489–2496

Engelhardt B, Carare RO, Bechmann I, Flugel A, Laman JD, Weller RO (2016) Vascular, glial, and lymphatic immune gateways of the central nervous system. Acta Neuropathol 132:317–338. doi:10.1007/s00401-016-1606-5. (in press)

Erickson MA, Banks WA (2013) Blood-brain barrier dysfunction as a cause and consequence of Alzheimer's disease. J Cereb Blood Flow Metab 33:1500–1513

Fagan AM, Mintun MA, Mach RH et al (2006) Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Abeta42 in humans. Ann Neurol 59:512–519. doi:10.1002/ana.20730

Farris W, Schutz SG, Cirrito JR et al (2007) Loss of neprilysin function promotes amyloid plaque formation and causes cerebral amyloid angiopathy. Am J Pathol 171:241–251. doi:10.2353/ajpath.2007.070105

Gandhi GK, Cruz NF, Ball KK, Dienel GA (2009a) Astrocytes are poised for lactate trafficking and release from activated brain and for supply of glucose to neurons. J Neurochem 111:522–536. doi:10.1111/j.1471-4159.2009.06333.x

Gandhi GK, Cruz NF, Ball KK, Theus SA, Dienel GA (2009b) Selective astrocytic gap junctional trafficking of molecules involved in the glycolytic pathway: impact on cellular brain imaging. J Neurochem 110:857–869. doi:10.1111/j.1471-4159.2009.06173.x

Ghiso J, Shayo M, Calero M et al (2004) Systemic catabolism of Alzheimer's Abeta40 and Abeta42. J Biol Chem 279:45897–45908. doi:10.1074/jbc.M407668200

Groothuis DR, Vavra MW, Schlageter KE et al (2007) Efflux of drugs and solutes from brain: the interactive roles of diffusional transcapillary transport, bulk flow and capillary transporters. J Cereb Blood Flow Metab 27:43–56. doi:10.1038/sj.jcbfm.9600315

Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat Rev Mol Cell Biol 8:101–112. doi:10.1038/nrm2101

Haass C, Kaether C, Thinakaran G, Sisodia SS (2012) Trafficking and proteolytic processing of APP. Cold Spring Harb Perspect Med 2:a006270. doi:10.1101/cshperspect.a006270

Harper JD, Lansbury PT Jr (1997) Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. Annu Rev Biochem 66:385–407. doi:10.1146/annurev.biochem.66.1.385

Hartz AMS, Miller DS, Bauer B (2010) Restoring blood-brain barrier P-glycoprotein reduces brain amyloid-beta in a mouse model of Alzheimer's disease. Mol Pharmacol 77:715–723. doi:10.1124/mol.109.061754

Hawkes CA, Hartig W, Kacza J, Schliebs R, Weller RO, Nicoll JA, Carare RO (2011) Perivascular drainage of solutes is impaired in the ageing mouse brain and in the presence of cerebral amyloid angiopathy. Acta Neuropathol 121:431–443

Hawkes CA, Sullivan PM, Hands S, Weller RO, Nicoll JAR, Carare RO (2012) Disruption of arterial perivascular drainage of amyloid-beta from the brains of mice expressing the human APOE epsilon 4 allele. PLoS One 7:e41636. doi:10.1371/journal.pone.0041636

Hawkins RA (2009) The blood-brain barrier and glutamate. Am J Clin Nutr 90:867S–874S

Hawkins RA, Miller AL, Nielsen RC, Veech RL (1973) The acute action of ammonia on rat brain metabolism in vivo. Biochem J 134:1001–1008

Hawkins RA, O'Kane RL, Simpson IA, Viña JR (2006) Structure of the blood-brain barrier and its role in the transport of amino acids. J Nutr 136:218S–226S

Hawkins RA, Viña JR, Mokashi A et al (2013) Synergism between the two membranes of the blood-brain barrier: glucose and amino acid transport. Am J Neurosci Res 1:201300168

Hladky SB, Barrand MA (2014) Mechanisms of fluid movement into, through and out of the brain: evaluation of the evidence. Fluids Barriers CNS 11:26. doi:10.1186/2045-8118-11-26

Hladky SB, Barrand MA (2016) Fluid and ion transfer across the blood–brain and blood–cerebrospinal fluid barriers; a comparative account of mechanisms and roles. Fluids Barriers CNS 13:19

Hortschansky P, Schroeckh V, Christopeit T, Zandomeneghi G, Fandrich M (2005) The aggregation kinetics of Alzheimer's beta-amyloid peptide is controlled by stochastic nucleation. Protein Sci 14:1753–1759. doi:10.1110/ps.041266605

Hulse BK, Landsness EC, Sarasso S, Ferrarelli F, Guokas JJ, Wanger T, Tononi G (2011) A postsleep decline in auditory evoked potential amplitude reflects sleep homeostasis. Clin Neurophysiol 122:1549–1555. doi:10.1016/j.clinph.2011.01.041

Ichimura T, Fraser PA, Cserr HF (1991) Distribution of extracellular tracers in perivascular spaces of the rat brain. Brain Res 545:103–113

Iliff JJ, Wang M, Liao Y et al (2012) A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. Sci Transl Med 4:147ra111. doi:10.1126/scitranslmed.3003748

Iliff JJ, Wang MH, Zeppenfeld DM et al (2013) Cerebral arterial pulsation drives paravascular CSF-interstitial fluid exchange in the murine brain. J Neurosci 33:18190–18199. doi:10.1523/jneurosci.1592-13.2013

Ito S, Matsumiya K, Ohtsuki S, Kamiie J, Terasaki T (2013) Contributions of degradation and brain-to-blood elimination across the blood-brain barrier to cerebral clearance of human amyloid-beta peptide(1-40) in mouse brain. J Cereb Blood Flow Metab 33:1770–1777. doi:10.1038/jcbfm.2013.125

Iwata N, Tsubuki S, Takaki Y et al (2000) Identification of the major Abeta1-42-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition. Nat Med 6:143–150. doi:10.1038/72237

Iwata N, Tsubuki S, Takaki Y et al (2001) Metabolic regulation of brain Aβ by neprilysin. Science 292:1550–1552. doi:10.1126/science.1059946

Iwata N, Higuchi M, Saido TC (2005) Metabolism of amyloid-beta peptide and Alzheimer's disease. Pharmacol Ther 108:129–148. doi:10.1016/j.pharmthera.2005.03.010

Jaeger LB, Dohgu S, Hwang MC et al (2009) Testing the neurovascular hypothesis of Alzheimer's disease: LRP-1 antisense reduces blood-brain barrier clearance, increases brain levels of amyloid-beta protein, and impairs cognition. J Alzheimers Dis 17:553–570. doi:10.3233/jad-2009-1074

Jin B-J, Smith AJ, Verkman AS (2016) Spatial model of convective solute transport in brain extracellular space does not support a "glymphatic" mechanism. J Gen Physiol 148:489–501. doi:10.1085/jgp.201611684

Ju Y-ES, Lucey BP, Holtzman DM (2014) Sleep and Alzheimer disease pathology – a bidirectional relationship. Nat Rev Neurol 10:115–119. doi:10.1038/nrneurol.2013.269

Kandimalla KK, Curran GL, Holasek SS, Gilles EJ, Wengenack TM, Poduslo JF (2005) Pharmacokinetic analysis of the blood-brain barrier transport of 125I-amyloid beta protein 40 in wild-type and Alzheimer's disease transgenic mice (APP,PS1) and its implications for amyloid plaque formation. J Pharmacol Exp Ther 313:1370–1378. doi:10.1124/jpet.104.081901

Kanekiyo T, Bu G (2014) The low-density lipoprotein receptor-related protein 1 and amyloid-beta clearance in Alzheimer's disease. Front Aging Neurosci 6:93. doi:10.3389/fnagi.2014.00093

Kanekiyo T, Liu CC, Shinohara M, Li J, Bu GJ (2012) LRP1 in brain vascular smooth muscle cells mediates local clearance of Alzheimer's amyloid-β. J Neurosci 32:16458–16465

Kanekiyo T, Cirrito JR, Liu C-C et al (2013) Neuronal clearance of amyloid-β by endocytic receptor LRP1. J Neurosci 33:19276–19283. doi:10.1523/jneurosci.3487-13.2013

Keable A, Fenna K, Yuen HM et al (2016) Deposition of amyloid beta in the walls of human leptomeningeal arteries in relation to perivascular drainage pathways in cerebral amyloid angiopathy. BBA-Mol Basis Dis 1862:1037–1046. doi:10.1016/j.bbadis.2015.08.024

Kennedy C, Gillin JC, Mendelson W et al (1981) Local cerebral glucose utilization in slow-wave sleep. Trans Am Neurol Assoc 106:25–28

Kervezee L, Hartman R, van den Berg DJ, Shimizu S, Emoto-Yamamoto Y, Meijer JH, de Lange ECM (2014) Diurnal variation in p-glycoprotein-mediated transport and cerebrospinal fluid turnover in the brain. AAPS J 16:1029–1037. doi:10.1208/s12248-014-9625-4

Knudsen GM, Paulson OB, Hertz MM (1991) Kinetic analysis of the human blood-brain barrier transport of lactate and its influence by hypercapnia. J Cereb Blood Flow Metab 11:581–586

Kress BT, Iliff JJ, Xia M et al (2014) Impairment of paravascular clearance pathways in the aging brain. Ann Neurol 76:845–861. doi:10.1002/ana.24271

Lambert MP, Barlow AK, Chromy BA et al (1998) Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. Proc Natl Acad Sci U S A 95:6448–6453

Lante F, Toledo-Salas J-C, Ondrejcak T, Rowan MJ, Ulrich D (2011) Removal of synaptic Ca(2)+-permeable AMPA receptors during sleep. J Neurosci 31:3953–3961. doi:10.1523/jneurosci.3210-10.2011

Lazarov O, Lee M, Peterson DA, Sisodia SS (2002) Evidence that synaptically released beta-amyloid accumulates as extracellular deposits in the hippocampus of transgenic mice. J Neurosci 22:9785–9793

Lee W-J, Hawkins RA, Vina JR, Peterson DR (1998) Glutamine transport by the blood-brain barrier: a possible mechanism for nitrogen removal. Am J Phys Cell Phys 274:C1101–C1107

Leissring MA, Farris W, Chang AY et al (2003) Enhanced proteolysis of β-amyloid in APP transgenic mice prevents plaque formation, secondary pathology, and premature death. Neuron 40:1087–1093

Levick JR (1987) Flow through interstitium and other fibrous matrices. Q J Exp Physiol 72:409–437

Liu Z-W, Faraguna U, Cirelli C, Tononi G, Gao X-B (2010) Direct evidence for wake-related increases and sleep-related decreases in synaptic strength in rodent cortex. J Neurosci 30:8671–8675. doi:10.1523/jneurosci.1409-10.2010

Lomakin A, Chung DS, Benedek GB, Kirschner DA, Teplow DB (1996) On the nucleation and growth of amyloidβ-protein fibrils: detection of nuclei and quantitation of rate constants. Proc Natl Acad Sci U S A 93:1125–1129

Louveau A, Smirnov I, Keyes TJ et al (2015) Structural and functional features of central nervous system lymphatic vessels. Nature 523:337–341. doi:10.1038/nature14432

Lucey BP, Bateman RJ (2014) Amyloid-beta diurnal pattern: possible role of sleep in Alzheimer's disease pathogenesis. Neurobiol Aging 35(Suppl 2):S29–S34. doi:10.1016/j.neurobiolaging.2014.03.035

Lundgaard I, Lu ML, Yang E et al (2016) Glymphatic clearance controls state-dependent changes in brain lactate concentration. J Cereb Blood Flow Metab 37:2112–2124. doi:10.1177/0271678x16661202. (in press)

Madsen PL, Cruz NF, Sokoloff L, Dienel GA (1999) Cerebral oxygen/glucose ratio is low during sensory stimulation and rises above normal during recovery: excess glucose consumption during stimulation is not accounted for by lactate efflux from or accumulation in brain tissue. J Cereb Blood Flow Metab 19:393–400. doi:10.1097/00004647-199904000-00005

Mathiisen TM, Lehre KP, Danbolt NC, Ottersen OP (2010) The perivascular astroglial sheath provides a complete covering of the brain microvessels: an electron microscopic 3D reconstruction. Glia 58:1094–1103

Mawuenyega KG, Sigurdson W, Ovod V et al (2010) Decreased clearance of CNS beta-amyloid in Alzheimer's disease. Science 330:1774. doi:10.1126/science.1197623

McIntee FL, Giannoni P, Blais S, Sommer G, Neubert TA, Rostagno A, Ghiso J (2016) In vivo differential brain clearance and catabolism of monomeric and oligomeric Alzheimer's Abeta protein. Front Aging Neurosci 8:223. doi:10.3389/fnagi.2016.00223

Miners JS, Barua N, Kehoe PG, Gill S, Love S (2011) Abeta-degrading enzymes: potential for treatment of Alzheimer disease. J Neuropathol Exp Neurol 70:944–959. doi:10.1097/NEN.0b013e3182345e46

Morris AWJ, Sharp MM, Albargothy NJ et al (2016) Vascular basement membranes as pathways for the passage of fluid into and out of the brain. Acta Neuropathol 131:725–736. doi:10.1007/s00401-016-1555-z

Naylor E, Aillon DV, Barrett BS et al (2012) Lactate as a biomarker for sleep. Sleep 35:1209–1222. doi:10.5665/sleep.2072

Nedergaard M (2013) Neuroscience. Garbage truck of the brain. Science 340:1529–1530. doi:10.1126/science.1240514

Nelson AR, Sweeney MD, Sagare AP, Zlokovic BV (2016) Neurovascular dysfunction and neurodegeneration in dementia and Alzheimer's disease. BBA-Mol Basis Dis 1862:887–900. doi:10.1016/j.bbadis.2015.12.016

Netchiporouk L, Shram N, Salvert D, Cespuglio R (2001) Brain extracellular glucose assessed by voltammetry throughout the rat sleep-wake cycle. Eur J Neurosci 13:1429–1434

Nicholson C (1980) Dynamics of the brain cell microenvironment. Neurosci Res Program Bull 18:175–322

Nicholson C (2001) Diffusion and related transport mechanisms in brain tissue. Rep Prog Phys 64:815–884

Nicholson C, Sykova E (1998) Extracellular space structure revealed by diffusion analysis. Trends Neurosci 21:207–215

Nisbet RM, Polanco J-C, Ittner LM, Gotz J (2015) Tau aggregation and its interplay with amyloid-beta. Acta Neuropathol 129:207–220. doi:10.1007/s00401-014-1371-2

Ohtsuki S, Ito S, Terasaki T (2010) Is P-glycoprotein involved in amyloid-β elimination across the blood-brain barrier in Alzheimer's disease? Clin Pharmacol Ther 88:443–445. doi:10.1038/clpt.2010.160

Pan WH, Kastin AJ (2014) Can sleep apnea cause Alzheimer's disease? Neurosci Biobehav Rev 47:656–669. doi:10.1016/j.neubiorev.2014.10.019

Pan W, Kastin AJ (2016) The blood-brain barrier: regulatory roles in wakefulness and sleep. Neuroscientist:1–13. doi:10.1177/1073858416639005

Pappolla M, Sambamurti K, Vidal R, Pacheco-Quinto J, Poeggeler B, Matsubara E (2014) Evidence for lymphatic Abeta clearance in Alzheimer's transgenic mice. Neurobiol Dis 71:215–219. doi:10.1016/j.nbd.2014.07.012

Patlak CS, Fenstermacher JD (1975) Measurements of dog blood-brain transfer constants by ventriculocisternal perfusion. Am J Physiol 229:877–884

Peng W, Achariyar TM, Li B et al (2016) Suppression of glymphatic fluid transport in a mouse model of Alzheimer's disease. Neurobiol Dis 93:215–224. doi:10.1016/j.nbd.2016.05.015

Petit JM, Magistretti PJ (2016) Regulation of neuron-astrocyte metabolic coupling across the sleep-wake cycle. Neuroscience 323:135–156. doi:10.1016/j.neuroscience.2015.12.007

Pflanzner T, Janko MC, Andre-Dohmen B et al (2011) LRP1 mediates bidirectional transcytosis of amyloid-beta across the blood-brain barrier. Neurobiol Aging 32(2323):e2321–e2311. doi:10.1016/j.neurobiolaging.2010.05.025

Plog BA, Dashnaw ML, Hitomi E et al (2015) Biomarkers of traumatic injury are transported from brain to blood via the glymphatic system. J Neurosci 35:518–526. doi:10.1523/jneurosci.3742-14.2015

Pollay M (2010) The function and structure of the cerebrospinal fluid outflow system. Cerebrospinal Fluid Res 7:9. doi:10.1186/1743-8454-7-9

Porkka-Heiskanen T, Kalinchuk AV (2011) Adenosine, energy metabolism and sleep homeostasis. Sleep Med Rev 15:123–135. doi:10.1016/j.smrv.2010.06.005

Potter R, Patterson BW, Elbert DL et al (2013) Increased in vivo amyloid-beta42 production, exchange, and loss in presenilin mutation carriers. Sci Transl Med 5:189ra177. doi:10.1126/scitranslmed.3005615

Ramanathan A, Nelson AR, Sagare AP, Zlokovic BV (2015) Impaired vascular-mediated clearance of brain amyloid beta in Alzheimer's disease: the role, regulation and restoration of LRP1. Front Aging Neurosci 7:136. doi:10.3389/fnagi.2015.00136

Rennels ML, Blaumanis OR, Grady PA (1990) Rapid solute transport throughout the brain via paravascular fluid pathways. Adv Neurol 52:431–439

Ries M, Sastre M (2016) Mechanisms of Aβ clearance and degradation by glial cells. Front Aging Neurosci 8:160. doi:10.3389/fnagi.2016.00160

Roberts KF, Elbert DL, Kasten TP et al (2014) Amyloid-beta efflux from the central nervous system into the plasma. Ann Neurol 76:837–844. doi:10.1002/ana.24270

Roh JH, Huang Y, Bero AW, Kasten T, Stewart FR, Bateman RJ, Holtzman DM (2012) Disruption of the sleep-wake cycle and diurnal fluctuation of beta-amyloid in mice with Alzheimer's disease pathology. Sci Transl Med 4:150ra122. doi:10.1126/scitranslmed.3004291

Roh JH, Jiang H, Finn MB et al (2014) Potential role of orexin and sleep modulation in the pathogenesis of Alzheimer's disease. J Exp Med 211:2487–2496. doi:10.1084/jem.20141788

Saido TC, Iwata N (2006) Metabolism of amyloid beta peptide and pathogenesis of Alzheimer's disease. Towards presymptomatic diagnosis, prevention and therapy. Neurosci Res 54:235–253. doi:10.1016/j.neures.2005.12.015

Saido T, Leissring MA (2012) Proteolytic degradation of amyloidβ-protein. Cold Spring Harb Perspect Med 2:a006379. doi:10.1101/cshperspect.a006379

Selkoe DJ (2001) Clearing the brain's amyloid cobwebs. Neuron 32:177–180

Sharma VK, Sharma P, Deshmukh R, Singh R (2015) Age associated sleep loss: a trigger for Alzheimer's disease. Bull Clin Psychopharmacol 25:78–88. doi:10.5455/bcp.20140909070449

Sherpa AD, Xiao F, Joseph N, Aoki C, Hrabetova S (2016) Activation of beta-adrenergic receptors in rat visual cortex expands astrocytic processes and reduces extracellular space volume. Synapse 70:307–316. doi:10.1002/syn.21908

Shibata M, Yamada S, Kumar SR et al (2000) Clearance of Alzheimer's amyloid-β(1-40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. J Clin Investig 106:1489–1499

Shiiki T, Ohtsuki S, Kurihara A et al (2004) Brain insulin impairs amyloid-beta(1-40) clearance from the brain. J Neurosci 24:9632–9637

Shirotani K, Tsubuki S, Iwata N et al (2001) Neprilysin degrades both amyloid β peptides 1-40 and 1-42 most rapidly and efficiently among thiorphan- and phosphoramidon-sensitive endopeptidases. J Biol Chem 276:21895–21901. doi:10.1074/jbc.M008511200

Siesjö BK (1978) Brain energy metabolism. Wiley, Chichester

Simon MJ, Iliff JJ (2016) Regulation of cerebrospinal fluid (CSF) flow in neurodegenerative, neurovascular and neuroinflammatory disease. BBA-Mol Basis Dis 1862:442–451. doi:10.1016/j.bbadis.2015.10.014

Sonnewald U (2014) Glutamate synthesis has to be matched by its degradation – where do all the carbons go? J Neurochem 131:399–406. doi:10.1111/jnc.12812

Spira AP, Chen-Edinboro LP, Wu MN, Yaffe K (2014) Impact of sleep on the risk of cognitive decline and dementia. Curr Opin Psychiatry 27:478–483. doi:10.1097/YCO.0000000000000106

Sprecher KE, Bendlin BB, Racine AM et al (2015) Amyloid burden is associated with self-reported sleep in nondemented late middle-aged adults. Neurobiol Aging 36:2568–2576. doi:10.1016/j.neurobiolaging.2015.05.004

Storck SE, Meister S, Nahrath J et al (2016) Endothelial LRP1 transports amyloid-beta(1-42) across the blood-brain barrier. J Clin Investig 126:123–136. doi:10.1172/JCI81108

Strazielle N, Ghersi-Egea JF (2013) Physiology of blood-brain interfaces in relation to brain disposition of small compounds and macromolecules. Mol Pharm 10:1473–1491

Sykova E, Nicholson C (2008) Diffusion in brain extracellular space. Physiol Rev 88:1277–1340. doi:10.1152/physrev.00027.2007

Szentistvanyi I, Patlak CS, Ellis RA, Cserr HF (1984) Drainage of interstitial fluid from different regions of rat brain. Am J Physiol 246:F835–F844

Tanzi RE, Moir RD, Wagner SL (2004) Clearance of Alzheimer's Aβ peptide: the many roads to perdition. Neuron 43:605–608. doi:10.1016/j.neuron.2004.08.024

Tarasoff-Conway JM, Carare RO, Osorio RS et al (2015) Clearance systems in the brain-implications for Alzheimer disease. Nat Rev Neurol 11:457–470. doi:10.1038/nrneurol.2015.119

Tononi G, Cirelli C (2014) Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. Neuron 81:12–34. doi:10.1016/j.neuron.2013.12.025

Van Uden E, Mallory M, Veinbergs I, Alford M, Rockenstein E, Masliah E (2002) Increased extracellular amyloid deposition and neurodegeneration in human amyloid precursor protein transgenic mice deficient in receptor-associated protein. J Neurosci 22:9298–9304

Vyazovskiy VV, Cirelli C, Pfister-Genskow M, Faraguna U, Tononi G (2008a) Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. Nat Neurosci 11:200–208. doi:10.1038/nn2035

Vyazovskiy VV, Cirelli C, Tononi G, Tobler I (2008b) Cortical metabolic rates as measured by 2-deoxyglucose-uptake are increased after waking and decreased after sleep in mice. Brain Res Bull 75:591–597. doi:10.1016/j.brainresbull.2007.10.040

Vyazovskiy VV, Olcese U, Lazimy YM et al (2009) Cortical firing and sleep homeostasis. Neuron 63:865–878. doi:10.1016/j.neuron.2009.08.024

Weller RO, Massey A, Newman TA, Hutchings M, Kuo YM, Roher AE (1998) Cerebral amyloid angiopathy: amyloid beta accumulates in putative interstitial fluid drainage pathways in Alzheimer's disease. Am J Pathol 153:725–733

Weller RO, Massey A, Kuo YM, Roher AE (2000) Cerebral amyloid angiopathy: accumulation of A beta in interstitial fluid drainage pathways in Alzheimer's disease. Ann N Y Acad Sci 903:110–117

Weller RO, Djuanda E, Yow H-Y, Carare RO (2009) Lymphatic drainage of the brain and the pathophysiology of neurological disease. Acta Neuropathol 117:1–14. doi:10.1007/s00401-008-0457-0

Xie L, Kang H, Xu Q et al (2013) Sleep drives metabolite clearance from the adult brain. Science 342:373–377. doi:10.1126/science.1241224

Yaffe K, Falvey CM, Hoang T (2014) Connections between sleep and cognition in older adults. Lancet Neurol 13:1017–1028. doi:10.1016/s1474-4422(14)70172-3

Yamada S, DePasquale M, Patlak CS, Cserr HF (1991) Albumin outflow into deep cervical lymph from different regions of rabbit brain. Am J Physiol 261:H1197–H1204

Yamaguchi H, Yamazaki T, Lemere CA, Frosch MP, Selkoe DJ (1992) Beta amyloid is focally deposited within the outer basement membrane in the amyloid angiopathy of Alzheimer's disease. An immunoelectron microscopic study. Am J Pathol 141:249–259

Yan P, Bero AW, Cirrito JR et al (2009) Characterizing the appearance and growth of amyloid plaques in APP/PS1 mice. J Neurosci 29:10706–10714. doi:10.1523/jneurosci.2637-09.2009

Ye L, Fritschi SK, Schelle J et al (2015) Persistence of Abeta seeds in APP null mouse brain. Nat Neurosci 18:1559–1561. doi:10.1038/nn.4117

Yin K-J, Cirrito JR, Yan P et al (2006) Matrix metalloproteinases expressed by astrocytes mediate extracellular amyloid-beta peptide catabolism. J Neurosci 26:10939–10948. doi:10.1523/jneurosci.2085-06.2006

Zekonyte J, Sakai K, Nicoll JAR, Weller RO, Carare RO (2016) Quantification of molecular interactions between ApoE, amyloid-beta (A beta) and laminin: relevance to accumulation of A beta in Alzheimer's disease. BBA-Mol Basis Dis 1862:1047–1053. doi:10.1016/j.bbadis.2015.08.025

Zerbinatti CV, Wozniak DF, Cirrito J et al (2004) Increased soluble amyloid-beta peptide and memory deficits in amyloid model mice overexpressing the low-density lipoprotein receptor-related protein. Proc Natl Acad Sci U S A 101:1075–1080

Zervas NT, Liszczak TM, Mayberg MR, Black PM (1982) Cerebrospinal fluid may nourish cerebral vessels through pathways in the adventitia that may be analogous to systemic vasa vasorum. J Neurosurg 56:475–481. doi:10.3171/jns.1982.56.4.0475

Zhao Z, Sagare AP, Ma Q et al (2015) Central role for PICALM in amyloid-β blood-brain barrier transcytosis and clearance. Nat Neurosci 18:978–987. doi:10.1038/nn.4025

Functional Interactions Between Sleep and Circadian Rhythms in Learning and Learning Disabilities

H. Craig Heller and Norman F. Ruby

Contents

Abstract

The propensity for sleep is timed by the circadian system. Many studies have shown that learning and memory performance is affected by circadian phase. And, of course it is well established that critical processes of memory consolidation occur during and depend on sleep. This chapter presents evidence that sleep and circadian rhythms do not just have separate influences on learning and memory that happen to coincide because of the circadian timing of sleep, but rather sleep and circadian systems have a critical functional interaction in the processes of memory consolidation. The evidence comes primarily from research on two models of learning disability: Down's syndrome model mice and Siberian hamsters. The Down's syndrome model mouse (Ts65Dn) has severe learning disability that has been shown to be due to GABAergic over-inhibition. Short-term, chronic therapies with $GABA_A$ antagonists restore learning ability in these mice long-term, but only if the antagonist treatments are given during the dark or

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sleep phase of the daily rhythm. The Siberian hamster is a model circadian animal except for the fact that a light treatment that gives the animal a phase advance on one day and a phase delay on the next day can result in total circadian arrhythmia for life. Once arrhythmic, the hamsters cannot learn. Learning, but not rhythmicity, is restored by short-term chronic treatment with GABA antagonists. Like many other species, if these hamsters are made arrhythmic by SCN lesion, their learning is unaffected. However, if made arrhythmic and learning disabled by the light treatment, subsequent lesions of their SCNs restore learning. SCN lesions also appear to restore learning in the Ts65Dn mice. The collective work on these two animal models of learning disability suggests that the circadian system modulates neuroplasticity. Our hypothesis is that a previously unrecognized function of the circadian system is to dampen neuroplasticity during the sleep phase to stabilize memory transcripts during their transfer to long-term memory. Thus, sleep and circadian systems have integrated roles to play in memory consolidation and do not just have separate but coincident influences on that process.

Keywords
Down's syndrome · GABA · Memory · Siberian hamsters · Suprachiasmatic nuclei

1 Interactions Between Sleep and Circadian Systems Are Extensive

The timing of the daily sleep/wake cycle is a fundamental functional interaction between the circadian system and sleep homeostasis that is widely recognized (Borbely 1982; Daan et al. 1984; Dijk and Czeisler 1995). The purpose of this chapter is to emphasize the need to understand functional interactions between circadian and sleep processes at many levels that go beyond the timing of sleep and wake. The master pacemaker of the mammalian circadian system is the suprachiasmatic nucleus (SCN) that uses transcriptional/translational feedback loops of clock genes to produce cellular oscillations (Buhr and Takahashi 2013). When these cellular rhythms are synchronized, they generate a circadian output signal that is projected largely to other areas of the hypothalamus including the ventrolateral preoptic area (VLPO) that is a sleep control nucleus. SCN projections also go to many areas beyond the hypothalamus such as the lateral septum, the paraventricular nucleus of the thalamus, the habenula, and the periaqueductal gray (Kriegsfeld et al. 2004). In addition to neural connectivity, the SCN may also release diffusible signals (Silver et al. 1996). Thus, the central pacemaker influences directly or indirectly a diversity of brain regions that control many physiological and behavioral functions. Because endocrine controls have such widely spread effects in the body, the regulation of neuroendocrine systems is of special significance (Kriegsfeld and Silver 2006). In addition, clock genes are expressed in a wide variety of peripheral tissues providing temporal organization to molecular, cellular,

biochemical, and physiological processes in most tissues and organs of the body (Zhang et al. 2014).

Coincidently, many functions in peripheral tissues are influenced by sleep and wake as shown by a plethora of experiments on animals and humans involving sleep deprivation. These functions include behavioral (e.g., Jackson et al. 2013), metabolic (e.g., Van Cauter et al. 2008), immune system (e.g., Ackermann et al. 2012), stem cell (e.g., Rolls et al. 2015), endocrine (e.g., Van Cauter et al. 2004), oxidative stress (e.g., Everson et al. 2014), bone metabolism (e.g., Everson et al. 2012), and many others including overall macromolecular biosynthesis (Mackiewicz et al. 2007).

There is a large overlap between processes/functions affected by circadian rhythms and by sleep. One way to conceptualize these overlaps is that the central and peripheral circadian timing mechanisms coordinate and facilitate sleep functions by controlling the underlying cellular and molecular processes so that necessary substrates and enzymes are provided at the right times, and by putting tissues and organs in proper states for maximum benefit from whatever function is being carried out. A consequence of that conceptual view is that circadian and sleep roles in many or most processes are not simply separate factors influencing the same processes but are integrated in their functions. Most studies of sleep or circadian influences on variables of interest focus on one or the other. Sleep studies usually treat circadian phase as a variable to be controlled for, and circadian studies try to avoid sleep confounds through sleep deprivation or constant routine protocols. Rarely do investigators ask whether there are important functional relationships between the sleep and the circadian influences on their system of interest. In this chapter we review the evidence that sleep and the circadian system play complementary and critically integrated roles in one set of functions of interest to both fields of study – learning and memory.

Both sleep and circadian phase have strong influences on learning and memory in humans and other mammals as has been documented in two extensive reviews (Rasch and Born 2014; Smarr et al. 2014). Both of those reviews barely touch upon the involvement of the other system. Recent studies, however, reveal that sleep and circadian systems do not simply have separate influences on learning and memory, rather they work in concert to achieve fidelity in memory consolidation. Our views on this interaction derive principally from studies of two animal models of learning disability: Down syndrome (DS) model mice and circadian-arrhythmic Siberian hamsters.

2 Learning Disability of Down Syndrome Involves GABAergic Over-Inhibition with Circadian and Sleep Components

The Ts65Dn mouse model of Down syndrome has severe impairments of long-term memory formation as shown by performance on the Morris water maze and radial arm maze tests (Seregaza et al. 2006) as well as on the novel object recognition (NOR) task (Dere et al. 2007; Fernandez et al. 2007; Colas et al. 2013). Possible

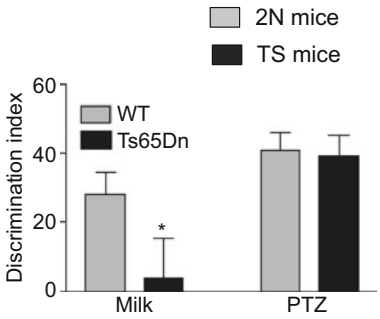

Fig. 1 Effects of short, chronic course of PTZ dosing on performance of Ts65Dn mice and their 2N littermates in the novel object recognition (NOR) test. Mice received daily doses of 3 mg/kg of PTZ in milk or just milk alone for 2 weeks. After that treatment ended, the mice were subjected to NOR testing with a 24-h delay between training and testing. If the mice spend equal times with the novel and the familiar object, the discrimination score is 0. Deviations from 0 indicate that the mouse spent more time exploring the new object than the previously experienced object. Whereas the PTZ treatment had no effect on the performance of the 2N mice, it normalized the performance of the TS mice to the levels shown by the 2N controls (Modified from Fernandez et al. 2007)

cellular bases for cognitive deficits in Ts65Dn mice have been reported in several studies ranging from neuroanatomical (Belichenko et al. 2004) to electrophysiological long-term potentiation (LTP) studies (Siarey et al. 1997; Kleschevnikov et al. 2004; Costa and Grybk 2005; Fernandez et al. 2007). A general inference from this body of work was that GABAergic over-inhibition is a possible cause of cognitive deficits in Ts65Dn mice.

Pursuing the GABAergic over-inhibition hypothesis, Fernandez et al. (2007) discovered that low, chronic doses of GABA antagonists, picrotoxin, bilobilide, and pentylenetetrazole (PTZ) restored the ability of Ts65Dn mice to perform as well as their 2N littermates in both NOR and spontaneous alternation (SA) tasks. The remarkable aspect of these studies is that a chronic treatment regime of low daily doses of the drugs for only 2 weeks results in a long-term normalization of the performance of the Ts65Dn mice that lasts for months (Fernandez et al. 2007) (Fig. 1). This study also showed that the PTZ treatment normalized the hippocampal LTP in the Ts65Dn mice. As with the behavioral tests, short-term (2-week) courses of daily dosing with GABA antagonists resulted in long-term (greater than 2 months) normalization of LTP.

Further characterization of the PTZ therapy in Ts65Dn mice produced additional interesting findings (Colas et al. 2013) (Fig. 2). First, the drug is effective at extremely low doses. Whereas the dose used in the Fernandez et al. study was 3 mg/kg, Colas et al. showed that 0.03 mg/kg was effective (Fig. 2b). Second, the therapy worked in young, middle-aged, and old Ts65Dn mice (Fig. 2b, c); thus, it was not working through a developmental effect nor a mitigation of senescence effect. Third, and most important for this discussion, the effectiveness of PTZ depended on time of day of dosing. If the drug was administered during the dark phase of the daily cycle, it had no effect (Fig. 2d). In addition, if the drug was

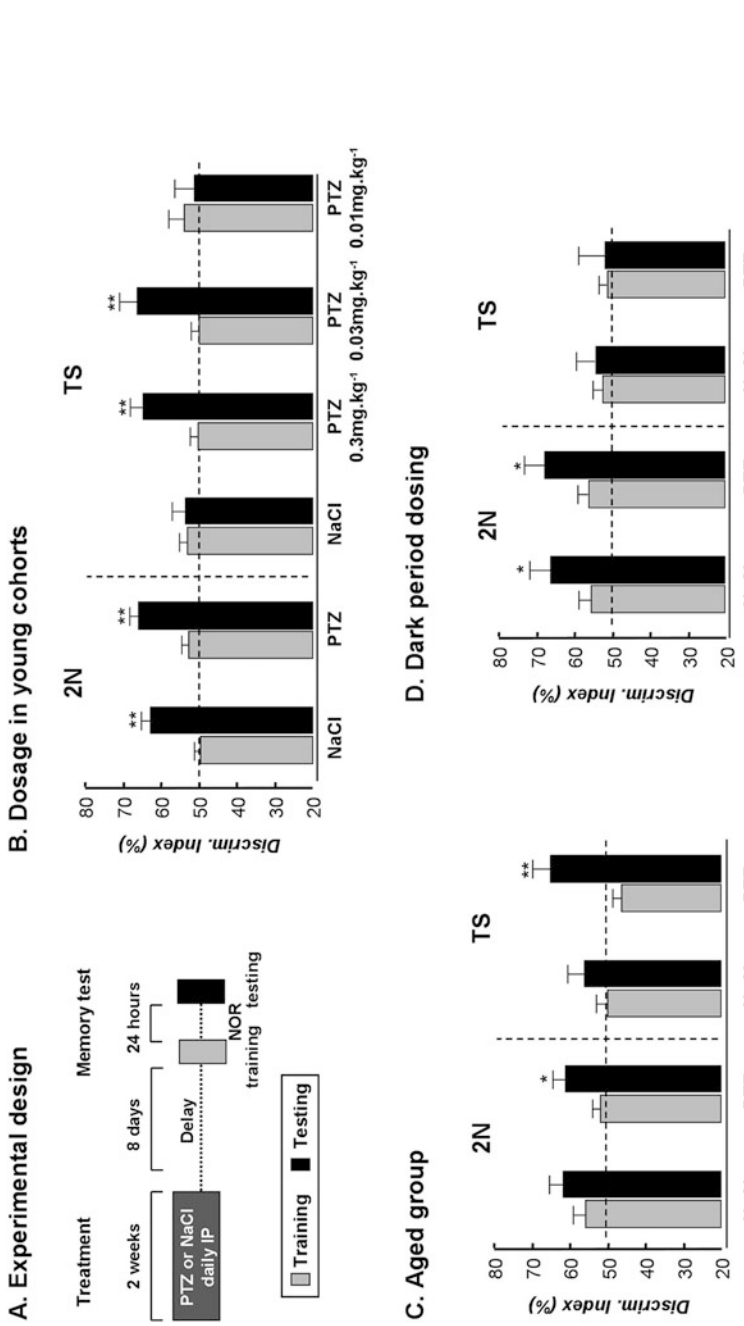

Fig. 2 Chronic treatment of Ts65Dn and 2N mice with daily injections of PTZ followed in 1 week with NOR testing reveal effective dose range, effect on young and aged mice, and sensitivity of the treatment to time of day. In these experiments a discrimination index of 50 means that the test animals spent equal time with the two objects. (**a**) Experimental design, (**b**) results showing that neither young Ts65Dn nor 2N mice favor one of two objects during training that 2N but not Ts65Dn mice recognize the novel object during testing if they received dosing with saline. The Ts65Dn mice receiving PTZ at the 0.3 or 0.03 mg/kg dosages, but not the 0.01 mg/kg dosage, showed performance on the NOR test equivalent to that of the 2N mice. (**c**) Older Ts65Dn mice (12–15 months) also show improved performance on the NOR test after receiving a 2-week course of daily IP injections of PTZ at 0.3 mg/kg. (**d**) PTZ treatment of Ts65Dn mice is only effective if delivered during the daily light phase (From Colas et al. 2013)

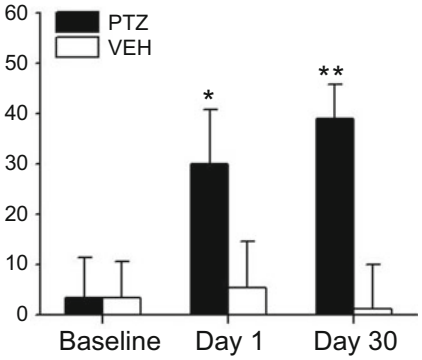

Fig. 3 Siberian hamsters made circadian-arrhythmic by a disruptive phase shift are not able to perform on the NOR test, but if they receive a 2-week course of daily injections of PTZ (1 mg/kg), they perform well on the NOR test on the day after the treatment ends and also 1 month later without any additional dosing with PTZ (modified from Ruby et al. 2013)

administered during the light phase, but the mice were sleep deprived for 1.5 h following dosing, the drug had no effect (Heller et al. 2014). Thus, GABAergic antagonism with PTZ was effective in reducing the cognitive disability of Ts65Dn mice, but its effectiveness was dependent on both circadian and sleep factors.

In a subsequent study, flumazenil, a benzodiazepine competitive antagonist, also was shown to be effective in long-term restoration of learning and memory in Ts65Dn mice (Colas et al. 2017). The flumazenil results are all the more remarkable because of the very short half-life of this drug. A critical question is how a short-term chronic treatment with GABAergic receptor antagonist drugs can produce an extremely long-lasting improvement in learning and memory.

Early on, the potential importance of the circadian system in the learning disability of DS led us to characterize the circadian rhythms of Ts65Dn mice in comparison to their 2N littermates. Contrary to expectations, the Ts65Dn mice had more robust circadian rhythms than their 2N littermates (Ruby et al. 2010). PTZ treatment did not influence any of the measured circadian parameters. At the time, we were surprised to see the robust circadian rhythms of the Ts65Dn animals, but subsequent work discussed below supports the possibility that stronger signals from the SCN in Ts65Dn mice may be a factor in their learning disability.

3 Circadian Arrhythmicity Can Cause Learning Disability in Hamsters

The apparent lack of circadian dysfunction in the Ts65Dn mice was in striking contrast to our previous work with another model of learning disability. We discovered that circadian timing could be eliminated in Siberian hamsters by exposing them to light for 2 h late at night, followed by a 3-h delay in onset of the next dark phase

(i.e., 3-h delay of the light-dark cycle; Ruby et al. 1996, 2004). This method has been termed the disruptive phase-shift (DPS) protocol (Prendergast et al. 2012). Being able to render hamsters circadian-arrhythmic with a simple light treatment gave us the opportunity to ask questions about the functions of circadian rhythms without doing invasive procedures such as lesions of the SCN, or genetic manipulations such as clock gene knockouts, or exposure of the animals to stressful conditions such as constant light. The first important finding with hamsters made circadian-arrhythmic by the DPS method was that once arrhythmic, they exhibited substantial deficits in recognition memory (Ruby et al. 2008), but as in the Ts65Dn mice, memory was restored by a 14-day injection regimen of PTZ (Ruby et al. 2013) (Fig. 3). PTZ treatment did not, however, restore the hamsters' circadian rhythms. Thus, whereas learning disabled Ts65Dn mice had robust circadian rhythms, learning disability in the hamster was associated with arrhythmia. The opposite results from these two models of learning disability raised the question of how both circadian arrhythmicity and stronger circadian rhythms could impair learning and memory.

4 Animals Made Arrhythmic by SCN Lesions Are Not Learning Disabled

Many studies have demonstrated that rodents continue to perform well in learning and memory tests after they have been made arrhythmic by SCN lesions. These studies included passive avoidance tests (Stephan and Kovacevic 1978; Cain et al. 2012), recognition memory and water maze learning (Mistlberber et al. 1996; Phan et al. 2011), spontaneous alternation and novel object recognition (Fernandez et al. 2014), and even conditioned time-place preference (Cain and Ralph 2009). Rodents made arrhythmic by clock gene knockouts also show mild or no deficits in spatial working memory, contextual fear memory, and object recognition memory (Mulder et al. 2013; Van der Zee et al. 2011; Wardlaw et al. 2014). However, Cry genes appear to be required for time-place memory (Van der Zee et al. 2011). Two studies in this assemblage caught our attention for a feature of the published data not noted by the authors (Stephan and Kovacevic 1978; Mistlberber et al. 1996). After the SCN was surgically ablated, animals seemed to improve performance in both passive avoidance and discrimination tasks. These and other studies raise the possibility that elimination of the SCN might have removed a circadian dampening of cognitive performance.

5 An Output of the SCN Modulates Neuroplasticity

The difference between the hamsters made arrhythmic by the DPS treatment and the rodents in SCN lesion studies is that the SCN remains functional in the hamsters even though it no longer generates a circadian signal. In contrast, the lesioned animals no longer had any SCN tissue. Measurements of clock gene RNAs in the arrhythmic hamsters showed that circadian arrhythmia was due to a loss of molecular

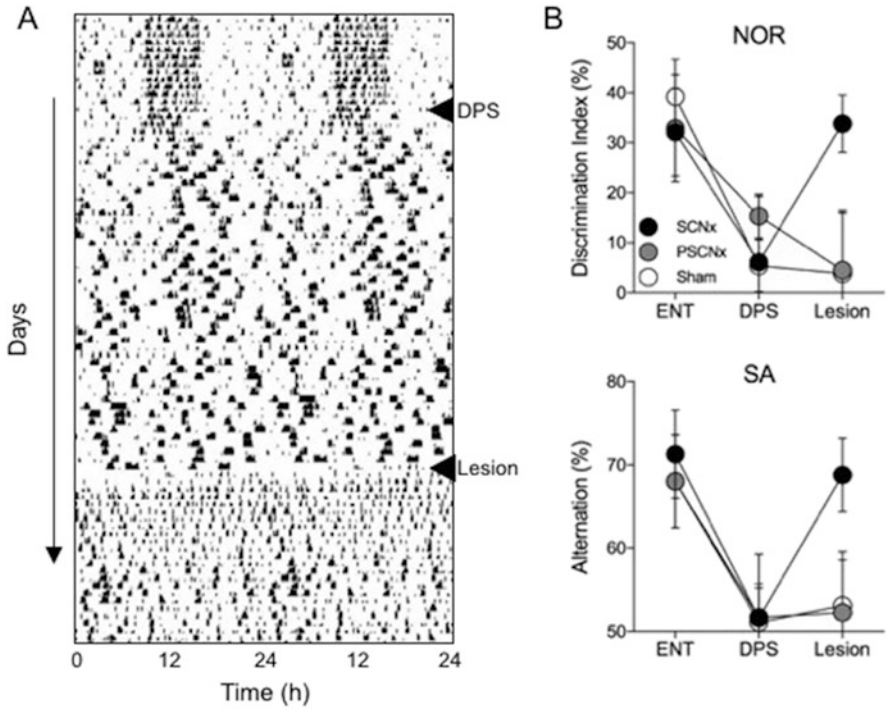

Fig. 4 Siberian hamsters made circadian-arrhythmic by a disruptive phase shift (a) lose the ability to perform normally (b) on either a test of declarative memory (NOR) or a test of working memory (SA). A complete lesion of the SCN, but not a partial lesion, restores their ability to perform normally on these two tests of learning ability (modified from Fernandez et al. 2014)

rhythms in the SCN (Grone et al. 2011). One potential inference from these studies is that SCN activity modulates neuroplasticity in learning and memory. Could it be that a continuous output from the SCN in the arrhythmic hamsters reduces neuroplasticity necessary for learning at all times of day?

The SCN is a GABAergic nucleus (Moore and Speh 1993; Belenky et al. 2008), and GABA receptor antagonism normalizes LTP in the dentate gyrus of hippocampal slices from learning disabled Ts65Dn mice (Siarey et al. 1997; Kleschevnikov et al. 2004; Costa and Grybk 2005; Fernandez et al. 2007). The hippocampus is essential for the formation of long-term declarative memories and has multiple inputs from different brain regions. The SCN does not project directly to the hippocampus, but it does innervate one of the major inputs to the hippocampus, the septal nuclei (Morin et al. 1994; Watts et al. 1987). Injections of $GABA_A$ receptor agonists into the medial septum disrupt performance in hippocampal-dependent learning tasks (Brioni et al. 1990; Parent et al. 1997; Degroot and Parent 2001; Krebs and Parent 2005). Thus, memory impairment in arrhythmic hamsters may be due to continuous release of GABA from the SCN into the septum (Ruby et al. 2008).

To test this hypothesis, hamsters were made circadian-arrhythmic by the DPS treatment, which impaired both recognition and spatial working memory (Fernandez et al. 2014). Next, these animals were subjected to stereotaxic thermolytic lesions of the SCN. Of course, these animals were still circadian-arrhythmic, but when subsequently retested for memory, hamsters with complete lesions of the SCN regained memory function and performed as well as rhythmic controls in tests of recognition and spatial memory (Fig. 4). Those animals that were still learning-impaired either had sham lesions or incomplete lesions that spared some SCN tissue (Fernandez et al. 2014). These results are strong support for the idea that the SCN is directly or indirectly responsible for the memory impairments found in hamsters made arrhythmic by the DPS protocol.

If a continuously active SCN can impair learning and memory in the arrhythmic hamsters, could an overly active SCN be responsible for the learning disability of the Ts65Dn mice? We are currently investigating this possibility by testing the NOR performance of Ts65Dn mice that were subjected to stereotaxic SCN lesions. Following recovery from the lesion surgery, the circadian rhythmicity of each animal is evaluated through actimetry under a light-dark cycle and in constant dark. This project is still in progress, but preliminary results to date show that the SCN lesions have no effect on the performance of the 2N mice in the NOR test, but the Ts65Dn mice rendered arrhythmic by the lesion surgery performed better than unlesioned Ts65Dn mice. Thus, in both animal models, the SCN might be directly or indirectly responsible for the GABAergic over-inhibition causing learning impairment in the Ts65Dn mice.

The idea that an intact SCN in Ts65Dn mice might interfere with memory as does an arrhythmic SCN in the hamster model led us to hypothesize that a normal function of the circadian system is to reduce or dampen neuroplasticity at a particular circadian phase. Why should that be so? We propose that the answer resides in a critical functional interaction and interdependency between the sleep and the circadian system in the process of memory consolidation.

6 Memory Consolidation Occurs Predominantly During Sleep

The foundation for mechanistic understanding of the role of the hippocampus in declarative learning and memory was the discovery of hippocampal neurons that fired selectively in response to spatial location of a rat (O'Keefe and Dostrovsky 1971; O'Keefe and Nadel 1978) and were therefore called place cells. The involvement of sleep in this learning process was pioneered by Wilson and McNaughton (1994). They showed that sequences of place cell activity recorded when a rat ran a maze were repeated when the rat was in subsequent NREM sleep. The authors hypothesized from their results: "...initial storage of event memory occurs through rapid synaptic modification, primarily within the hippocampus. During subsequent slow-wave sleep, synaptic modification within the hippocampus itself is suppressed, and the neuronal states encoded within the hippocampus are 'played back' as part of a consolidation process by which hippocampal information is gradually transferred

to the neocortex." Extensive subsequent work (briefly reviewed in Chapter "The Function(s) of Sleep") has characterized aspects of the last component of the Wilson and McNaughton hypothesis, the transfer of newly acquired information from the hippocampus to the neocortex (Siapas et al. 2005; Ji and Wilson 2007; Fujisawa and Buzsaki 2011; Kitamura et al. 2017). This body of work fits well with a model proposed by Born and Wilhelm (2012) suggesting that both the hippocampus and the cortex acquire information during wake, but the cortical representation is weaker than the hippocampal. During sleep, the hippocampus tutors the cortex in a process referred to as memory consolidation making the cortical engram stronger ensuring long-term memory as the hippocampal record eventually weakens.

The first part of the Wilson and McNaughton hypothesis stating that during sleep "the synaptic modification within the hippocampus itself is suppressed" probably had its origin in an earlier study by Barnes et al. (1977) that measured in rats and monkeys the sensitivity of cells in the *fascia dentata* of the hippocampus to stimulation of their afferent fibers. Their finding was that this measure of synaptic excitability had a daily rhythm. In the rat it was higher during the dark phase than the light phase, but in the monkey, it was higher during the light phase than the dark phase. They ruled out light level as being a causative factor, and they also ruled out sleep as a causative factor as all recordings were from awake moving animals. They also confirmed that the daily rhythm was circadian by conducting the recordings on a blinded rat. Many subsequent studies of hippocampal sensitivity and LTP have shown circadian variation. Many of these studies have been cited in an excellent report on the circadian rhythms of LTP in mouse hippocampal slices (Chaudhury et al. 2005). More recently a circadian rhythm of LTP has been demonstrated in freely behaving rats (Bowden et al. 2012). The unavoidable conclusion from this entire body of work is that the synaptic excitability of both diurnal and nocturnal animals is lowest during the sleep phases of their circadian rhythms. We propose from our work on learning disabled mice and hamsters that the dampening of hippocampal excitability, and presumably neuroplasticity, during the sleep phase is the functional contribution of the circadian system to the process of memory consolidation.

7 Hypothesis: During Sleep the Circadian System Stabilizes Memory Consolidation

Why should suppression of hippocampal neuroplasticity be necessary for memory consolidation? We suggest that the reason is to prevent alteration of the hippocampal engrams and thereby to ensure the fidelity of the information being transferred into long-term memory. The importance of memory reactivation and reconsolidation during sleep has been clearly established by studies in which specific elements of learning can be associated with a conditioned stimulus such as a tone or an odor, and then the subject can be exposed to those conditioned stimuli during subsequent sleep. The learning elements associated with the conditioned stimuli reintroduced

during sleep are recalled better during the following waking phase (Rolls et al. 2013; Rudoy et al. 2009; Rasch et al. 2007).

The interpretation of these studies is that experiencing the conditioned stimuli during sleep reactivated the memory and that memory was then strengthened by the subsequent reconsolidation. Presumably there are many different hippocampal engrams reactivated, replayed, and reconsolidated during sleep. What is preventing them from becoming mixed and thereby altered? Elegant experiments in the Tonegawa lab at MIT have shown that engrams formed in a neutral context can be labeled and then reactivated optogenetically. If such a neutral engram is activated during a fear conditioning process in a different context, the initial neutral context subsequently elicits strong fear responses (Ramirez et al. 2013). Thus, the mixing of engrams in the awake animal can create a false memory. During sleep, the mixing of engrams might be more likely because they are not delineated by sensory input, but mixing is prevented by the dampening of hippocampal neuroplasticity.

The vulnerability for memories to modification during sleep has been demonstrated by stimulating reactivation and replay during sleep. As discussed in Chap. 1, Rolls et al. (2013) showed that the emotional valence of fear conditioning could be enhanced by re-exposure of the animal to the conditioning stimulus during sleep. In this experiment, mice were subjected to foot shock following the conditioning stimulus – a puff of a distinctive odor. During subsequent sleep they were re-exposed to puffs of the conditioning odor or a control odor. When subsequently tested in a novel context during wake, the mice that received the conditioning odor showed more freezing (a sign of anxiety) than the mice that received the control odor. The emotional valence of the memory had been altered. The converse experiment took advantage of the fact that the reconsolidation of reactivated memories requires protein synthesis. The sleep phase was preceded by injections of a protein synthesis inhibitor into the amygdala, and then during sleep the mice were exposed either to the conditioning odor or to a control odor. The mice exposed to the conditioning odor, but not the control odor, showed a decrease in the emotional valence of the memory when exposed to the conditioning stimulus during subsequent wake in a novel context. Interestingly, if the conditioning stimulus were presented only during wake episodes, there was no change in the emotional valence of the memory. Presumably, if the conditioning stimulus induces replay of a memory during sleep, that memory is strengthened unless a protein synthesis inhibitor is present to interfere with the memory reconsolidation process. Thus, a memory is vulnerable to being modified during sleep. We propose that the functional contribution of the circadian system to the sleep-related process of memory consolidation is to stabilize the hippocampal engram while it is being transferred to the cortex, thus ensuring high fidelity of long-term memory.

8 Summary and Conclusions

The goal of this chapter is to encourage more integrated research on the interrelations between sleep and circadian rhythms. Sleep researchers interested in exploring the functions of sleep generally do not give consideration to circadian rhythms other than to control for their influence. Similarly, circadian researchers rarely take sleep into account other than efforts to eliminate it as a variable in their experiments. Control of the timing of sleep by the circadian system is generally recognized, but surely circadian rhythms have many more functional interactions with sleep. Searches for sleep functions have included abundant molecular, cellular, physiological, and behavioral variables. If all of those possible or putative sleep functions are grouped as one component of a Venn diagram, and all circadian processes constitute another component of a Venn diagram, there is huge overlap between them. All cells have clock genes, and expression of those genes synchronized by the central pacemaker in the SCN control many cellular functions. We should expect, therefore, that many sleep functions are facilitated by being integrated with circadian processes. As an example, this chapter describes evidence suggesting a previously unrecognized role that the circadian system plays in learning and memory – the stabilization of the memory consolidation process.

Insights came from studies of two animal models of learning disability, a Down syndrome model mouse (Ts65Dn) and Siberian hamsters rendered circadian-arrhythmic by a noninvasive, one-time light treatment called a disruptive phase shift. Prior work on the Ts65Dn mouse showed that the learning disability was due to GABAergic over-inhibition and was reversible with a short-term chronic daily treatment with low-dose GABAergic receptor antagonists. The same treatment restored learning and memory in the arrhythmic hamsters as well without restoring their circadian rhythms. Prior work on many species including humans has shown circadian rhythms in learning and memory, and the SCN is largely a GABAergic nucleus. Thus, there is the possibility that the SCN is the direct or indirect source of the GABAergic over-inhibition of the hippocampal circuits in the Ts65Dn mouse. That suggestion is strengthened by the finding that the GABA receptor antagonist dosing was only effective when delivered during the light phase of the daily rhythm of the Ts65Dn mice. However, a serious difference exists between the two model systems. The hamster learning disability was associated with circadian arrhythmia, whereas the Ts65Dn mice had intact circadian rhythms. A possible explanation was that the learning disabilities could be caused by excessive GABAergic inhibition at a specific time of day. If the SCN is creating the GABAergic over-inhibition, eliminating the SCN should restore learning abilities, which it does in the arrhythmic Siberian hamster. Complete SCN lesions in the arrhythmic hamsters normalize their ability to learn and remember. And, in preliminary experiments, the same appears to be true of the Ts65Dn mouse.

The questions that arise out of this sequence of research findings is whether a normal function of the circadian system is to dampen neuroplasticity responsible for the formation of memories, and if so, what is its adaptive significance. An extensive and excellent literature has developed on the neurophysiology of the process of

consolidation of short-term to long-term memory during sleep. For declarative memories, this involves replay of hippocampal engrams and their sharing with the cortex. Presumably this process involves many engrams shared between the hippocampus and the cortex over the course of a sleep phase. We have shown that individual engrams produced by fear conditioning can be altered during sleep, and the Tonegawa lab at MIT has shown that even during wake, engrams can become falsely associated. Therefore, we propose that the circadian system interacts with the sleep processes of memory consolidation to insure the fidelity of the information being consolidated into long-term memory.

In conclusion, we hope that this example of a functional interaction between sleep and the circadian system that goes beyond the simple issue of timing will encourage other sleep and circadian researchers to seek examples and mechanisms of integration rather than just treating one or the other system as a variable to be controlled.

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References

Ackermann K, Revell VL, Lao O, Rombouts EJ, Skene DJ, Kayser M (2012) Diurnal rhythms in blood cell populations and the effect of acute sleep deprivation in healthy young men. Sleep 35(7):933–934

Barnes CA, McNaughton BL, Goddard GV, Douglas RM, Adamec R (1977) Circadian rhythm of synaptic excitability in rat and monkey central nervous system. Science 197(4298):91–92

Belenky MA, Yarom Y, Pickard GE (2008) Heterogeneous expression of gamma-aminobutyric acid-associated receptors and transporters in the rat suprachiasmatic nucleus. J Comp Neurol 506(4):708–732

Belichenko PV, Masliah E, Kleschevnikov AM, Villar AJ, Epstein CJ, Salehi A, Mobley WC (2004) Synaptic structural abnormalities in the Ts65Dn mouse model of Down Syndrome. J Comp Neurol 480:281–298

Borbely AA (1982) A two-process model of sleep regulation. Hum Neurobiol 1:195–204

Born J, Wilhelm I (2012) System consolidation of memory during sleep. Psychol Res 76:192–203

Bowden JB, Abraham WC, Harris KM (2012) Differential effects of strain, circadian cycle, and stimulation pattern on LTP and concurrent LTD in the dentate gyrus of freely moving rats. Hippocampus 22(6):1363–1367

Brioni JD, Decker MW, Gamboa LP, Izquierdo I, McGaugh JL (1990) Muscimol injections in the medial septum impair spatial learning. Brain Res 522:227–234

Buhr ED, Takahashi JS (2013) Molecular components of the mammalian circadian clock. Handb Exp Pharmacol 217:3–27

Cain SW, Ralph MR (2009) Circadian modulation of conditioned place preference in hamsters does not require the suprachiasmatic nucleus. Neurobiol Learn Mem 91:81–84

Cain SW, Chalmers JA, Ralph MR (2012) Circadian modulation of passive avoidance is not eliminated in arrhythmic hamsters with suprachiasmatic nucleus lesions. Behav Brain Res 230 (1):288–290

Chaudhury D, Wang LM, Colwell CS (2005) Circadian regulation of hippocampal long-term potentiation. J Biol Rhythm 20:225–236

Colas D, Chuluun B, Warrier D, Blank M, Wetmore DZ, Buckmaster P, Garner CC, Heller HC (2013) Short-term treatment with the $GABA_A$ antagonist pentylenetetrazole produces a sustained procognitive benefit in a mouse model of Down's syndrome. Br J Pharmacol 169:763–773

Colas D, Chuluun B, Garner CC, Heller HC (2017) Short-term treatment with flumazenil restores long-term object memory in a mouse model of Down syndrome. Neurobiol Learn Mem 140:11–16

Costa ACS, Grybk MJ (2005) Deficits in hippocampal CA1 LTP induced by TBS but not HFS in the Ts65Dn mouse: a model of Down syndrome. Neurosci Lett 382:317–322

Daan S, Beersma DGM, Borbely AA (1984) Timing of human sleep: recovery process gated by a circadian pacemaker. Am J Phys 246:R161–RR178

Degroot A, Parent MB (2001) Infusions of physostigmine into the hippocampus or the entorhinal cortex attenuate avoidance retention deficits produced by intra-septal infusions of the GABA agonist muscimol. Brain Res 920(1–2):10–18

Dere E, Huston JP, De Souza Silva MA (2007) The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. Neurosci Biobehav Rev 31(5):673–704

Dijk DJ, Czeisler CA (1995) Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. J Neurosci 15:3526–3538

Everson CA, Folley AE, Toth JM (2012) Chronically inadequate sleep results in abnormal bone formation and abnormal bone marrow in rats. Exp Biol Med 237(9):1101–1109

Everson CA, Henchen CJ, Szabo A, Hogg N (2014) Cell injury and repair resulting from sleep loss and sleep recovery in laboratory rats. Sleep 37(12):1929–1940

Fernandez F, Morishita W, Zuniga E, Nguyen J, Blank M, Malenka RC, Garner CC (2007) Pharmacotherapy for cognitive impairment in a mouse model of Down syndrome. Nat Neurosci 10(4):411–413

Fernandez F, Lu D, Ha P, Costacurta P, Chavez R, Heller HC, Ruby NF (2014) Dysrhythmia in the suprachiasmatic nucleus inhibits memory processing. Science 346:854–857

Fujisawa S, Buzsaki G (2011) A 4 Hz oscillation adaptively synchronizes prefrontal, VTA, and hippocampal activities. Neuron 72(1):153–165

Grone BP, Chang D, Bourgin P, Cao V, Fernald RD, Heller HC, Ruby NF (2011) Acute light exposure suppresses circadian rhythms in clock gene expression. J Biol Rhythm 26:78–81

Heller HC, Ruby NF, Rolls A, Makam M, Colas D (2014) Adaptive and pathological inhibition of neuroplasticity associated with circadian rhythms and sleep. Behav Neurosci 128:273–282

Jackson ML, Gunzelmann G, Whitney P, Hinson JM, Belenky G, Rabat A, Van Dongen HP (2013) Deconstructing and reconstructing cognitive performance in sleep deprivation. Sleep Med Rev 17(3):215–225

Ji D, Wilson MA (2007) Coordinated memory replay in the visual cortex and hippocampus during sleep. Nat Neurosci 10:100–107

Kitamura T, Ogawa SK, Roy DS, Okuyama T, Morrissey MD, Smith LM, Redondo RL, Tonegawa S (2017) Engrams and circuits crucial for systems consolidation of a memory. Science 356(6333):73–78

Kleschevnikov AM, Belichenko PV, Villar AJ, Epstein CJ, Malenka RC, Mobley WC (2004) Hippocampal long-term potentiation suppressed by increased inhibition in the Ts65Dn mouse, a genetic model of Down syndrome. J Neurosci 24:8153–8160

Krebs DL, Parent MB (2005) Hippocampal infusions of pyruvate reverse the memory-impairing effects of septal muscimol infusions. Eur J Pharmacol 520(1–3):91–99

Kriegsfeld LJ, Silver R (2006) The regulation of neuroendocrine function: timing is everything. Horm Behav 49(5):557–574

Kriegsfeld LJ, Leak RK, Yackuli DB, LeSauter J, Silver R (2004) Organization of suprachiasmatic nucleus projections in Syrian hamsters (Mesocricetus auratus): an anterograde and retrograde analysis. J Comp Neurol 468(3):361–379

Mackiewicz M, Shickley KR, Romer MA, Galante RJ, Zimmerman JE, Naidoo N, Baldwin DA, Jensen ST, Churchill GA, Pack AI (2007) Macromolecule biosynthesis – a key function of sleep. Physiol Genomics 31:441

Mistlberber RE, de Groot MH, Bossert JM, Marchant EG (1996) Discrimination of circadian phase in intact and suprachiasmatic nuclei-ablated rats. Brain Res 739(1–2):12–18

Moore RY, Speh JC (1993) GABA is the principal neurotransmitter of the circadian system. Neurosci Lett 150(1):112–116

Morin LP, Goodless-Sanchez N, Smale L (1994) Projections of the suprachiasmatic nuclei, subparaventricular zone and retrochiasmatic area in the golden hamster. J Neurosci 61(2):391–410

Mulder C, Van Der Zee EA, Hut RA, Gerkema MP (2013) Time-place learning and memory persist in mice lacking functional Per1 and Per2 clock genes. J Biol Rhythm 28(6):367–379

O'Keefe J, Dostrovsky J (1971) The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain Res 34(1):171–175

O'Keefe J, Nadel L (1978) The hippocampus as a cognitive map. Clarendon Press, Oxford 570 pp

Parent MB, Laurey PT, Wilkniss S, GoldP E (1997) Intraseptal infusions of muscimol impair spontaneous alternation performance: infusions of glucose into the hippocampus, but not the medial septum, reverse the deficit. Neurobiol Learn Mem 68(1):75–85

Phan TX, Chan GC, Sindreu CB, Eckel-Mahan KL, Storm DR (2011) The diurnal oscillation of MAP (mitogen-activated protein) kinase and adenylyl cyclase activities in the hippocampus depends on the suprachiasmatic nucleus. J Neurosci 31:10640–10647

Prendergast BJ, Cisse YM, Cable EJ, Zucker I (2012) Dissociation of ultradian and circadian phenotypes in female and male Siberian hamsters. J Biol Rhythm 27:287–298

Ramirez S, Liu X, Lin R, Suh J, Pignatelli M, Redondo R, Ryan T, Tonegawa S (2013) Creating a false memory in the hippocampus. Science 341:387–389

Rasch B, Born J (2014) About sleep's role in memory. Physiol Rev 93(2):681–766

Rasch B, Buchel C, Gais S, Born J (2007) Odor cues during slow-wave sleep prompt declarative memory consolidation. Science 315:1426–1429

Rolls A, Makam M, Kroeger D, Colas D, de Lecea L, Heller HC (2013) Sleep to forget: interference of fear memories during sleep. Mol Psychiatry 18:1166

Rolls A, Pang WW, Ibarrra I, Colas D, Bonnavion P, Korin B, Heller HC, Weissman IL, deLecea L (2015) Sleep disruption impairs haematopoietic stem cell transplantation in mice. Nat Commun 6:8516

Ruby N, Sara A, Kang T, Franken P, Heller HC (1996) Siberian hamsters free run or become arrhythmic after a phase delay of the photocycle. Am J Phys 40(4):R881–R876

Ruby NF, Barakat MT, Heller HC (2004) Phenotypic differences in reentrainment behavior and sensitivity to nighttime light pulses in Siberian hamsters. J Biol Rhythm 19:1–12

Ruby NF, Hwang C, Wessells C, Fernandez F, Zhang P, Sapolsky R, Heller HC (2008) Hippocampal-dependent learning requires a functional circadian system. Proc Natl Acad Sci U S A 105(40):15593–15598

Ruby NF, Fernandez F, Zhang P, Klima J, Heller HC, Garner CC (2010) Circadian locomotor rhythms are normal in Ts65Dn "Down syndrome" mice and unaffected by pentylenetetrazole. J Biol Rhythm 25:63–66

Ruby NF, Fernandez F, Garrett A, Klima J, Zhang P, Sapolsky R, Heller HC (2013) Spatial memory and long-term object recognition are impaired by circadian arrhythmia and restored by the $GABA_A$ antagonist pentylenetetrazole. PLoS One 8(8):e72433

Rudoy J, Voss JL, Westerberg CE, Paller KA (2009) Strengthening individual memories by reactivating them during sleep. Science 326:1079

Seregaza S, Roubertoux PL, Jamon M, Sounireu-Mourat B (2006) Mouse models of cognitive disorders in trisomy 21: a review. Behav Genet 36:387–404

Siapas AG, Lubenov EV, Wilson MA (2005) Prefrontal phase locking to hippocampal theta oscillations. Neuron 45(1):141–151

Siarey RJ, Stoll J, Rapoport SI, Galdzicki Z (1997) Altered long-term potentiation in the young and old Ts65Dn mouse, a model for Down Syndrome. Neuropharmacology 36(11–12):1549–1554

Silver R, LeSauter J, Tresco PA, Lehman MN (1996) A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms. Nature 382(6594):810–813

Smarr BL, Jennings KJ, Driscoll JR, Kriegsfeld LJ (2014) A time to remember: the role of circadian clocks in learning and memory. Behav Neurosci 128(3):283–303

Stephan FK, Kovacevic NS (1978) Multiple retention deficit in passive avoidance in rats is eliminated by suprachiasmatic lesions. Behav Biol 22(4):456–462

Van Cauter E, Latta F, Nedeltcheva A, Spiegel K, Leproult R, Vandenbril C, Weiss R, Mockel J, Legros JJ, Copinschi G (2004) Reciprocal interactions between the GH axis and sleep. Growth Hormon IGF Res 14(Suppl A):S10–S17

Van Cauter E, Spiegel K, Tasali E, Leproult R (2008) Metabolic consequences of sleep and sleep loss. Sleep Med 9(Suppl 1):S23–S28

Van der Zee EA, Havekes R, Bar RP, Hut RA, Nijholt IM, Jacobs EH, Gerkema MP (2011) Circadian time-place learning in mice depends on Cry genes. Curr Biol 18:844–848

Wardlaw SM, Phan TX, Saraf A, Chen X, Storm DR (2014) Genetic disruption of the core circadian clock impairs hippocampus-dependent memory. Learn Mem 21(8):417–423

Watts AG, Swanson LW, Sanchez-Watts G (1987) Efferent projections of the suprachiasmatic nucleus: I. Studies using anterograde transport of Phaseolus vulgaris leucoagglutinin in the rat. J Comp Neurol 258:204–229

Wilson M, McNaughton B (1994) Reactivation of hippocampal ensemble memories during sleep. Science 265:676–679

Zhang R, Lahens NF, Ballance HI, Hughes ME, Hogenesch JB (2014) A circadian gene expression atlas in mammals: implications for biology and medicine. Proc Natl Acad Sci U S A 111(45):16219–16224

Sleep Physiology, Circadian Rhythms, Waking Performance and the Development of Sleep-Wake Therapeutics

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Abstract

Disturbances of the sleep-wake cycle are highly prevalent and diverse. The aetiology of some sleep disorders, such as circadian rhythm sleep-wake disorders, is understood at the conceptual level of the circadian and homeostatic regulation of sleep and in part at a mechanistic level. Other disorders such as insomnia are more difficult to relate to sleep regulatory mechanisms or sleep physiology. To further our understanding of sleep-wake disorders and the potential of novel therapeutics, we discuss recent findings on the neurobiology of sleep regulation and circadian rhythmicity and its relation with the subjective experience of sleep and the quality of wakefulness. Sleep continuity and to some extent REM sleep emerge as determinants of subjective sleep quality and waking performance. The effects of insufficient sleep primarily concern subjective and objective sleepiness as well as vigilant attention, whereas performance on higher cognitive functions appears to be better preserved albeit at the cost of increased effort. We discuss age-related, sex and other trait-like differences in sleep physiology and sleep need and compare the effects of existing pharmacological and non-pharmacological sleep- and wake-promoting treatments. Successful non-pharmacological approaches such as sleep restriction for insomnia and light and melatonin treatment for circadian rhythm sleep disorders target processes such as sleep homeostasis or circadian rhythmicity. Most pharmacological treatments of sleep disorders target specific signalling pathways with no well-established role in either sleep homeostasis or circadian rhythmicity. Pharmacological sleep therapeutics induce changes in sleep structure and the sleep EEG which are specific to the mechanism of action of the drug. Sleep- and wake-promoting therapeutics often induce residual effects on waking performance and sleep, respectively. The need for novel therapeutic approaches continues not at least because of the societal demand to sleep and be awake out of synchrony with the natural light-dark cycle, the high prevalence of sleep-wake disturbances in mental health disorders and in neurodegeneration. Novel approaches, which will provide a more comprehensive description of sleep and allow for large-scale sleep and circadian physiology studies in the home environment, hold promise for continued improvement of therapeutics for disturbances of sleep, circadian rhythms and waking performance.

Keywords

Circadian · Drug discovery · REM sleep · Sleep continuity · Sleep quality · Slow-wave sleep · Wake quality

1 Quantifying the Quality of Sleep and Wakefulness

1.1 A Multivariate Phenomenology

Sleep is a major determinant of well-being, mental and physical health and understanding sleep-health relations, and sleep disorders are dependent on adequate quantification of sleep. Sleep and wake states are characterised by constellations of variables representing nearly all levels of system organisation ranging from gene expression (O'Callaghan et al. 2018), neuronal firing patterns (McKillop and Vyazovskiy 2018), neurotransmitter release (Zant et al. 2016; Luppi and Fort 2018), endocrine and autonomic nervous system status (Morris et al. 2012; Fink et al. 2018), body and brain temperature (Landolt et al. 1995), responsiveness to external stimuli (Ermis et al. 2010), motor behaviour (Horner and Peever 2017) to changes in consciousness (Casali et al. 2013). Which variables are essential to sleep quality and how these variables contribute to sleep's associations with well-being, physical health and brain function remain, however, largely unknown even though several hypotheses have been proposed.

Here we will summarise some recent developments in research on sleep physiology, circadian rhythms and waking performance and their interrelations in humans without sleep complaints and discuss some implications for the understanding and development of non-pharmacological and pharmacological therapeutics for sleep and circadian disorders.

1.1.1 Sleep

Sleep has a rich phenomenology consisting of both subjective and objective aspects, and new facets continue to be reported. In humans, sleep comprises the subjective experience of the cessation of consciousness (falling asleep), the presence of dreams and nightmares or the feeling of not having slept all night. After waking up, the sleep experience can be reported as sleep quality and sleep depth. At another level sleep is a state of the brain and the body which in humans and animals can be quantified by objective behavioural criteria such as immobility and arousal thresholds and by a wide range of physiological variables. The electroencephalogram, electromyogram, electrooculogram, electrocardiogram, endocrine parameters, and respiratory parameters are among the physiological variables most commonly recorded in laboratory studies of human sleep. A recording of these state variables is referred to as a polysomnogram (PSG) (Berry et al. 2017) (see Fig. 1). Currently sleep is primarily quantified by sleep staging based on EEG, EMG, and EOG signals. Sleep is subdivided into non-rapid eye movement (NREM) sleep and rapid eye movement sleep (REM). In the current sleep staging guidelines for humans, NREM sleep will be further segmented in N1–N3 with N3 often referred to as slow-wave sleep (SWS). Previously NREM sleep was subdivided in stages 1–4 with stages 3 + 4 being combined as SWS. After sleep staging the sleep process is quantified by variables such as the latency to sleep onset, the duration of the various sleep stages, the relative contribution of sleep stages to total sleep time or the transitions between sleep stages. NREM sleep typically precedes REM sleep, and the ultradian cycle of the alternation

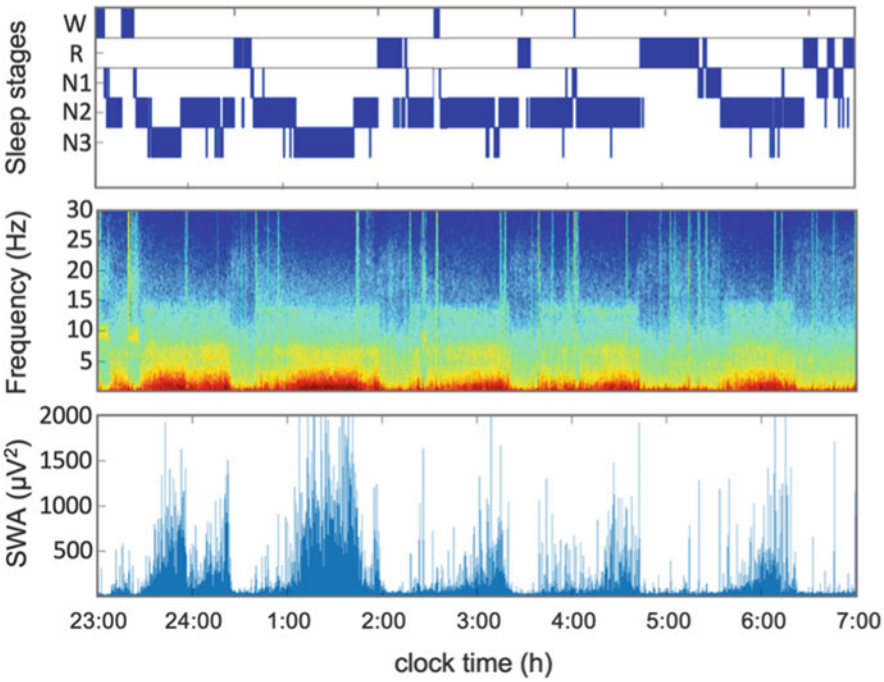

Fig. 1 Nocturnal sleep in a young adult. Hypnogram, spectrogram and time course of slow-wave activity (SWA = EEG power in the 0.75–4.5 Hz range). *W* wakefulness, *R* REM sleep, *N1–N3* NREM sleep stages 1–3

of NREM and REM sleep is one characteristic of interest. The duration of the NREM-REM cycle scales in a lawful manner to body and brain size sleep suggesting that it is closely linked to a primordial but as yet unknown function of sleep (Frank and Heller 2019). Sleep can be further characterised by its continuity, i.e. are sleep and wake episodes consolidated or are they interrupted by brief intrusions of the other state. Sleep continuity can be quantified as sleep efficiency (i.e. total sleep time/time in bed), wake after sleep onset, the number of awakenings, EEG arousals or the distribution of the duration of uninterrupted sleep bouts as assessed by survival analysis or other methods (Klerman et al. 2013; Svetnik et al. 2018).

Signal analysis-based approaches such as spectral analysis have been used to further characterise sleep although these approaches are still not standard in most research and clinical studies. Quantification of specific electrophysiological phenomena such as slow waves and sleep spindle oscillations (Dijk et al. 1993; Lazar et al. 2015), muscle tone (Brunner et al. 1990a; Jeppesen et al. 2018) or heart rate variability (Yang et al. 2018; Viola et al. 2008a) and changes thereof across the sleep episode or in response to pharmacological and non-pharmacological sleep manipulations have provided new insights into the sleep process. Quantification of slow waves through spectral analysis as power density in the low-frequency range

(0.75–4.5 Hz) and commonly referred to as slow-wave activity (SWA) has formed the basis for quantification of the homeostatic process in the two-process model of sleep regulation (Borbély 1982; Daan et al. 1984). Topographical analyses using multi-electrode EEG recordings have identified local aspects of sleep which may be relevant to local recovery processes (Kattler et al. 1994; Huber et al. 2006), memory consolidation (Rasch and Born 2013), the dream process (Siclari et al. 2017) or maybe the lack of cessation of consciousness despite the presence of global sleep as observed in insomnia (Riedner et al. 2016). Analyses of synchronisation across multiple EEG derivations and EEG frequencies have shown that the three vigilance states (Wakefulness, NREM and REM) are very different with REM sleep being the most synchronised state (Achermann et al. 2016), even though the REM sleep EEG is commonly described as desynchronised. Analyses of the temporal characteristics of the EEG have revealed phenomena such as phase locking between slow oscillations and sleep spindles (Klinzing et al. 2016) and ultra-slow oscillatory processes in spindle activity with periods of approximately 50–75 s (Lecci et al. 2017; Lazar et al. 2018).

1.1.2 Wakefulness

Sleep research tacitly assumes that the quality of wakefulness relates, at least in part, to the quality of sleep, although the reverse pathway is also plausible. Wakefulness is subjectively experienced, with alertness, sleepiness and fatigue being commonly used words to describe the quality of wakefulness. The subjective quality of wakefulness in terms of perceived alertness can be quantified with the Karolinska sleepiness scale (Akerstedt et al. 2017) or the Epworth sleepiness scale (Johns 1991) or objectively assessed as the ability to stay awake using the maintenance of wakefulness test or the propensity to fall asleep by the multiple sleep latency test (Sullivan and Kushida 2008). The quality of wakefulness can be characterised by absence or intrusion of sleep like electroencephalographic features such as theta activity (Greneche et al. 2008; Cajochen et al. 1995). The quality of wakefulness can be measured as the ability to remain vigilant and respond to stimuli in simple, and rather boring, test conditions such as in the psychomotor vigilance test (Lim and Dinges 2008). Finally, the quality of wakefulness may be assessed by the performance on tasks probing more complicated brain functions like working memory, executive function tests and the effort it takes to perform these tasks (Lo et al. 2012; Groeger et al. 2014) and tasks related to our professional and social life, e.g. driving (Shekari Soleimanloo et al. 2017).

The quality of wakefulness immediately following sleep is of special interest. Following the transition from sleep to wakefulness as defined by the PSG, it takes the brain some time to reach full waking performance (Balkin et al. 2002; Santhi et al. 2013). This process, referred to as sleep inertia, is relevant in operational conditions, for example, when pilots take naps on long haul flights, and may be exacerbated by non-pharmacological (e.g. sleep restriction) (Balkin and Badia 1988) and pharmacological (Boyle et al. 2012a; Cohen et al. 2010a) treatments of sleep disturbances.

Understanding how the different dimensions of sleep and wakefulness are interrelated, e.g. which physiologic aspect of sleep predicts the subjective quality of sleep, how sleep physiology relates to the quality of waking and how sleep- and wake-promoting therapeutics affect wake and sleep quality, respectively, is one of the challenges of sleep research and sleep medicine.

We will first discuss how sleep physiology is regulated in response to sleep loss.

2 Homeostatic Regulation of Sleep Physiology

Homeostatic regulation of sleep physiology has been investigated since the early days of modern sleep research. Most frequently used protocols are total sleep deprivation, e.g. not sleeping at all for 40 or 64 h, repeated partial sleep deprivation (e.g. a week of only 6 h of sleep per day), sleep extension, selective sleep deprivation (e.g. suppression of SWS) and nap studies. Sleep propensity and sleep structure of recovery sleep was the primary outcome measure in many of these studies. A major limitation of most of these studies is that time in bed during recovery sleep was restricted and sleep termination was thus not spontaneous. As a consequence the homeostatic regulation of human sleep duration is not well documented.

2.1 Sleep Propensity and Sleep Continuity

Total sleep deprivation, repeated partial sleep deprivation and selective disruption of SWS all lead to an increase in subjective and objective sleep propensity, i.e. subjective sleepiness and a reduction in the latency to sleep onset. These interventions also lead to a reduction in wake after sleep onset and the number of awakenings, i.e. sleep continuity increases with increasing homeostatic sleep pressure. Increasing time in bed, i.e. extending the nocturnal sleep opportunity (Bei et al. 2014, 2017; Skorucak et al. 2018) or taking naps and in particular naps in the later part of the day (Werth et al. 1996), leads to increases in the objective and subjective latency to sleep onset and also leads to a reduction in sleep continuity. Remarkably, a large-scale observational study in the community indicated that short sleep is associated with lower sleep efficiency and more awakenings, i.e. a pattern opposite to that observed in interventional laboratory studies (Akerstedt et al. 2019). This probably implies that short sleep in the community is caused by 'sleep problems' rather than imposed sleep restriction.

2.2 NREM Sleep, SWS and SWA

In humans NREM sleep is subdivided into N1, N2 and slow-wave sleep. Slow-wave sleep and slow-wave activity, i.e. EEG power density in the low-frequency range content of naps increases as naps are taken later in the day (Dijk et al. 1987a), nocturnal slow-wave sleep is increased after acute total sleep deprivation

(Borbély et al. 1981), and slow-wave sleep in nocturnal sleep is decreased after a nap taken later in the day (Werth et al. 1996). Slow-wave sleep is only marginally increased after repeated partial sleep deprivation (Brunner et al. 1993). Slow-wave sleep deprivation leads to an increase of SWS in subsequent undisturbed sleep (Dijk et al. 1987b; Dijk and Beersma 1989). These responses are consistent with a saturating exponential increase of slow-wave sleep pressure with time awake (Dijk et al. 1990a) and an approximately exponential decline of slow-wave sleep pressure during sleep (Dijk et al. 1990b; Achermann et al. 1993). The time constants of these functions are such that most of the dynamic range of slow-wave sleep is covered within a normal 24-h sleep-wake cycle (Skorucak et al. 2018; Rusterholz et al. 2010). This implies that SWS is primarily responsive to acute variation in sleep and wakefulness. Some data suggest that increasing the intensity of wakefulness through physical exercise or exposure to particular waking experiences may lead to global or local enhancement of slow-wave sleep (Horne and Moore 1985; Melancon et al. 2015; Huber et al. 2004).

2.3 REM Sleep

The homeostatic regulation of REM sleep is different from NREM sleep. Acute total sleep deprivation has no major effect on REM sleep during subsequent recovery sleep when the duration of recovery sleep is experimentally constrained (Borbély et al. 1981). Repeated partial sleep deprivation, which is not associated with a loss of SWS but is associated with a loss of REM sleep, leads to a REM sleep rebound during recovery sleep (Brunner et al. 1990b; Skorucak et al. 2018). REM sleep deprivation in the beginning of the sleep period leads to a rebound in the second half of the same sleep period (Beersma et al. 1990). A typical characteristic of REM sleep deprivation is that attempts to initiate REM sleep occur in clusters and increases in the course of REM sleep deprivation (Endo et al. 1998). A pronounced REM sleep rebound was also observed during withdrawal from REM sleep suppressing antidepressants (Landolt and de Boer 2001). Some authors have suggested that REM sleep's homeostatic regulation is primarily related to NREM sleep preceding REM sleep rather than preceding wakefulness (see Frank and Heller 2019).

It is often tacitly assumed that sleep after sleep loss is good sleep which implies that more SWS, shorter sleep latencies and fewer awakenings are the hallmarks of good sleep. Whether more SWS or more REM sleep is a positive characteristic depends on whether one considers total sleep deprivation or partial sleep deprivation the more natural challenge of sleep regulatory systems.

2.4 Age- and Sex-Related Differences in Sleep Physiology

Slow-wave sleep and slow-wave activity decline during the adult life span but so do sleep spindle activity and REM sleep (Schwarz et al. 2017; Della Monica et al. 2018). Age-related reductions in REM sleep are probably underestimated because as

mentioned, in most studies, time in bed was limited. Under ad libitum sleep conditions, age-related reductions in REM sleep may be as large as reductions in SWS (Klerman and Dijk 2008). Apart from changes in SWS and REM sleep, reductions in sleep efficiency and increases in the number of awakenings are consistently observed in healthy ageing. At the same time, the subjective quality of sleep as reported by the sleeper, rather than construed by sleep researchers, may not change much in healthy ageing (Akerstedt et al. 2016; Della Monica et al. 2018), and this is commonly interpreted as a habituation to reduced objective sleep quality.

Sex differences in sleep physiology primarily concern slow-wave sleep with women spending more time in slow-wave sleep and higher slow-wave activity (Dijk et al. 1989a; Svetnik et al. 2017). The age-related decline in SWA appears shallower in women than in men. Sleep continuity measures are not markedly different between the sexes (Carrier et al. 2017; Della Monica et al. 2018).

2.5 Sleep Physiology and the Subjective Quality of Sleep

A personal experience-oriented approach to sleep may define good sleep as sleep associated with sleep satisfaction or satisfactory alertness during the subsequent waking day. Sleep research may aim to understand how subjective sleep quality relates to objective aspects of sleep. When we only consider recent observational studies in which sleep in participants without sleep complaints were assessed by polysomnography, the consensus conclusion across these cross-sectional studies is that sleep efficiency/continuity is a significant determinant of reported sleep quality and the feeling of being refreshed upon awakening (Akerstedt et al. 2016; Kaplan et al. 2017a, b; Della Monica et al. 2018). This association is present across the adult life span and according to one study is much stronger in women than in men (Della Monica et al. 2018). Remarkably, these studies imply that deep sleep, i.e. SWS or N3, is not a main determinant of subjective measures of sleep quality. In one study REM sleep duration was positively associated with subjective sleep quality (Della Monica et al. 2018) (see Fig. 2). It should be noted that even though significant PSG predictors were identified, they explained only a small portion of the variance in subjective sleep quality.

2.6 Sleep Physiology and the Quality of Wakefulness

2.6.1 Observational Studies

How sleep is related to the quality of subsequent wakefulness is a key question when we consider sleep as a process of recovery and preparation for the next wake episode. Total sleep time is a correlate of subsequent waking quality such that in healthy participants, self-reported and objectively assessed total sleep time associates with alertness the next day as assessed by the multiple sleep latency test (MSLT) (Klerman and Dijk 2005). Longitudinal studies have demonstrated that the night-to-night variation in sleep duration is associated with subjective sleepiness

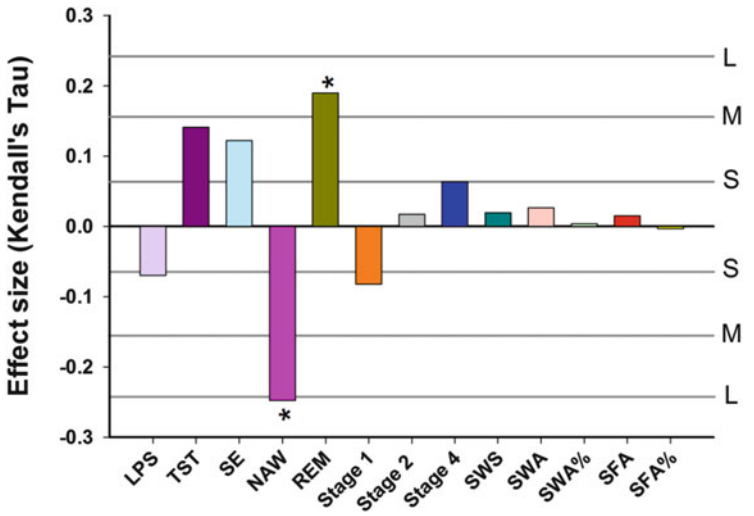

Fig. 2 Contribution of polysomnographically determined sleep variables to subjective sleep quality. Effect size is quantified with Kendall's tau after correcting for age and sex. Vertical lines represent large, medium and small effect sizes. *NAW* number of awakenings, *REM* rapid eye movement sleep, *TST* total sleep time, *SEFF* sleep efficiency, *LPS* latency to persistent sleep, *SWS* slow-wave sleep, *SWA* slow-wave activity, *SFA* sleep spindle frequency activity. Modified from Della Monica et al. (2018)

(Akerstedt et al. 2013). When we consider performance on specific tasks as an indicator of the quality of wakefulness, the picture emerging from observational studies in which sleep duration was assessed by self-report becomes more complex. For example, in a large-scale Internet-based study, it was found that both short and long sleep were associated with reduced performance on reasoning and verbal skills and no relationship between sleep duration and short-term memory performance was observed (Wild et al. 2018). Similarly, in an analysis of UK biobank data, both short and long sleep (self-reported) were associated with impaired performance, and the complaint of insomnia was not associated with reduced performance (Kyle et al. 2017).

These observations imply that good sleep and waking function require an optimal balance between sleep and wake duration. If finding the right sleep-wake balance is indeed essential for obtaining good quality sleep and waking function, then a number of questions emerge. What is 'normal/optimal' sleep duration and how to assess sufficiency of sleep on an individual basis? In this context, individual differences in sleep need and changes in sleep need across the adult life span may be considered.

Whether and how specific sleep stages predict the quality of wakefulness or brain function, i.e. cognition, has been investigated in young and older participants. In a comprehensive review of the literature addressing this question, it was concluded that a specific contribution of a specific sleep stage to cognition has not been firmly established (Scullin and Bliwise 2015). In one recent study, the association between

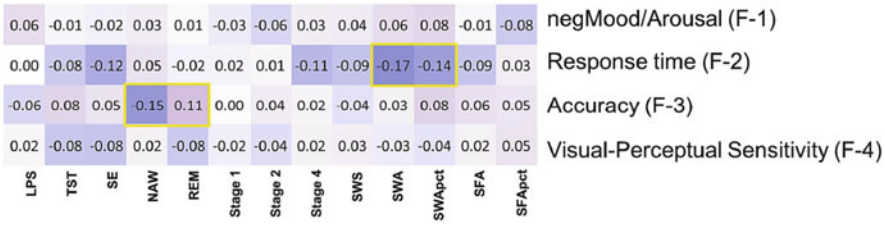

Fig. 3 Contribution of polysomnographically determined sleep variables to waking performance. Performance factors were derived from a factor analysis of performance measures. Colour coding represents Kendall's correlation coefficient, after controlling for sex and age. Note the negative correlation between SWA measures and response time, i.e. more SWA implies faster response times. A number of awakenings are negatively and REM sleep is positively correlated with accuracy. Modified from Della Monica et al. (2018)

nocturnal sleep and waking function was quantified across the healthy adult life span by an extensive battery of subjective and objective measures. After a factor analysis of the waking performance measures, it was found that SWS relates to a latent factor labelled 'Speed' of performance and REM sleep to 'Accuracy'. Importantly, sleep continuity was found to be the strongest predictor of 'Accuracy', which contains elements of executive function and working memory performance (Della Monica et al. 2018) (see Fig. 3).

Several observational studies have focused on contributions of specific aspects of sleep to other aspects of waking/brain function such as the association between sleep spindles and memory consolidation (Lafortune et al. 2014) and slow-wave sleep and metabolic clearance (e.g. Ju et al. 2018; Hladky and Barrand 2017). In the specific contexts of these studies, evidence for the contribution of slow-wave sleep, sleep spindles, REM sleep and sleep continuity to brain function has been reported although the magnitude of these effects and relative importance of various aspects of sleep remains to be established.

2.6.2 Interventional Studies

Interventional studies may provide more definitive insights into the relation between sleep and waking function. Acute total sleep deprivation, selective disruption of SWS and chronic sleep restriction all lead to an increase in subjective sleepiness and a reduction in objectively or subjectively assessed latency to sleep onset, measured at either habitual bedtime or during the daytime in the multiple sleep latency test (Carskadon and Dement 1987; Dijk et al. 2010a; Lo et al. 2012). Many studies have investigated the effects of sleep loss on aspects of brain function beyond sleepiness. Most studies implemented acute total sleep deprivation, but some used chronic partial sleep deprivation or selective disruption of SWS or sleep continuity (Lowe et al. 2017; Lo et al. 2012; Skorucak et al. 2018; Groeger et al. 2014). The most consistent finding is that sleep loss leads to impairment of vigilant attention. Many other aspects of waking performance are also affected. These include working memory, executive function, etc. Several studies have reported effects on memory

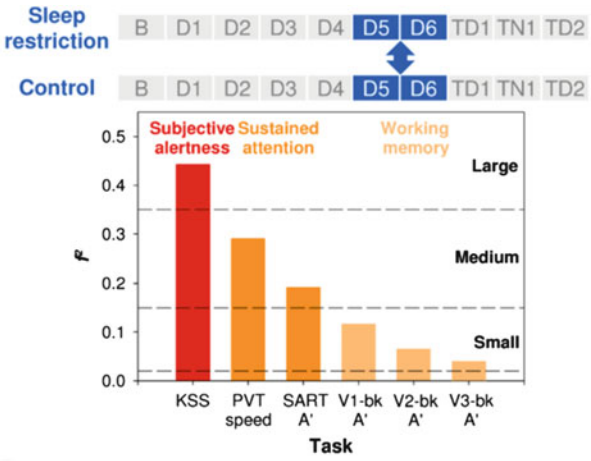

Fig. 4 Effects on 1 week of sleep restriction (6 h time in bed) on the Karolinska sleepiness scale (KSS), the 10% slowest reaction times on the psychomotor vigilance task (PVT), accuracy on the sustained attention to response task (SART) and accuracy of the verbal 1-, 2-, and 3-back. Modified from Lo et al. (2012)

consolidation as well. Repeated partial sleep deprivation studies have established that there is a progressive deterioration of waking performance and in particular of sustained attention suggesting that adaptation to sleep restriction does not occur (Van Dongen et al. 2003; Lo et al. 2012). It should be noted that repeated partial sleep deprivation does not lead to a large deficit in SWS but primarily to a deficit in REM sleep and N2, implying that the cognitive deficits cannot be attributed to SWS, but should be attributed to TST or REM sleep (Skorucak et al. 2018). It has been hypothesised that the cognitive deficits of sleep restriction were related to extension of the wake period beyond 16 h (Van Dongen et al. 2003), but this hypothesis was dismissed in a forced desynchrony study in which sleep-wake ratio was manipulated within 'days' shorter than 20 h. Despite the fact that wake episodes were never longer than 16 h, increasing the wake-sleep ratio nevertheless led to deficits in vigilant attention (McHill et al. 2018). This implies that it is simply the balance between wake and sleep duration that determines waking function and not the absolute duration of wakefulness.

Few studies have simultaneously assessed multiple aspects of waking function but those studies that did have shown that higher cognitive functions are less affected than relatively simple functions (e.g. sustained attention on the PVT) (see Fig. 4). In fact, it appears that if the sleep-deprived brain is able to engage with a task, deficits are relatively small. However, at the same time, the self-reported effort markedly increases. This suggests that insufficient sleep, at least with the time span of a week or so, does not lead to a fundamental inability of the waking brain to function even when, for example, working memory tasks with a high executive load are to be performed (Lo et al. 2012). The brain primarily struggles to stay awake and allocates more effort to maintain performance. Increases in subjective sleepiness and objective sleepiness and measures of effort are among the first signs of experimentally induced insufficient sleep and are among the dependent variables with the largest effect sizes (Balkin et al. 2004; Groeger et al. 2014; Lo et al. 2012). One major limitation of

these studies is that assessment of higher brain function in a realistic context was often not implemented. The implication is that functions such as creativity, complex decision-making and planning may still be markedly affected by insufficient sleep.

2.6.3 Individual Differences in Sleep Need and Response to Sleep Loss

Habitual long and short sleepers of approximately the same age differ in sleep propensity and sleep need when we consider total sleep time as the relevant measure of sleep propensity. However, nocturnal sleep latencies are longer in long sleepers, and short sleepers show more signs of intrusion of sleepiness-related theta activity during wakefulness (Aeschbach et al. 1996, 2001). Apparently long and short sleepers differ in their 'preferred' or 'tolerated' level of sleepiness. Whether and how this relates to homeostatic aspects of sleep regulation or to the longer circadian biological night observed in long sleepers remain unclear (Aeschbach et al. 2003).

Ageing is a major determinant of sleep propensity. Self-reported sleep duration and polysomnographically assessed total sleep time decline with age while at the same time daytime sleepiness decreases (Roenneberg 2013; Dijk et al. 2010a; Akerstedt et al. 2018). The most parsimonious explanation for these observations is that sleep need, at least as measured by sleep propensity, declines with age (Skeldon et al. 2016). An age-related reduction in the maximum capacity for sleep and reduction in sleep propensity has been demonstrated convincingly in protocols in which older and young adults spent 16 h in bed and this for several days. Older people slept 1.5 h less than young adults (who slept 8.7 h) but nevertheless were less sleepy (Klerman and Dijk 2008). That this reduction in the TST doesn't just reflect a reduced capacity for sleep but also for sleep need is supported by interventional studies which demonstrate that in older people the detrimental effects of total acute and repeated partial sleep deprivation are much smaller than in young people (Adam et al. 2006; Landolt et al. 2012; Zitting et al. 2018; Schwarz et al. 2018). Observational studies have not always confirmed these observations (Wild et al. 2018).

Individual differences in the deterioration of waking performance in response to total sleep deprivation and repeated partial sleep deprivation are also observed when only young adults are considered (Van Dongen et al. 2004; Rupp et al. 2012).

Several genetic variants associated with individual variation in sleep duration, sleep timing and sleep structure have been identified (Doherty et al. 2018; Rhodes et al. 2018; Viola et al. 2007; Archer et al. 2018). How genetic variants may affect response to sleep manipulations and pharmacological agents for wake and sleep promotion has been reviewed elsewhere (Bachmann et al. 2012; Holst et al. 2014; Landolt et al. 2018).

2.7 Effects of Insufficient Sleep on Peripheral Physiology, Endocrinology and the Blood Transcriptome

Insufficient sleep not only affects brain function and waking performance but also peripheral systems. This has been assessed by monitoring physiology during recovery sleep from total or chronic partial sleep deprivation and during wakefulness

following insufficient sleep. Autonomic tone changes in response to sleep loss such that during recovery sleep, there is shift to parasympathetic dominance as reflected in measures of heart rate variability (Viola et al. 2008a). Several hormones respond to insufficient sleep such that testosterone is suppressed, and appetite-regulating hormones ghrelin and leptin are up- and downregulated, respectively (Hanlon and Van Cauter 2011). Several markers of immune function respond to insufficient sleep such that immune function appears impaired (Irwin and Opp 2017). Finally genome-wide assessment of transcripts in whole blood has shown that sleep loss leads to changes in transcripts implicated in chromatin modification, gene expression regulation, macromolecular metabolism and inflammatory, immune and stress responses (Moller-Levet et al. 2013). Some of these changes may explain why and how insufficient sleep leads to adverse health outcomes such as obesity.

2.8 Sleep Physiology and Sleep Homeostasis as a Target for Pharmacological and Non-pharmacological Therapeutics

2.8.1 Sleep Promotion

Improving sleep quality in general focuses on latency to sleep onset, total sleep time and sleep continuity (maintenance) aspects such as wake after sleep onset or specific aspects of sleep structure such as slow-wave sleep. Peripheral correlates of sleep quality are in general not considered as target for sleep improvement.

Increasing homeostatic sleep pressure can improve latency to sleep onset, sleep continuity and total sleep time within a given time in bed period. This can be accomplished by increasing wake duration prior to sleep or by increasing the intensity of wakefulness through either physical or maybe mental activity. Such an approach may be useful in ageing and insomnia since in these conditions, optimal homeostatic sleep pressure may not be achieved because of daytime napping or excessive time in bed (Cross et al. 2015). Indeed, the recommended treatment of insomnia, which is the complaint of difficulties initiating and maintaining sleep, is cognitive behavioural therapy, the main component of which is thought to be sleep restriction (Maurer et al. 2018). An alternative approach to increase homeostatic sleep pressure may be to pharmacologically target the molecular signalling pathways of sleep homeostasis. Emerging pathways are adenosine and prostaglandins (Holst and Landolt 2018; Urade 2017; Korkutata et al. 2019) although approved drugs for these targets are not yet available.

Available pharmacological approaches of insomnia may target wake after sleep onset or sleep latency, but in general these approaches do not target the (largely unknown) molecular signalling pathways of sleep homeostasis but instead focus on inhibiting mechanisms related to 'arousal' and 'wake promotion', e.g. GABAergic, histaminergic and orexinergic mechanisms (Landolt et al. 2018).

Since SWA/SWS is accurately regulated in acute sleep manipulation protocols, is often considered a key indicator of sleep homeostasis, shows a marked age-related decline and has been implicated in memory consolidation, it has been and remains a target for sleep promotion and improvement (Mander et al. 2017; Wilckens et al.

2018). Non-pharmacological approaches include exercise and neuromodulation (Kredlow et al. 2015; Wilckens et al. 2018). In one type of neuromodulation, acoustic stimuli are phase locked to spontaneous slow waves. This has been reported to enhance slow waves in young people, but not in older people (Garcia-Molina et al. 2018). Whether this enhancement improves subjective sleep quality and waking function has not been firmly established. Rocking stimulation has recently been reported to increase SWS, spindle density in SWS and memory consolidation in healthy young adults (Perrault et al. 2019).

Pharmacological enhancement of slow-wave sleep has been accomplished by compounds that have different mechanisms of action. Agonists of the extra-synaptic $GABA_A$ receptor such as gaboxadol, also known as THIP, reliably induce SWS and SWA in healthy participants at baseline, in a model of transient insomnia (traffic noise, Dijk et al. 2012), a model of sleep onset insomnia (Mathias et al. 2001), a circadian phase advance model (Walsh et al. 2007), older participants (Lancel et al. 2001) and insomnia patients (Lankford et al. 2008). Interestingly, the effects of gaboxadol on sleep are much stronger in women than in men (Dijk et al. 2010b; Ma et al. 2011; Roth et al. 2010).

These enhancements of SWS were accompanied by modest improvement in sleep continuity and subjective sleep quality and in one report an improvement of daytime sleepiness and waking performance (Walsh et al. 2008). Gaboxadol was not fully developed as a treatment for insomnia, but its unique action on $GABA_A$ receptors containing the delta subunit and its effects on sleep and the EEG remain intriguing.

The most commonly used drugs for the treatment of insomnia are allosteric modulators of the $GABA_A$ receptor. In general these drugs do have hypnotic effects, e.g. improve wake after sleep onset (WASO), but do not enhance SWS, although zolpidem can enhance visually scored SWS (Bettica et al. 2012). Spectral analysis of the sleep EEG has revealed that the effects of all $GABA_A$ receptor allosteric modulators lead to a very similar spectral profile with reductions in delta and theta activity and enhancement in sigma activity (Arbon et al. 2015; Brunner et al. 1991; Trachsel et al. 1990) (see Fig. 5). This profile is very different from the spectral changes induced by physiological enhancement of homeostatic sleep pressure through sleep deprivation. The increase in visually scored SWS which is sometimes reported following zolpidem administration appears to be related to an increase in activity in very low-frequency slow waves (Landolt et al. 2000).

The hypnotic and EEG effects of $GABA_A$ receptor allosteric modulators can be separated, i.e. they are mediated by different receptor systems, and the rich variety of $GABA_A$ receptors remains a target for the pharmacological improvement of sleep (Wisden et al. 2017). In addition to $GABA_A$, $GABA_B$ receptors also appear to be involved in sleep regulation. $GABA_B$ receptors mediate some of the effects of gamma-hydroxybutyrate (GHB) and its sodium salt: sodium oxybate (SO). SO is the current first-line treatment of excessive daytime sleepiness and cataplexy in narcolepsy type 1. GHB induces SWS (Van Cauter et al. 1997; Dornbierer et al. 2019) and sleep onset REM periods (Vienne et al. 2012) and has been reported to improve performance (Walsh et al. 2010).

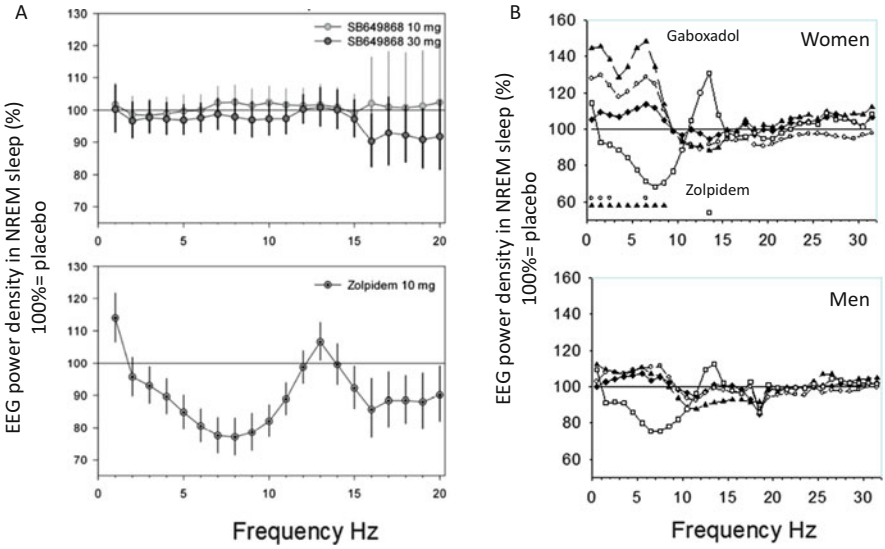

Fig. 5 (**a**) Effects of zolpidem and a dual orexin antagonist (SB-649868) on EEG power spectra in NREM sleep (modified from Bettica et al. 2012). (**b**) Sex differences in the effects of zolpidem (10 mg) and gaboxadol (5, 10, 15 mg) on EEG power spectra in NREM sleep. Modified from Dijk et al. (2010b)

Antagonists of serotonergic 5-HT_{2A} and histaminergic H_1 receptors the latter being widely used as nonprescription drugs for the treatment of sleep disturbances also enhance SWS. This maps on to the serotonergic and histaminergic pathways implicated in sleep-wake regulation generation (Landolt et al. 2018; Yu et al. 2018). The spectral profile of these effects is much more similar, although not identical, to the effects of sleep deprivation than the effects $GABA_A$ receptor allosteric modulators, i.e. these antagonists induce an increase in delta and theta activity (Dijk et al. 1989b; Landolt and Wehrle 2009). The pharmacological enhancement of SWS by 5-HT_{2A} antagonists is in general not accompanied by a shortening of sleep latency, an improvement in sleep continuity or a subjective sleep quality (Landolt et al. 1999). Alcohol is by many used as a sleep facilitator. It indeed hastens sleep onset but leads to more wakefulness in the second half of the sleep period, especially in older participants (Landolt et al. 1996). The effects of alcohol on EEG power spectra are different from those induced by $GABA_A$ allosteric modulators (Dijk et al. 1992a).

Sleep spindles are not a target in drug development for insomnia, even though several benzodiazepines and z-drugs enhance sleep spindle activity, and these changes have been related to changes in plasticity (Wisden et al. 2017).

Even though REM sleep deficits have been reported in insomnia (Baglioni et al. 2014) and in other conditions such as Alzheimer's disease (Winsky-Sommerer et al. 2018) and REM sleep is a positive predictor of subjective sleep quality and waking

performance (Della Monica et al. 2018), REM sleep enhancement does not appear to have been a target for pharmacological treatments. In fact REM sleep enhancement and reduced latencies to REM sleep observed after administration of orexin antagonists, e.g. (Bettica et al. 2012), are considered as an unwanted effect because of its potential link with narcolepsy and cataplexy (Jacobson et al. 2017).

Negative effects of hypnotics on waking performance after drug-enhanced sleep remain widespread despite continuing efforts to reduce these residual effects. For example, in one detailed analysis of the effects of zopiclone and eszopiclone, it was found that for some aspects of performance residual effects lasted as long as 11 h after dosing, i.e. until mid-late morning (Boyle et al. 2012a).

2.8.2 Wake Promotion

Extending sleep is one approach to reduce sleepiness caused by sleep restriction although this approach is obviously not realistic in idiopathic hypersomnolence (Baumann 2018).

Non-pharmacological approaches, other than extra sleep, to improve waking function include light exposure. Light has now been shown in a large number of experiments (both in humans and animals) to have direct alerting effects (Pachito et al. 2018; Viola et al. 2008b; Gaggioni et al. 2014). The photoreceptor and molecular and neuroanatomical signalling pathways have in part been elucidated (LeGates et al. 2014). Considering characteristics of light as an important non-pharmacological approach to improving alertness is relevant in view of the time we spend indoors where we are exposed primarily to artificial light.

Pharmacological approaches to improve alertness/wakefulness include caffeine (Wyatt et al. 2004) which through its adenosinergic action is thought to interact with sleep homeostatic mechanisms and indeed disrupts sleep (Clark and Landolt 2017). Although widely used, some of the effects of caffeine remain poorly understood. For example, and somewhat paradoxical, caffeine consumption may be associated with sleepiness (Wyatt et al. 2004). Patterns of caffeine consumption may suggest that it is primarily used to reduce sleep inertia and new delivery systems for this application have been developed (Newman et al. 2013).

Pharmacological approaches target the main wake-promoting systems such as the dopaminergic and histaminergic (H3) systems (Landolt et al. 2018). More recently the glutamatergic (AMPA receptor) system has been targeted for wake promotion. In experimental medicine type of conditions, these compounds have been shown to exert wake promotion as evidenced by longer latencies on the maintenance of wakefulness test or reduction of theta activity in the wake EEG (Boyle et al. 2012b; James et al. 2011; Iannone et al. 2010). At the same time little evidence for enhancement of cognition was observed. Furthermore, analyses of sleep following administration of compounds to promote wakefulness have documented ‘residual’ effects such as prolonged sleep latency or changes in sleep structure and the sleep EEG. Remarkably these effects were not observed after administration of modafinil (James et al. 2011; Bodenmann et al. 2012).

3 Circadian Rhythmicity: Sleep and Waking Performance

Circadian rhythmicity together with sleep homeostasis shapes the timing and structure of the sleep-wake cycle in young and older people and in men and women (Dijk and Czeisler 1995; Dijk et al. 1999; Wyatt et al. 1999; Santhi et al. 2016). Humans are a diurnal species, and in adult humans, sleep normally occurs at night and wakefulness during the day, although in modern industrialised societies with access to artificial light and the many social constraints it may be unclear where the day ends and the night begins (Skeldon et al. 2017; Walch et al. 2016). Sleep is not always monophasic, and daytime naps occur in normal and pathological conditions. Sleep timing can be measured against clock time, relative to the natural light-dark cycle and relative to preferred sleep timing, and imposed work schedules but also in relation to the many rhythmic circadian processes such as the hormones cortisol, melatonin, core body temperature, etc. (Dijk and Lockley 2002).

The timing and duration of sleep are among the most accessible characteristics of sleep, and dysregulation of these aspects may be related to circadian rhythm sleep disorders, insomnia, hypersomnia, etc. (see below). The two-process model of sleep regulation describes sleep timing as the result of the interaction of a circadian process and a homeostatic process (Daan et al. 1984; Borbély et al. 2016). The homeostatic aspect of sleep regulation relates to the contribution of sleep debt – as accrued during wakefulness and dissipating during sleep – to overall sleep propensity. The circadian process represents a clock-like intrinsically generated rhythm in sleep propensity. Sleep timing is also strongly influenced by social constraints such as work schedules and social obligations. Age-related and genetically determined differences in these processes and their interactions as well as psychological phenomena such as rumination and apprehension will shape individual differences in sleep-wake timing and the risk for sleep disturbances and sleep disorders (see Fig. 6).

3.1 Circadian Regulation of Sleep and Wakefulness

3.1.1 Circadian Rhythm of Sleep-Wake Propensity

The circadian process drives a rhythm in sleep-wake propensity and persists in the absence of sleep. The sleep-wake propensity rhythm is closely associated with the melatonin rhythm and the core body temperature rhythm. The waveform and timing of this sleep-wake propensity rhythm are at first glance somewhat paradoxical (Dijk and Czeisler 1994). Multiple protocols have established that in participants without sleep disturbances, the circadian drive for wakefulness increases during the waking day and reaches a maximum in the evening hours. The zone of maximum circadian drive for wakefulness, located just before the nocturnal (9–11 p.m.) increase in plasma melatonin concentrations, has been referred to as the wake maintenance zone (Strogatz et al. 1987). This drive for wakefulness then dissipates rapidly after the rise of melatonin (see Fig. 7).

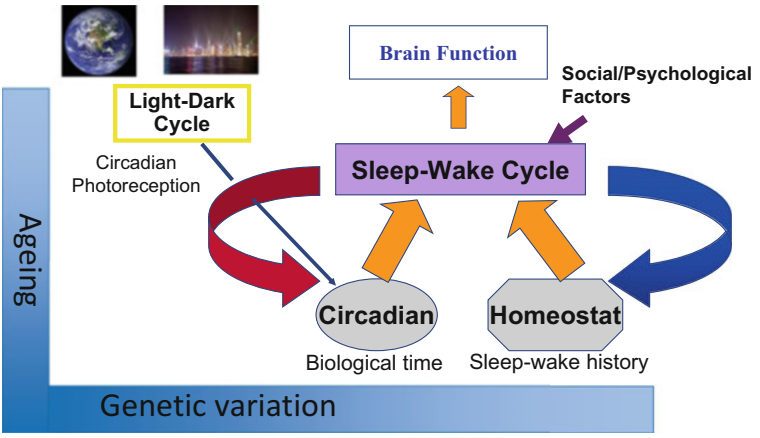

Fig. 6 Schematic representation of the interaction between circadian rhythmicity, sleep homeostasis and the light-dark cycle in the regulation of the sleep-wake cycle. Modified from Dijk and Lockley (2002)

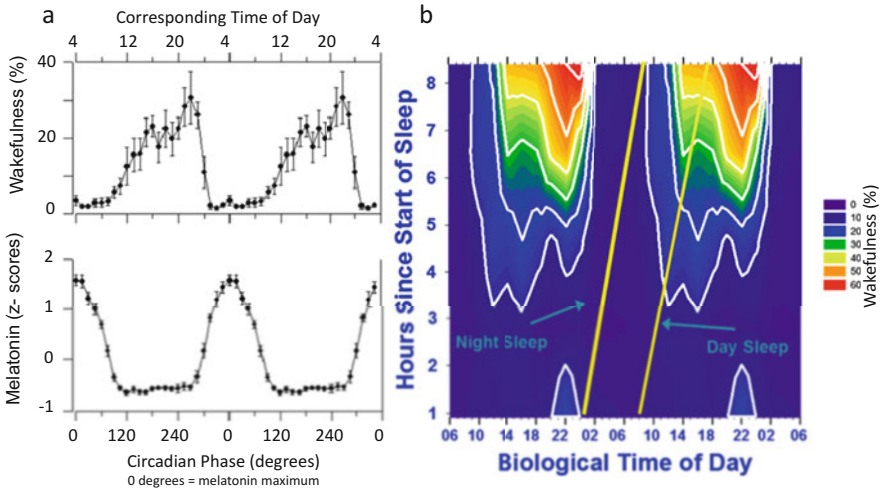

Fig. 7 Circadian and homeostatic regulation of sleep. (**a**) Association between wakefulness in sleep episode and the plasma melatonin rhythm. Modified from Dijk et al. (1997). (**b**) Sleep disruption (% wakefulness) as a function of hours since start of sleep episode and circadian phase. The two yellow trajectories represent a typical nocturnal and diurnal sleep episode. Modified from Dijk and Czeisler (1994)

The maximum circadian drive for sleep is located close to the nadir of the body temperature rhythm which in healthy participant occurs at around 4–6 a.m. and has been referred to as the sleep maintenance zone. When this circadian-driven sleep-

wake propensity rhythm is dysfunctional, a consolidated sleep-wake cycle is no longer observed (Dijk and von Schantz 2005; Czeisler and Gooley 2007).

3.1.2 Circadian Contribution to Sleep Structure, Sleep Continuity and the Sleep EEG

The circadian pacemaker actively promotes REM sleep such that even when expressed as a percentage of total sleep time, REM sleep propensity is maximal at around 2 h after the nadir of the core body temperature rhythm (Dijk and Czeisler 1995). The density of rapid eye movement is highest when sleep occurs in the evening hours, i.e. during the wake maintenance zone (Khalsa et al. 2002). Sleep continuity or sleep consolidation is an important determinant of sleep quality. The circadian process has a strong impact on this characteristic of sleep such that the duration of awakenings is much shorter during the biological night than during the biological day (Dijk et al. 2001).

The circadian pacemaker also modulates the EEG within sleep such that it actively promotes sleep spindle activity at night. The duration of sleep spindles is longer, their frequency is lower, and their amplitude is higher when sleep occurs at night (Wei et al. 1999). It has been hypothesised that this circadian modulation of sleep spindles may contribute to sleep consolidation (Dijk et al. 1997).

Slow waves are to a lesser extent modulated by circadian phase such that slow-wave activity is slightly lower and their slope is shallower when sleep occurs at night. The circadian modulation of the sleep and wake EEG, relative to the sleep-related modulation, is dependent on topography such that it is largest in occipital and smallest in frontal derivations (Lazar et al. 2015).

3.1.3 Intrinsic Circadian Period and Sleep Timing

Basic circadian rhythm research has established that the timing of the circadian clock relative to the 24 h cycle is determined by the strength (i.e. amplitude) of the light-dark cycle and the intrinsic, largely genetically determined, period of the circadian clock. Assessment of the intrinsic period of the circadian clock requires specialised protocols in which light input to the clock is absent (as in blind people) or distributed evenly across the circadian cycle in protocols in which the sleep-wake cycle is scheduled to a period several hours shorter or longer than the intrinsic circadian period and light levels during waking are very low (Duffy et al. 2011). These protocols have shown that on average, the intrinsic period is 24.15 h with a standard deviation of 0.2 h. Longer intrinsic periods are associated with later timing of the sleep-wake cycle, body temperature cycle, melatonin cycle and an evening-type diurnal preference and longer time in bed during weekends (Wright et al. 2001; Duffy et al. 2011; Lazar et al. 2013). Conversely, shorter periods are associated with earlier sleep timing, morning preference, etc.

Circadian rhythmicity is present not only in the suprachiasmatic nucleus (SCN) which hosts the master pacemaker driving the circadian sleep-wake propensity rhythm but also in the periphery. Intrinsic periods assessed through circadian reporter systems in cell cultures of fibroblasts have a weak association with SCN periods (Hasan et al. 2012).

3.1.4 Light Input

Under normal conditions, the circadian process is synchronised to the 24 h cycle, primarily by light. Exposure to light in the evening delays (slows down) the clock, i.e. clock-timed events like the rise of melatonin will occur later. Light in the morning will advance (speed up) the clock, and events such as the peak of the cortisol rhythm will occur earlier (Duffy and Wright 2005). The human circadian timing system is very sensitive to light, and ordinary room light exerts approximately 50% of the maximum effect of light (Zeitzer et al. 2000; Santhi et al. 2012). The discovery of melanopsin-expressing light-sensitive ganglion cells has drawn attention to the spectral composition of light as a determinant of the circadian effectiveness of light (Lucas et al. 2014). Blue light has been shown to be very effective, and the increasing blue light content of our LED home lighting and the blue light content of light emitted by the screens of our gadgets are now considered to be a determinant of sleep timing (Czeisler 2013; Gringras et al. 2015).

The timing of the circadian clock is determined by the overall 24-h pattern of light exposure, and sufficient exposure to daylight is now jeopardised because we spend most of our day indoors. Low levels of exposure to daylight, especially in the winter, will increase the delaying effects of artificial evening light.

3.1.5 Circadian Regulation of Waking Function

The circadian clock also modulates waking function. Thus many aspects of performance are impaired when performance is assessed in the early morning hours, at around the core temperature nadir, whereas in the evening hours, performance is at its maximum, even when homeostatic sleep pressure at these circadian phases is identical (Dijk et al. 1992b; Wyatt et al. 1999). Importantly, the effects of circadian phase on waking function interact with homeostatic sleep pressure. Deterioration of performance in the early morning is severe when it is combined with high homeostatic sleep pressure, either caused by acute sleep loss (Dijk et al. 1992b; Wyatt et al. 1999, 2004) or chronic sleep loss (Cohen et al. 2010b). These experiments have also shown that during the wake maintenance zone, the brain appears to be resilient against the effects of sleep loss. Brain correlates of the circadian and the wake duration-dependent deterioration of vigilant attention have been documented (Muto et al. 2016).

Although circadian misalignment affects many aspects of performance, effects are strongest for sleepiness, vigilant attention and effort (Santhi et al. 2016) although others have emphasised that the circadian system may significantly modulate aspects of higher cognitive function as well (Burke et al. 2015b).

3.1.6 Circadian Aspects of Sleep Disorders

The role of the circadian timing system in sleep disorders is well recognised. Circadian rhythm sleep-wake disorders are a separate diagnostic category in both the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (DSM-5) and the *International Classification of Sleep Disorders, Third Edition*. These disorders include delayed, advanced, irregular, non-24-h, shift work and jet lag disorders. Causes of all of these disorders may be intrinsic, environmental or

behavioural and social, e.g. work schedules. The contribution of the circadian timing system to sleep disturbances in shift work and jet lag is obvious and in essence is caused by inertia of the circadian pacemaker. The circadian clock takes longer to shift than the acute displacement of the sleep-wake schedule imposed by either shift work or rapid travel across time zones. As a consequence people attempt to sleep during the biological day and to be awake and work during the biological night.

3.1.7 External Factors: Modern Light Exposure Patterns as an Environmental and Lifestyle Factor Contributing to Sleep-Wake Disturbances

Reduced or inappropriate light exposure or changes in light input pathways to the clock are obvious putative mechanisms of circadian sleep-wake disorders. Indeed, the contribution of the circadian timing system to the non-24-h sleep-wake disorders often observed in the blind is now well documented with 63% of blind individuals displaying circadian rhythms which are not appropriately synchronised to the 24-h day (Flynn-Evans et al. 2014). Inadequate or inappropriate light exposure, such as excessive evening light, may contribute to delayed sleep and evidence for this is now emerging. Indeed exposure to ordinary room light in the evening has been shown to suppress melatonin and sleepiness, delay bedtime and prolong sleep latency (Gooley et al. 2011; Santhi et al. 2012). Abnormal light exposure patterns are observed in delayed sleep-wake disorders (Van der Maren et al. 2018; Wilson et al. 2018). Furthermore individual differences in the circadian sensitivity to light are emerging (Santhi et al. 2012) and increased sensitivity to light may be a characteristic of delayed sleep-wake phase syndrome (DSPS) (Watson et al. 2018).

A major roadblock in the full exploration of the contribution of light exposure to circadian sleep-wake disturbances is the lack of adequate and acceptable longitudinal sensing systems for the intensity and spectral composition of retinal light exposure. Nevertheless, some studies combining light exposure measurements and mathematical modelling thereof have already shown that light exposure is a good predictor of circadian phase (Woelders et al. 2017).

3.1.8 Intrinsic Factors: Genetic Variants and Differences in Circadian Period Contributing to Sleep-Wake Disturbances

Genetic Variants

Evidence for the involvement of circadian rhythmicity in sleep timing abnormalities may be derived from the various polymorphisms and mutations in genes which are part of the molecular machinery generating circadian rhythms. Variations in the period genes have been associated with familial advanced sleep phase syndrome and DSPS. Some of these mutations and polymorphisms have been shown to shorten or lengthen circadian period when introduced in animal or cell culture models (Shi et al. 2017). Maybe somewhat surprising, other clock gene variants do not seem to affect circadian period even though they associate with variations in sleep timing (see below). Many clock gene variants associate with variation in diurnal preference (Kalmbach et al. 2017; Jones et al. 2016; Lane et al. 2016), and extremes of diurnal

preferences may translate to circadian sleep timing disorders. The effects of circadian variants to aspects of sleep other than its timing remain poorly characterised. It is of interest that several studies have now shown that circadian variants not only affect sleep timing but also sleep structure. For example, variants of the period genes are associated with differences in N3, i.e. slow-wave sleep (Hasan et al. 2014; Archer et al. 2018; Chang et al. 2016; Dijk and Archer 2010).

Circadian Period

Direct evidence for a contribution of *intrinsic* circadian factors such as abnormally long or short intrinsic circadian periods to circadian sleep-wake disorders is growing. Assessments of intrinsic circadian period in sleep disorders are rare, but it has been reported that circadian period is longer in DSPS and non-24-h sleep-wake disorder (Micic et al. 2016). It is often implied that developmental and age-related changes in circadian period contribute to late sleep timing in adolescence and early sleep timing in old age, but there is no solid evidence that the intrinsic circadian periods change with age (Dijk et al. 2000; Crowley et al. 2018; Skeldon et al. 2016). However, differences in circadian period as small as a few minutes may lead to substantial difference in entrained phase (Wright et al. 2005), and few studies with the statistical power to detect these small differences have been conducted. One example of small differences in circadian period concerns sex differences with circadian period in women being approximately 6 min shorter than in men (Duffy et al. 2011). This sex difference in intrinsic period may explain the earlier preferred timing of bedtime relative to clock time in women (Diurnal preference) and the earlier timing of the melatonin and core body temperature rhythm relative to sleep in women (Cain et al. 2010).

Circadian Phase

Indirect evidence for a contribution of the circadian timing system to variation in sleep timing stems from the observed associations between the timing of the melatonin rhythm and the timing of sleep in people without sleep timing complaints and in DSPS (Archer et al. 2008). Although this association implies that later timing of the melatonin rhythm may lead to later sleep timing, it cannot be excluded that the later sleep timing and associated light exposure lead to the delay of the melatonin rhythm. Recent data show that the intrinsic timing of the melatonin rhythm, as assessed by the dim light melatonin onset, is not delayed in almost half of DSPD patients (Murray et al. 2017).

The circadian timing system may also contribute to non-circadian sleep disorders. Insomnia with the prominent and persistent symptoms of difficulties initiating and maintaining sleep is in general not considered a circadian sleep disorder. Yet excessive strength of the evening wake maintenance zone or reduced circadian promotion of sleep during the night may contribute to these symptoms. Recently it was reported that in 10–22% of insomnia patients, the timing of sleep relative to the melatonin rhythm is abnormal such that sleep is attempted to be initiated at an early ‘melatonin time’ (Flynn-Evans et al. 2017).

3.1.9 Circadian Approaches to Treatment of Sleep-Wake Disturbances

In cases in which the cause of sleep-wake disturbances is clearly circadian, such as in jet lag disorder or non-24-h sleep-wake disorders in the blind, treatments are typically directed at the circadian timing system. Currently, abnormal timing of the circadian sleep-wake propensity rhythm may be corrected by either timed light exposure and light avoidance or administration of melatonin.

Light Treatment

Effects of time light exposure have been most extensively investigated in DSPS. Exposure to bright light in the morning, sometimes combined with instructions to avoid light in the evening, which theoretically should advance the sleep propensity rhythm, has been shown to be effective in DSPS (Richardson et al. 2018; Auger et al. 2015a, b). Optimisation of light treatment will require further quantitative understanding of the effects of light exposure patterns on the phase and amplitude of the circadian sleep-wake propensity rhythm. Current models for the effects of light do neither account for the spectral composition of light or the effects of prior light history on the sensitivity to light. Successful implementation of light therapy may also require assessment of circadian phase because effects of light depend on circadian phase, and, in some situation, like jet lag, circadian phase is not easily predicted. Light treatment also requires light delivery or light input manipulation systems that are easy to use and acceptable to users. Recent light treatment approaches have focused on reducing the blue light content of light to which the retina is exposed (Zerbini et al. 2018), and further development of methods by which the 24-h light exposure pattern, at the work place and at home, can be manipulated is a promising avenue for the treatment of some circadian rhythm sleep-wake disorders.

Development of novel therapeutics focusing on the light input pathway to the circadian system could target the anatomical and molecular pathways involved in entrainment.

Melatonin Therapy

Melatonin therapy has been successfully implemented in circadian rhythm sleep-wake disorders (Auger et al. 2015a, b) although the need for more research is recognised (Auger et al. 2015a, b). Melatonin is in the first instance an output of the circadian timing system. Its synthesis in the pineal is driven by sympathetic input, the rhythmicity of which is driven by the SCN such that in both nocturnal and diurnal species plasma melatonin concentrations are high during the biologically night and low during the biological day. This rhythm persists in constant darkness, but light at night suppresses melatonin. Melatonin administration leads to an increase in sleep propensity and shift of the sleep propensity rhythm, when it is administered when endogenous levels are low. This has been demonstrated in forced desynchrony protocols (Wyatt et al. 2006) and daytime sleep experiments (Rajaratnam et al. 2004) in participants without melatonin deficiency or sleep timing complaints. This effect is commonly referred to as the direct sleep-facilitating effect of melatonin. Melatonin can also induce a change in the timing of the endogenous

phase of rhythms driven by the SCN. Some of these phase markers include the cortisol, core body temperature and endogenous melatonin rhythm (Rajaratnam et al. 2003). The direction of the shift depends on the endogenous circadian phase at which melatonin is administered (Lewy et al. 1998). Circadian rhythms will be advanced when melatonin is administered several hours before the endogenous rise of melatonin, although the optimal timing has not been explored in great detail. Evidence for melatonin's effectiveness in delaying endogenous circadian rhythms is more limited. The phase-advancing effects of melatonin have been successfully exploited in several disorders such as DSPS (Sletten et al. 2018). Melatonin is also used to treat sleep disturbances in developmental disorders such as autism (Gringras et al. 2017). In all of these conditions, the largest effects are observed for latency to sleep onset. The effectiveness of melatonin in these conditions may be related to a combination of its direct sleep-facilitating and phase-shifting effects. Compared to allosteric modulators of the $GABA_A$ receptor, melatonin has very minor effects on slow-wave activity and EEG power spectra (Arbon et al. 2015).

Daily administration of melatonin can entrain non-synchronised rhythms, such as observed in the blind, to the 24 h day (Lockley et al. 2000). Melatonin treatment has also been advocated for the treatment of insomnia and in particular insomnia in older people (Lemoine and Zisapel 2012). The rationale for this approach is that endogenous melatonin levels may be too low in this particular patient group. The evidence for melatonin deficiency in insomnia is however rather limited although timing of melatonin may be abnormal in insomnia (see above).

Efforts have been made to optimise the dose of melatonin as well as the kinetics by, for example, slow-release preparations for oral or dermal delivery (Lemoine and Zisapel 2012; Aeschbach et al. 2009).

Effects of melatonin are mediated by M1 and M2 melatonin receptors which are abundant in the SCN but also in other brain areas. Melatonin receptor agonists have been developed for the treatment of insomnia (ramelteon) (Liu and Wang 2012) and non-24 h sleep-wake disorders (tasimelteon) (Lockley et al. 2015). Whether these agonists which have been approved are more effective than melatonin itself has not been investigated.

Further development of the melatonin-based treatment of sleep-wake disorders may benefit from new methods to assess endogenous circadian phase, which now requires collection of multiple samples under carefully controlled conditions. Several promising approaches based on analyses of the blood transcriptome have recently been developed (Laing et al. 2017; Wittenbrink et al. 2018).

3.1.10 Other Circadian Outputs and Novel Therapeutics for Sleep-Wake Disturbances

Further exploitation of the profound circadian influence on the sleep-wake propensity rhythm will require a better understanding of the neuroanatomical and molecular pathways by which the circadian system interacts with sleep executive systems generating sleep and wakefulness. An interesting example of interactions between sleep and circadian mechanisms relates to adenosinergic mechanisms. Caffeine, well known to affect sleep, also appears to affect the circadian timing system such that it

delays phase, lengthens circadian period (Burke et al. 2015a) and affects light sensitivity of the circadian timing system (van Diepen et al. 2014). Another example relates to orexin. The orexin system is under circadian control but is also affected by sleep deprivation (Deboer et al. 2004) and is implicated in sleep-wake regulation. Orexin antagonists have been successfully developed for the treatment of insomnia (Herring et al. 2018).

4 Novel Therapeutics and Sleep-Wake Regulation: Outlook

4.1 Continued Need and Opportunities

Given the high prevalence of sleep-wake disturbances and the imperfection of current treatments of these disturbances, efforts to develop novel therapeutics will continue and are warranted. These efforts will be informed by a better understanding of the multifaceted nature of sleep-wake regulation and disturbances thereof. The need for novel therapeutics is likely to increase because of the continued high prevalence of shift work and associated sleep-wake disorders, demographic changes with more people living longer and the increase in the number of people living with dementia and associated disruption of sleep-wake cycles. The multidimensional phenomenology of sleep and wakefulness implies that the treatment of disorders of sleep and wakefulness, and the development of new sleep-wake therapeutics, continues to require the input from a variety of scientific disciplines ranging from molecular biology to clinical and cognitive psychology.

4.2 A More Comprehensive Phenomenology of Sleep and Sleep Disturbances

Understanding the subjective complaints about sleep quality or complaints of waking function attributed to poor sleep will remain a first point of entry for sleep medicine and the development of novel therapeutics. A better understanding of how sleep complaints relate to sleep and circadian physiology will require a more comprehensive phenomenology of healthy sleep and disturbed sleep. This extended phenomenology may open up new avenues for the development of novel therapeutics. Examples of this are already available. The complaint of not being able to fall asleep is common to insomnia and circadian rhythm sleep-wake disorders (delayed). The observation that insomnia patients experience difficulties sleeping irrespective of time of day, whereas in delayed or advanced sleep phase syndromes, there are times of day at which sleep-ability is normal, distinguishes the two disorders. Although this may now appear to be trivial, it is only the recognition of the importance of the circadian timing system in sleep-wake regulation that allowed for this distinction and development of specific treatments. Within this context it may be relevant to point out that in the treatment of circadian rhythm sleep-wake disorders, sleep remains the relevant outcome measure, but the target for the

pharmacological and non-pharmacological therapeutics is far removed from classical sleep neurotransmitter systems. By contrast, in insomnia, neurotransmitter systems directly involved in sleep-wake regulation and associated processes such as arousal remain the target. Early on in these programmes, sleep physiology measures such as latency to sleep onset and wake after sleep onset are primary outcome measures, even though in at least a sizable fraction of insomnia patients, physiologically determined total sleep time is not severely abnormal (Vgontzas et al. 2013), and the complaint appears to be more related to the persistent mentation in the presence of physiological sleep. A better understanding of the sleep experience or the absence thereof and new EEG measures that better capture the sleep experience are needed. Efforts in this direction are already underway and can be guided by a simple question: what is the relation between sleep physiology and the sleep experience?

No doubt the discovery of other novel therapeutics will be parsimonious and not informed by any biased approach. Examples of current parsimoniously discovered therapeutics include the wake-promoting compound modafinil. It is only now that the important role of the dopaminergic system in wake (and sleep) regulation is recognised, providing a rational basis for modafinil's effects.

4.3 Short-Term vs Long-Term Treatment and Consequences of Short and Long Sleep as a Risk Factor for Ill Health

It is understandable that approaches to the development of novel therapeutics are primarily targeting acute and ongoing complaints about sleep. Successful treatments of these complaints may not only resolve the acute complaints but also have long-term health implications. Epidemiological studies indeed show that, for example, the complaint of insomnia is associated with adverse physical and mental health outcomes such as depression in particular (Sivertsen et al. 2014). Population-based epidemiological studies also show that not only short sleep duration but also long self-reported sleep duration predict negative health outcomes (Jike et al. 2018). Whereas the sleep research field, in which the 'sleep as a recovery process' is the prevailing view, can easily envision pathways from short sleep to ill health, although even these remain poorly defined, the pathways from long sleep to ill health remain unclear. In fact, it currently remains uncertain whether long sleep is an early consequence of covert ill health or a cause. Whereas approaches to lengthen short sleep can include advocating lifestyle changes, no currently accepted policy for long sleep including excessive napping, which, for example, is highly prevalent in dementia, is available.

A major limitation of the currently available epidemiology is that the exposure assessment (sleep duration) is to the larger extent based on self-report. Limitations of self-reported sleep duration are considerable, in particular, because the reporting error appears to be correlated with health outcomes (Miller et al. 2015).

4.4 Symptom Based vs Disorder (Nosology) Based

Traditionally the development of therapeutics was nosology or disorder based, and the underlying assumption is that specific disorders have a specific aetiology which can be targeted. This approach may work well in cases in which the disease or disorder and its aetiology are clearly defined. However, many disorders may be rather heterogenous, symptoms may be common to several disorders, and disorders may be closely related. For example, insomnia patients may display several anxiety-related symptoms. In fact GWAS studies of insomnia show that the genetic associations with insomnia and anxiety overlap to a considerable extent (Hammerschlag et al. 2017). Furthermore, insomnia disorder may consist of various subtypes with different aetiologies (Blanken et al. 2019). Thus insomnia with objectively reduced TST and insomnia without objectively reduced TST may have a different aetiology, different adverse consequences, etc. One approach to the development of therapeutics may be to target symptoms, e.g. anxiety, rather than a disorder, e.g. insomnia, and then target those insomnia patients who specifically suffer from anxiety symptoms. Precise and comprehensive phenomenology is obviously key to such an approach.

4.5 Sleep as a Whole-Body Phenomenon

Quantification of sleep often focuses on the brain. The EEG is a major source of information about the sleep process, but sleep is obviously much more than changed in neuronal firing patterns. Changes affecting both the brain and the body include temperature, cardiovascular, endocrine and many other variables. How peripheral physiology contributes to the sleep process and its recovery value remains somewhat under-investigated. For example, skin, body and brain temperatures are affected by sleep, and as early as 1967, Monroe reported body temperature during sleep to be higher in poor sleepers compared to good sleepers (Monroe 1967). Indeed, manipulating peripheral and central thermoregulatory processes have been shown to improve sleep in, for example, ageing (Te Lindert and Van Someren 2018). There is extensive epidemiology pointing to associations between sleep duration and obesity, and effects of sleep manipulation on appetite- and glucose-regulating hormones have been reported. Yet, relatively little is known about the effects of obesity or food intake on sleep. Future approaches to the development of novel therapeutics may exploit some of these peripheral phenomena to improve sleep for the brain and the body.

4.6 Interactions: Circadian Rhythmicity and Sleep-Wake History

The separate contribution of circadian rhythmicity and sleep-wake history (sleep homeostasis) to sleep propensity and waking functions is well recognised. However, the most important determinant of both sleep propensity and waking function is the

combined action of circadian rhythmicity and sleep homeostasis. Thus, waking performance at 6 a.m. close to the core body temperature nadir is not much impaired at all if the participant has had sufficient sleep prior to this time point. However, performance at this circadian phase is severely impaired when the participant is carrying an acute or chronic sleep debt. The implication for the development of novel therapeutics is that identification of the locus and mechanism of the interaction between circadian and homeostatic signals may offer new insights and targets. Some approaches are now emerging in preclinical research programmes in which local circadian clocks in arousal-related structures are manipulated and their effects on sleep-wake cycles are documented (Yu et al. 2014).

4.7 Monogenetic-Polygenetic, Monopharmacy vs Polypharmacy

Developing treatments of disorders of sleep and wakefulness necessarily implies the targeting of relevant circuits, receptor systems, etc. The ever more detailed description of the multitude of, for example, receptor subtypes offers opportunities for developing compounds with greater specificity for specific receptor subtypes. Likewise the ever more detailed description of the neuronal cell types regulating and contributing to specific aspects of sleep, e.g. sleep initiation vs REM sleep regulation, or EEG synchronisation. Such a specific targeted approach will be fruitful of specific receptor subtypes contributing to specific sleep disorders. This may be true in some cases, e.g. narcolepsy, even though even in this disorder, multiple neuromodulators have been implicated, but not in other cases. For example, it is unlikely that insomnia is caused by a deficiency in one particular $GABA_A$ receptor subtype. These states such as insomnia are likely to be associated with changes in constellations of biochemical signalling pathways. The implication is that a less specific treatment approach or drug development programme may be more effective.

4.8 The Sleep Environment

One defining aspect of sleep is partial disengagement from the environment, but the sleeper is not completely disconnected from its environment. Environmental influences on sleep and sleep disturbances may deserve more attention. Environmental variables that can be considered to be relevant include temperature, humidity, noise and light. With modern humans spending most of their time indoors, a closer look at the indoor environmental environment may be warranted. Sleep-wake cycles and their circadian regulation evolved in part as adaptation to environmental cycles related to the earth rotation around its axis. The light-dark cycle is one prominent example, but the natural darkness of the night is often hard to find in urban environments and the bedroom. Profound daily cycles in environmental temperature in the natural environment are to some extent mirrored in the circadian rhythm of body temperature which reaches a maximum during the later part of the day and a minimum close to dawn. Yet, temperature cycles in our home environments

including bedrooms are very different, and little is known about the potential impact of environmental temperature cycles on sleep-wake cycles and their quality.

4.9 Large-Scale Sleep Studies in the Home Environment

New developments in electronics, electrode technologies and signal analysis have enabled new recording and analyses approaches which have revealed novel aspects of sleep phenomenology. New recording technologies and new scoring approaches based on machine learning techniques using reduced montages will soon enable large-scale sleep studies in the home environment (Peake et al. 2018; Mikkelsen et al. 2019).

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References

Achermann P, Dijk DJ, Brunner DP, Borbély AA (1993) A model of human sleep homeostasis based on EEG slow-wave activity: quantitative comparison of data and simulations. Brain Res Bull 31(1–2):97–113

Achermann P, Rusterholz T, Durr R, Konig T, Tarokh L (2016) Global field synchronization reveals rapid eye movement sleep as most synchronized brain state in the human EEG. R Soc Open Sci 3(10):160201

Adam M, Rétey JV, Khatami R, Landolt HP (2006) Age-related changes in the time course of vigilant attention during 40 hours without sleep in men. Sleep 29(1):55–57

Aeschbach D, Cajochen C, Landolt H, Borbély AA (1996) Homeostatic sleep regulation in habitual short sleepers and long sleepers. Am J Phys 270(1 Pt 2):R41–R53

Aeschbach D, Postolache TT, Sher L, Matthews JR, Jackson MA, Wehr TA (2001) Evidence from the waking electroencephalogram that short sleepers live under higher homeostatic sleep pressure than long sleepers. Neuroscience 102(3):493–502

Aeschbach D, Sher L, Postolache TT, Matthews JR, Jackson MA, Wehr TA (2003) A longer biological night in long sleepers than in short sleepers. J Clin Endocrinol Metab 88(1):26–30

Aeschbach D, Lockyer BJ, Dijk DJ, Lockley SW, Nuwayser ES, Nichols LD, Czeisler CA (2009) Use of transdermal melatonin delivery to improve sleep maintenance during daytime. Clin Pharmacol Ther 86(4):378–382

Akerstedt T, Axelsson J, Lekander M, Orsini N, Kecklund G (2013) The daily variation in sleepiness and its relation to the preceding sleep episode – a prospective study across 42 days of normal living. J Sleep Res 22(3):258–265

Akerstedt T, Schwarz J, Gruber G, Lindberg E, Theorell-Haglow J (2016) The relation between polysomnography and subjective sleep and its dependence on age – poor sleep may become good sleep. J Sleep Res 25(5):565–570

Akerstedt T, Hallvig D, Kecklund G (2017) Normative data on the diurnal pattern of the Karolinska sleepiness scale ratings and its relation to age, sex, work, stress, sleep quality and sickness absence/illness in a large sample of daytime workers. J Sleep Res 26(5):559–566

Akerstedt T, Discacciati A, Miley-Akerstedt A, Westerlund H (2018) Aging and the change in fatigue and sleep – a longitudinal study across 8 years in three age groups. Front Psychol 9:234

Akerstedt T, Schwarz J, Gruber G, Theorell-Haglow J, Lindberg E (2019) Short sleep-poor sleep? A polysomnographic study in a large population-based sample of women. J Sleep Res 4:e12812

Arbon EL, Knurowska M, Dijk DJ (2015) Randomised clinical trial of the effects of prolonged-release melatonin, temazepam and zolpidem on slow-wave activity during sleep in healthy people. J Psychopharmacol 29(7):764–776

Archer SN, Viola AU, Kyriakopoulou V, von Schantz M, Dijk DJ (2008) Inter-individual differences in habitual sleep timing and entrained phase of endogenous circadian rhythms of BMAL1, PER2 and PER3 mRNA in human leukocytes. Sleep 31(5):608–617

Archer SN, Schmidt C, Vandewalle G, Dijk DJ (2018) Phenotyping of PER3 variants reveals widespread effects on circadian preference, sleep regulation, and health. Sleep Med Rev 40:109–126

Auger RR, Burgess HJ, Emens JS, Deriy LV, Sharkey KM (2015a) Do evidence-based treatments for circadian rhythm sleep-wake disorders make the GRADE? Updated guidelines point to need for more clinical research. J Clin Sleep Med 11(10):1079–1080

Auger RR, Burgess HJ, Emens JS, Deriy LV, Thomas SM, Sharkey KM (2015b) Clinical practice guideline for the treatment of intrinsic circadian rhythm sleep-wake disorders: advanced sleep-wake phase disorder (ASWPD), delayed sleep-wake phase disorder (DSWPD), non-24-hour sleep-wake rhythm disorder (N24SWD), and irregular sleep-wake rhythm disorder (ISWRD). An update for 2015: an American Academy of Sleep Medicine Clinical Practice Guideline. J Clin Sleep Med 11(10):1199–1236

Bachmann V, Klaus F, Bodenmann S, Schafer N, Brugger P, Huber S, Berger W, Landolt HP (2012) Functional ADA polymorphism increases sleep depth and reduces vigilant attention in humans. Cereb Cortex 22(4):962–970

Baglioni C, Regen W, Teghen A, Spiegelhalder K, Feige B, Nissen C, Riemann D (2014) Sleep changes in the disorder of insomnia: a meta-analysis of polysomnographic studies. Sleep Med Rev 18(3):195–213

Balkin TJ, Badia P (1988) Relationship between sleep inertia and sleepiness: cumulative effects of four nights of sleep disruption/restriction on performance following abrupt nocturnal awakenings. Biol Psychol 27(3):245–258

Balkin TJ, Braun AR, Wesensten NJ, Jeffries K, Varga M, Baldwin P, Belenky G, Herscovitch P (2002) The process of awakening: a PET study of regional brain activity patterns mediating the re-establishment of alertness and consciousness. Brain 125(Pt 10):2308–2319

Balkin TJ, Bliese PD, Belenky G, Sing H, Thorne DR, Thomas M, Redmond DP, Russo M, Wesensten NJ (2004) Comparative utility of instruments for monitoring sleepiness-related performance decrements in the operational environment. J Sleep Res 13(3):219–227

Baumann CR (2018) Clinical sleep-wake disorders I: focus on hypersomnias and movement disorders during sleep. Handb Exp Pharmacol. https://doi.org/10.1007/164_2018_126

Beersma DG, Dijk DJ, Blok CG, Everhardus I (1990) REM sleep deprivation during 5 hours leads to an immediate REM sleep rebound and to suppression of non-REM sleep intensity. Electroencephalogr Clin Neurophysiol 76(2):114–122

Bei B, Allen NB, Nicholas CL, Dudgeon P, Murray G, Trinder J (2014) Actigraphy-assessed sleep during school and vacation periods: a naturalistic study of restricted and extended sleep opportunities in adolescents. J Sleep Res 23(1):107–117

Bei B, Manber R, Allen NB, Trinder J, Wiley JF (2017) Too long, too short, or too variable? Sleep intraindividual variability and its associations with perceived sleep quality and mood in adolescents during naturalistically unconstrained sleep. Sleep 40(2):zsw067

Berry RB, Brooks R, Gamaldo C, Harding SM, Lloyd RM, Quan SF, Troester MT, Vaughn BV (2017) AASM scoring manual updates for 2017 (version 2.4). J Clin Sleep Med 13(5):665–666

Bettica P, Squassante L, Groeger JA, Gennery B, Winsky-Sommerer R, Dijk DJ (2012) Differential effects of a dual orexin receptor antagonist (SB-649868) and zolpidem on sleep initiation and consolidation, SWS, REM sleep, and EEG power spectra in a model of situational insomnia. Neuropsychopharmacology 37(5):1224–1233

Blanken TF, Benjamins JS, Borsboom D, Vermunt JK, Paquola C, Ramautar J, Dekker K, Stoffers D, Wassing R, Wei Y, Van Someren EJW (2019) Insomnia disorder subtypes derived from life history and traits of affect and personality. Lancet Psychiatry 6(2):151–163

Bodenmann S, Hohoff C, Freitag C, Deckert J, Rétey JV, Bachmann V, Landolt HP (2012) Polymorphisms of ADORA2A modulate psychomotor vigilance and the effects of caffeine on neurobehavioural performance and sleep EEG after sleep deprivation. Br J Pharmacol 165 (6):1904–1913

Borbély AA (1982) A two process model of sleep regulation. Hum Neurobiol 1(3):195–204

Borbély AA, Baumann F, Brandeis D, Strauch I, Lehmann D (1981) Sleep deprivation: effect on sleep stages and EEG power density in man. Electroencephalogr Clin Neurophysiol 51 (5):483–495

Borbély AA, Daan S, Wirz-Justice A, Deboer T (2016) The two-process model of sleep regulation: a reappraisal. J Sleep Res 25(2):131–143

Boyle J, Groeger JA, Paska W, Cooper JA, Rockett C, Jones S, Gandhi P, Scott J, Atzori G, Dijk DJ (2012a) A method to assess the dissipation of the residual effects of hypnotics: eszopiclone versus zopiclone. J Clin Psychopharmacol 32(5):704–709

Boyle J, Stanley N, James LM, Wright N, Johnsen S, Arbon EL, Dijk DJ (2012b) Acute sleep deprivation: the effects of the AMPAKINE compound CX717 on human cognitive performance, alertness and recovery sleep. J Psychopharmacol 26(8):1047–1057

Brunner DP, Dijk DJ, Borbély AA (1990a) A quantitative analysis of phasic and tonic submental EMG activity in human sleep. Physiol Behav 48(5):741–748

Brunner DP, Dijk DJ, Tobler I, Borbély AA (1990b) Effect of partial sleep deprivation on sleep stages and EEG power spectra: evidence for non-REM and REM sleep homeostasis. Electroencephalogr Clin Neurophysiol 75(6):492–499

Brunner DP, Dijk DJ, Munch M, Borbély AA (1991) Effect of zolpidem on sleep and sleep EEG spectra in healthy young men. Psychopharmacology 104(1):1–5

Brunner DP, Dijk DJ, Borbély AA (1993) Repeated partial sleep deprivation progressively changes in EEG during sleep and wakefulness. Sleep 16(2):100–113

Burke TM, Markwald RR, McHill AW, Chinoy ED, Snider JA, Bessman SC, Jung CM, O'Neill JS, Wright KP Jr (2015a) Effects of caffeine on the human circadian clock in vivo and in vitro. Sci Transl Med 7(305):305ra146

Burke TM, Scheer F, Ronda JM, Czeisler CA, Wright KP Jr (2015b) Sleep inertia, sleep homeostatic and circadian influences on higher-order cognitive functions. J Sleep Res 24(4):364–371

Cain SW, Dennison CF, Zeitzer JM, Guzik AM, Khalsa SB, Santhi N, Schoen MW, Czeisler CA, Duffy JF (2010) Sex differences in phase angle of entrainment and melatonin amplitude in humans. J Biol Rhythm 25(4):288–296

Cajochen C, Brunner DP, Krauchi K, Graw P, Wirz-Justice A (1995) Power density in theta/alpha frequencies of the waking EEG progressively increases during sustained wakefulness. Sleep 18 (10):890–894

Carrier J, Semba K, Deurveilher S, Drogos L, Cyr-Cronier J, Lord C, Sekerovick Z (2017) Sex differences in age-related changes in the sleep-wake cycle. Front Neuroendocrinol 47:66–85

Carskadon MA, Dement WC (1987) Daytime sleepiness: quantification of a behavioral state. Neurosci Biobehav Rev 11(3):307–317

Casali AG, Gosseries O, Rosanova M, Boly M, Sarasso S, Casali KR, Casarotto S, Bruno MA, Laureys S, Tononi G, Massimini M (2013) A theoretically based index of consciousness independent of sensory processing and behavior. Sci Transl Med 5(198):198ra105

Chang AM, Bjonnes AC, Aeschbach D, Buxton OM, Gooley JJ, Anderson C, Van Reen E, Cain SW, Czeisler CA, Duffy JF, Lockley SW, Shea SA, Scheer FA, Saxena R (2016) Circadian gene variants influence sleep and the sleep electroencephalogram in humans. Chronobiol Int 33 (5):561–573

Clark I, Landolt HP (2017) Coffee, caffeine, and sleep: a systematic review of epidemiological studies and randomized controlled trials. Sleep Med Rev 31:70–78

Cohen DA, Wang W, Klerman EB, Rajaratnam SM (2010a) Ramelteon prior to a short evening nap impairs neurobehavioral performance for up to 12 hours after awakening. J Clin Sleep Med 6 (6):565–571

Cohen DA, Wang W, Wyatt JK, Kronauer RE, Dijk DJ, Czeisler CA, Klerman EB (2010b) Uncovering residual effects of chronic sleep loss on human performance. Sci Transl Med 2 (14):14ra13

Cross N, Terpening Z, Rogers NL, Duffy SL, Hickie IB, Lewis SJ, Naismith SL (2015) Napping in older people 'at risk' of dementia: relationships with depression, cognition, medical burden and sleep quality. J Sleep Res 24(5):494–502

Crowley SJ, Wolfson AR, Tarokh L, Carskadon MA (2018) An update on adolescent sleep: new evidence informing the perfect storm model. J Adolesc 67:55–65

Czeisler CA (2013) Perspective: casting light on sleep deficiency. Nature 497(7450):S13

Czeisler CA, Gooley JJ (2007) Sleep and circadian rhythms in humans. Cold Spring Harb Symp Quant Biol 72:579–597

Daan S, Beersma DG, Borbély AA (1984) Timing of human sleep: recovery process gated by a circadian pacemaker. Am J Phys 246(2 Pt 2):R161–R183

Deboer T, Overeem S, Visser NA, Duindam H, Frolich M, Lammers GJ, Meijer JH (2004) Convergence of circadian and sleep regulatory mechanisms on hypocretin-1. Neuroscience 129(3):727–732

Della Monica C, Johnsen S, Atzori G, Groeger JA, Dijk DJ (2018) Rapid eye movement sleep, sleep continuity and slow wave sleep as predictors of cognition, mood, and subjective sleep quality in healthy men and women, aged 20-84 years. Front Psych 9:255

Dijk DJ, Archer SN (2010) PERIOD3, circadian phenotypes, and sleep homeostasis. Sleep Med Rev 14(3):151–160

Dijk DJ, Beersma DG (1989) Effects of SWS deprivation on subsequent EEG power density and spontaneous sleep duration. Electroencephalogr Clin Neurophysiol 72(4):312–320

Dijk DJ, Czeisler CA (1994) Paradoxical timing of the circadian rhythm of sleep propensity serves to consolidate sleep and wakefulness in humans. Neurosci Lett 166(1):63–68

Dijk DJ, Czeisler CA (1995) Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. J Neurosci 15(5 Pt 1):3526–3538

Dijk DJ, Lockley SW (2002) Integration of human sleep-wake regulation and circadian rhythmicity. J Appl Physiol 92(2):852–862

Dijk DJ, von Schantz M (2005) Timing and consolidation of human sleep, wakefulness, and performance by a symphony of oscillators. J Biol Rhythm 20(4):279–290

Dijk DJ, Beersma DG, Daan S (1987a) EEG power density during nap sleep: reflection of an hourglass measuring the duration of prior wakefulness. J Biol Rhythm 2(3):207–219

Dijk DJ, Beersma DG, Daan S, Bloem GM, Van den Hoofdakker RH (1987b) Quantitative analysis of the effects of slow wave sleep deprivation during the first 3 h of sleep on subsequent EEG power density. Eur Arch Psychiatry Neurol Sci 236(6):323–328

Dijk DJ, Beersma DG, Bloem GM (1989a) Sex differences in the sleep EEG of young adults: visual scoring and spectral analysis. Sleep 12(6):500–507

Dijk DJ, Beersma DG, Daan S, van den Hoofdakker RH (1989b) Effects of seganserin, a 5-HT2 antagonist, and temazepam on human sleep stages and EEG power spectra. Eur J Pharmacol 171 (2–3):207–218

Dijk DJ, Brunner DP, Beersma DG, Borbély AA (1990a) Electroencephalogram power density and slow wave sleep as a function of prior waking and circadian phase. Sleep 13(5):430–440

Dijk DJ, Brunner DP, Borbély AA (1990b) Time course of EEG power density during long sleep in humans. Am J Phys 258(3 Pt 2):R650–R661

Dijk DJ, Brunner DP, Aeschbach D, Tobler I, Borbély AA (1992a) The effects of ethanol on human sleep EEG power spectra differ from those of benzodiazepine receptor agonists. Neuropsychopharmacology 7(3):225–232

Dijk DJ, Duffy JF, Czeisler CA (1992b) Circadian and sleep/wake dependent aspects of subjective alertness and cognitive performance. J Sleep Res 1(2):112–117

Dijk DJ, Hayes B, Czeisler CA (1993) Dynamics of electroencephalographic sleep spindles and slow wave activity in men: effect of sleep deprivation. Brain Res 626(1–2):190–199

Dijk DJ, Shanahan TL, Duffy JF, Ronda JM, Czeisler CA (1997) Variation of electroencephalographic activity during non-rapid eye movement and rapid eye movement sleep with phase of circadian melatonin rhythm in humans. J Physiol 505(Pt 3):851–858

Dijk DJ, Duffy JF, Riel E, Shanahan TL, Czeisler CA (1999) Ageing and the circadian and homeostatic regulation of human sleep during forced desynchrony of rest, melatonin and temperature rhythms. J Physiol 516(Pt 2):611–627

Dijk DJ, Duffy JF, Czeisler CA (2000) Contribution of circadian physiology and sleep homeostasis to age-related changes in human sleep. Chronobiol Int 17(3):285–311

Dijk DJ, Duffy JF, Czeisler CA (2001) Age-related increase in awakenings: impaired consolidation of nonREM sleep at all circadian phases. Sleep 24(5):565–577

Dijk DJ, Groeger JA, Stanley N, Deacon S (2010a) Age-related reduction in daytime sleep propensity and nocturnal slow wave sleep. Sleep 33(2):211–223

Dijk DJ, James LM, Peters S, Walsh JK, Deacon S (2010b) Sex differences and the effect of gaboxadol and zolpidem on EEG power spectra in NREM and REM sleep. J Psychopharmacol 24(11):1613–1618

Dijk DJ, Stanley N, Lundahl J, Groeger JA, Legters A, Trap Huusom AK, Deacon S (2012) Enhanced slow wave sleep and improved sleep maintenance after gaboxadol administration during seven nights of exposure to a traffic noise model of transient insomnia. J Psychopharmacol 26(8):1096–1107

Doherty A, Smith-Byrne K, Ferreira T, Holmes MV, Holmes C, Pulit SL, Lindgren CM (2018) GWAS identifies 14 loci for device-measured physical activity and sleep duration. Nat Commun 9(1):5257

Dornbierer D, Baur DM, Stucky B, Quednow BB, Krämer T, Seifritz E, Bosch OG, Landolt H-P (2019) Neurophysiological signature of gamma-hydroxybutyrate augmented sleep in healthy male volunteers may reflect biomimetic sleep enhancement: a randomized controlled trial. Neuropsychopharmacology. (in press). https://doi.org/10.1038/s41386-019-0382-z

Duffy JF, Wright KP Jr (2005) Entrainment of the human circadian system by light. J Biol Rhythm 20(4):326–338

Duffy JF, Cain SW, Chang AM, Phillips AJ, Munch MY, Gronfier C, Wyatt JK, Dijk DJ, Wright KP Jr, Czeisler CA (2011) Sex difference in the near-24-hour intrinsic period of the human circadian timing system. Proc Natl Acad Sci U S A 108(Suppl 3):15602–15608

Endo T, Roth C, Landolt HP, Werth E, Aeschbach D, Achermann P, Borbély AA (1998) Selective REM sleep deprivation in humans: effects on sleep and sleep EEG. Am J Phys 274(4 Pt 2): R1186–R1194

Ermis U, Krakow K, Voss U (2010) Arousal thresholds during human tonic and phasic REM sleep. J Sleep Res 19(3):400–406

Fink AM, Bronas UG, Calik MW (2018) Autonomic regulation during sleep and wakefulness: a review with implications for defining the pathophysiology of neurological disorders. Clin Auton Res 28(6):509–518

Flynn-Evans EE, Tabandeh H, Skene DJ, Lockley SW (2014) Circadian rhythm disorders and melatonin production in 127 blind women with and without light perception. J Biol Rhythm 29 (3):215–224

Flynn-Evans EE, Shekleton JA, Miller B, Epstein LJ, Kirsch D, Brogna LA, Burke LM, Bremer E, Murray JM, Gehrman P, Rajaratnam SMW, Lockley SW (2017) Circadian phase and phase angle disorders in primary insomnia. Sleep 40(12):zsx163. https://doi.org/10.1093/sleep/zsx163

Frank MG, Heller HC (2019) The function(s) of sleep. Handb Exp Pharmacol. https://doi.org/10.1007/164_2018_140

Gaggioni G, Maquet P, Schmidt C, Dijk DJ, Vandewalle G (2014) Neuroimaging, cognition, light and circadian rhythms. Front Syst Neurosci 8:126

Garcia-Molina G, Tsoneva T, Jasko J, Steele B, Aquino A, Baher K, Pastoor S, Pfundtner S, Ostrowski L, Miller B, Papas N, Riedner B, Tononi G, White DP (2018) Closed-loop system to enhance slow-wave activity. J Neural Eng 15(6):066018

Gooley JJ, Chamberlain K, Smith KA, Khalsa SB, Rajaratnam SM, Van Reen E, Zeitzer JM, Czeisler CA, Lockley SW (2011) Exposure to room light before bedtime suppresses melatonin onset and shortens melatonin duration in humans. J Clin Endocrinol Metab 96(3):E463–E472

Greneche J, Krieger J, Erhardt C, Bonnefond A, Eschenlauer A, Muzet A, Tassi P (2008) EEG spectral power and sleepiness during 24 h of sustained wakefulness in patients with obstructive sleep apnea syndrome. Clin Neurophysiol 119(2):418–428

Gringras P, Middleton B, Skene DJ, Revell VL (2015) Bigger, brighter, bluer-better? Current light-emitting devices – adverse sleep properties and preventative strategies. Front Public Health 3:233

Gringras P, Nir T, Breddy J, Frydman-Marom A, Findling RL (2017) Efficacy and safety of pediatric prolonged-release melatonin for insomnia in children with autism spectrum disorder. J Am Acad Child Adolesc Psychiatry 56(11):948–957.e4

Groeger JA, Stanley N, Deacon S, Dijk DJ (2014) Dissociating effects of global SWS disruption and healthy aging on waking performance and daytime sleepiness. Sleep 37(6):1127–1142

Hammerschlag AR, Stringer S, de Leeuw CA, Sniekers S, Taskesen E, Watanabe K, Blanken TF, Dekker K, Te Lindert BHW, Wassing R, Jonsdottir I, Thorleifsson G, Stefansson H, Gislason T, Berger K, Schormair B, Wellmann J, Winkelmann J, Stefansson K, Oexle K, Van Someren EJW, Posthuma D (2017) Genome-wide association analysis of insomnia complaints identifies risk genes and genetic overlap with psychiatric and metabolic traits. Nat Genet 49 (11):1584–1592

Hanlon EC, Van Cauter E (2011) Quantification of sleep behavior and of its impact on the cross-talk between the brain and peripheral metabolism. Proc Natl Acad Sci U S A 108(Suppl 3):15609–15616

Hasan S, Santhi N, Lazar AS, Slak A, Lo J, von Schantz M, Archer SN, Johnston JD, Dijk DJ (2012) Assessment of circadian rhythms in humans: comparison of real-time fibroblast reporter imaging with plasma melatonin. FASEB J 26(6):2414–2423

Hasan S, van der Veen DR, Winsky-Sommerer R, Hogben A, Laing EE, Koentgen F, Dijk DJ, Archer SN (2014) A human sleep homeostasis phenotype in mice expressing a primate-specific PER3 variable-number tandem-repeat coding-region polymorphism. FASEB J 28 (6):2441–2454

Herring WJ, Roth T, Krystal AD, Michelson D (2018) Orexin receptor antagonists for the treatment of insomnia and potential treatment of other neuropsychiatric indications. J Sleep Res 28(2): e12782

Hladky SB, Barrand MA (2017) Metabolite clearance during wakefulness and sleep. Handb Exp Pharmacol. https://doi.org/10.1007/164_2017_37

Holst SC, Landolt HP (2018) Sleep-wake neurochemistry. Sleep Med Clin 13(2):137–146

Holst SC, Bersagliere A, Bachmann V, Berger W, Achermann P, Landolt HP (2014) Dopaminergic role in regulating neurophysiological markers of sleep homeostasis in humans. J Neurosci 34 (2):566–573

Horne JA, Moore VJ (1985) Sleep EEG effects of exercise with and without additional body cooling. Electroencephalogr Clin Neurophysiol 60(1):33–38

Horner RL, Peever JH (2017) Brain circuitry controlling sleep and wakefulness. Continuum (Minneap Minn) 23(4, Sleep Neurology):955–972

Huber R, Ghilardi MF, Massimini M, Tononi G (2004) Local sleep and learning. Nature 430 (6995):78–81

Huber R, Ghilardi MF, Massimini M, Ferrarelli F, Riedner BA, Peterson MJ, Tononi G (2006) Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. Nat Neurosci 9(9):1169–1176

Iannone R, Palcza J, Renger JJ, Calder N, Cerchio K, Gottesdiener K, Hargreaves R, Dijk DJ, Boyle J, Murphy MG (2010) Acute alertness-promoting effects of a novel histamine subtype-3 receptor inverse agonist in healthy sleep-deprived male volunteers. Clin Pharmacol Ther 88 (6):831–839

Irwin MR, Opp MR (2017) Sleep health: reciprocal regulation of sleep and innate immunity. Neuropsychopharmacology 42(1):129–155

Jacobson LH, Chen S, Mir S, Hoyer D (2017) Orexin OX2 receptor antagonists as sleep aids. Curr Top Behav Neurosci 33:105–136

James LM, Iannone R, Palcza J, Renger JJ, Calder N, Cerchio K, Gottesdiener K, Hargreaves R, Murphy MG, Boyle J, Dijk DJ (2011) Effect of a novel histamine subtype-3 receptor inverse agonist and modafinil on EEG power spectra during sleep deprivation and recovery sleep in male volunteers. Psychopharmacology 215(4):643–653

Jeppesen J, Otto M, Frederiksen Y, Hansen AK, Fedorova TD, Knudsen K, Nahimi A, Brooks DJ, Borghammer P, Sommerauer M (2018) Observations on muscle activity in REM sleep behavior disorder assessed with a semi-automated scoring algorithm. Clin Neurophysiol 129(3):541–547

Jike M, Itani O, Watanabe N, Buysse DJ, Kaneita Y (2018) Long sleep duration and health outcomes: a systematic review, meta-analysis and meta-regression. Sleep Med Rev 39:25–36

Johns MW (1991) A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 14(6):540–545

Jones SE, Tyrrell J, Wood AR, Beaumont RN, Ruth KS, Tuke MA, Yaghootkar H, Hu Y, Teder-Laving M, Hayward C, Roenneberg T, Wilson JF, Del Greco F, Hicks AA, Shin C, Yun CH, Lee SK, Metspalu A, Byrne EM, Gehrman PR, Tiemeier H, Allebrandt KV, Freathy RM, Murray A, Hinds DA, Frayling TM, Weedon MN (2016) Genome-wide association analyses in 128,266 individuals identifies new morningness and sleep duration loci. PLoS Genet 12(8): e1006125

Ju YS, Zangrilli MA, Finn MB, Fagan AM, Holtzman DM (2018) Obstructive sleep apnea treatment, slow wave activity, and amyloid-beta. Ann Neurol 85(2):291–295

Kalmbach DA, Schneider LD, Cheung J, Bertrand SJ, Kariharan T, Pack AI, Gehrman PR (2017) Genetic basis of chronotype in humans: insights from three landmark GWAS. Sleep 40(2): zsw048. https://doi.org/10.1093/sleep/zsw048

Kaplan KA, Hardas PP, Redline S, Zeitzer JM; Sleep Heart Health Study Research Group (2017a) Correlates of sleep quality in midlife and beyond: a machine learning analysis. Sleep Med 34:162–167

Kaplan KA, Hirshman J, Hernandez B, Stefanick ML, Hoffman AR, Redline S, Ancoli-Israel S, Stone K, Friedman L, Zeitzer JM, Osteoporotic Fractures in Men (MrOS), Study of Osteoporotic Fractures SOF Research Groups (2017b) When a gold standard isn't so golden: lack of prediction of subjective sleep quality from sleep polysomnography. Biol Psychol 123:37–46

Kattler H, Dijk DJ, Borbély AA (1994) Effect of unilateral somatosensory stimulation prior to sleep on the sleep EEG in humans. J Sleep Res 3(3):159–164

Khalsa SB, Conroy DA, Duffy JF, Czeisler CA, Dijk DJ (2002) Sleep- and circadian-dependent modulation of REM density. J Sleep Res 11(1):53–59

Klerman EB, Dijk DJ (2005) Interindividual variation in sleep duration and its association with sleep debt in young adults. Sleep 28(10):1253–1259

Klerman EB, Dijk DJ (2008) Age-related reduction in the maximal capacity for sleep – implications for insomnia. Curr Biol 18(15):1118–1123

Klerman EB, Wang W, Duffy JF, Dijk DJ, Czeisler CA, Kronauer RE (2013) Survival analysis indicates that age-related decline in sleep continuity occurs exclusively during NREM sleep. Neurobiol Aging 34(1):309–318

Klinzing JG, Molle M, Weber F, Supp G, Hipp JF, Engel AK, Born J (2016) Spindle activity phase-locked to sleep slow oscillations. Neuroimage 134:607–616

Korkutata M, Saitoh T, Cherasse Y, Ioka S, Duo F, Qin R, Murakoshi N, Fujii S, Zhou X, Sugiyama F, Chen JF, Kumagai H, Nagase H, Lazarus M (2019) Enhancing endogenous adenosine A2A receptor signaling induces slow-wave sleep without affecting body temperature and cardiovascular function. Neuropharmacology 144:122–132

Kredlow MA, Capozzoli MC, Hearon BA, Calkins AW, Otto MW (2015) The effects of physical activity on sleep: a meta-analytic review. J Behav Med 38(3):427–449

Kyle SD, Sexton CE, Feige B, Luik AI, Lane J, Saxena R, Anderson SG, Bechtold DA, Dixon W, Little MA, Ray D, Riemann D, Espie CA, Rutter MK, Spiegelhalder K (2017) Sleep and cognitive performance: cross-sectional associations in the UK Biobank. Sleep Med 38:85–91

Lafortune M, Gagnon JF, Martin N, Latreille V, Dube J, Bouchard M, Bastien C, Carrier J (2014) Sleep spindles and rapid eye movement sleep as predictors of next morning cognitive performance in healthy middle-aged and older participants. J Sleep Res 23(2):159–167

Laing EE, Moller-Levet CS, Poh N, Santhi N, Archer SN, Dijk DJ (2017) Blood transcriptome based biomarkers for human circadian phase. Elife 6:e20214

Lancel M, Wetter TC, Steiger A, Mathias S (2001) Effect of the GABAA agonist gaboxadol on nocturnal sleep and hormone secretion in healthy elderly subjects. Am J Physiol Endocrinol Metab 281(1):E130–E137

Landolt HP, de Boer LP (2001) Effect of chronic phenelzine treatment on REM sleep: report of three patients. Neuropsychopharmacology 25(5 Suppl):S63–S67

Landolt HP, Wehrle R (2009) Antagonism of serotonergic 5-HT2A/2C receptors: mutual improvement of sleep, cognition and mood? Eur J Neurosci 29(9):1795–1809

Landolt HP, Moser S, Wieser HG, Borbély AA, Dijk DJ (1995) Intracranial temperature across 24-hour sleep-wake cycles in humans. Neuroreport 6(6):913–917

Landolt HP, Roth C, Dijk DJ, Borbély AA (1996) Late-afternoon ethanol intake affects nocturnal sleep and the sleep EEG in middle-aged men. J Clin Psychopharmacol 16(6):428–436

Landolt HP, Meier V, Burgess HJ, Finelli LA, Cattelin F, Achermann P, Borbély AA (1999) Serotonin-2 receptors and human sleep: effect of a selective antagonist on EEG power spectra. Neuropsychopharmacology 21(3):455–466

Landolt HP, Finelli LA, Roth C, Buck A, Achermann P, Borbély AA (2000) Zolpidem and sleep deprivation: different effect on EEG power spectra. J Sleep Res 9(2):175–183

Landolt HP, Rétey JV, Adam M (2012) Reduced neurobehavioral impairment from sleep deprivation in older adults: contribution of adenosinergic mechanisms. Front Neurol 3:62

Landolt HP, Holst SC, Valomon A (2018) Clinical and experimental human sleep-wake pharmacogenetics. Handb Exp Pharmacol. https://doi.org/10.1007/164_2018_175

Lane JM, Vlasac I, Anderson SG, Kyle SD, Dixon WG, Bechtold DA, Gill S, Little MA, Luik A, Loudon A, Emsley R, Scheer FA, Lawlor DA, Redline S, Ray DW, Rutter MK, Saxena R (2016) Genome-wide association analysis identifies novel loci for chronotype in 100,420 individuals from the UK Biobank. Nat Commun 7:10889

Lankford DA, Corser BC, Zheng YP, Li Z, Snavely DB, Lines CR, Deacon S (2008) Effect of gaboxadol on sleep in adult and elderly patients with primary insomnia: results from two randomized, placebo-controlled, 30-night polysomnography studies. Sleep 31(10):1359–1370

Lazar AS, Santhi N, Hasan S, Lo JC, Johnston JD, Von Schantz M, Archer SN, Dijk DJ (2013) Circadian period and the timing of melatonin onset in men and women: predictors of sleep during the weekend and in the laboratory. J Sleep Res 22(2):155–159

Lazar AS, Lazar ZI, Dijk DJ (2015) Circadian regulation of slow waves in human sleep: topographical aspects. Neuroimage 116:123–134

Lazar ZI, Dijk DJ, Lazar AS (2018) Infraslow oscillations in human sleep spindle activity. J Neurosci Methods 316:22–34

Lecci S, Fernandez LM, Weber FD, Cardis R, Chatton JY, Born J, Luthi A (2017) Coordinated infraslow neural and cardiac oscillations mark fragility and offline periods in mammalian sleep. Sci Adv 3(2):e1602026

LeGates TA, Fernandez DC, Hattar S (2014) Light as a central modulator of circadian rhythms, sleep and affect. Nat Rev Neurosci 15(7):443–454

Lemoine P, Zisapel N (2012) Prolonged-release formulation of melatonin (Circadin) for the treatment of insomnia. Expert Opin Pharmacother 13(6):895–905

Lewy AJ, Bauer VK, Ahmed S, Thomas KH, Cutler NL, Singer CM, Moffit MT, Sack RL (1998) The human phase response curve (PRC) to melatonin is about 12 hours out of phase with the PRC to light. Chronobiol Int 15(1):71–83

Lim J, Dinges DF (2008) Sleep deprivation and vigilant attention. Ann N Y Acad Sci 1129:305–322

Liu J, Wang LN (2012) Ramelteon in the treatment of chronic insomnia: systematic review and meta-analysis. Int J Clin Pract 66(9):867–873

Lo JC, Groeger JA, Santhi N, Arbon EL, Lazar AS, Hasan S, von Schantz M, Archer SN, Dijk DJ (2012) Effects of partial and acute total sleep deprivation on performance across cognitive domains, individuals and circadian phase. PLoS One 7(9):e45987

Lockley SW, Skene DJ, James K, Thapan K, Wright J, Arendt J (2000) Melatonin administration can entrain the free-running circadian system of blind subjects. J Endocrinol 164(1):R1–R6

Lockley SW, Dressman MA, Licamele L, Xiao C, Fisher DM, Flynn-Evans EE, Hull JT, Torres R, Lavedan C, Polymeropoulos MH (2015) Tasimelteon for non-24-hour sleep-wake disorder in totally blind people (SET and RESET): two multicentre, randomised, double-masked, placebo-controlled phase 3 trials. Lancet 386(10005):1754–1764

Lowe CJ, Safati A, Hall PA (2017) The neurocognitive consequences of sleep restriction: a meta-analytic review. Neurosci Biobehav Rev 80:586–604

Lucas RJ, Peirson SN, Berson DM, Brown TM, Cooper HM, Czeisler CA, Figueiro MG, Gamlin PD, Lockley SW, O'Hagan JB, Price LL, Provencio I, Skene DJ, Brainard GC (2014) Measuring and using light in the melanopsin age. Trends Neurosci 37(1):1–9

Luppi PH, Fort P (2018) Neuroanatomical and neurochemical bases of vigilance states. Handb Exp Pharmacol. https://doi.org/10.1007/164_2017_84

Ma J, Dijk DJ, Svetnik V, Tymofyeyev Y, Ray S, Walsh JK, Deacon S (2011) EEG power spectra response to a 4-h phase advance and gaboxadol treatment in 822 men and women. J Clin Sleep Med 7(5):493–501A

Mander BA, Winer JR, Walker MP (2017) Sleep and human aging. Neuron 94(1):19–36

Mathias S, Steiger A, Lancel M (2001) The GABA(A) agonist gaboxadol improves the quality of post-nap sleep. Psychopharmacology 157(3):299–304

Maurer LF, Espie CA, Kyle SD (2018) How does sleep restriction therapy for insomnia work? A systematic review of mechanistic evidence and the introduction of the Triple-R model. Sleep Med Rev 42:127–138

McHill AW, Hull JT, Wang W, Czeisler CA, Klerman EB (2018) Chronic sleep curtailment, even without extended (>16-h) wakefulness, degrades human vigilance performance. Proc Natl Acad Sci U S A 115(23):6070–6075

McKillop LE, Vyazovskiy VV (2018) Sleep- and wake-like states in small networks in vivo and in vitro. Handb Exp Pharmacol. https://doi.org/10.1007/164_2018_174

Melancon MO, Lorrain D, Dionne IJ (2015) Sleep depth and continuity before and after chronic exercise in older men: electrophysiological evidence. Physiol Behav 140:203–208

Micic G, Lovato N, Gradisar M, Burgess HJ, Ferguson SA, Lack L (2016) Circadian melatonin and temperature taus in delayed sleep-wake phase disorder and non-24-hour sleep-wake rhythm disorder patients: an ultradian constant routine study. J Biol Rhythm 31(4):387–405

Mikkelsen KB, Ebajemito JK, Bonmati-Carrion MA, Santhi N, Revell VL, Atzori G, Della Monica C, Debener S, Dijk DJ, Sterr A, de Vos M (2019) Machine-learning-derived sleep-wake staging from around-the-ear electroencephalogram outperforms manual scoring and actigraphy. J Sleep Res 28(2):e12786

Miller CB, Gordon CJ, Toubia L, Bartlett DJ, Grunstein RR, D'Rozario AL, Marshall NS (2015) Agreement between simple questions about sleep duration and sleep diaries in a large online survey. Sleep Health 1(2):133–137

Moller-Levet CS, Archer SN, Bucca G, Laing EE, Slak A, Kabiljo R, Lo JC, Santhi N, von Schantz M, Smith CP, Dijk DJ (2013) Effects of insufficient sleep on circadian rhythmicity and expression amplitude of the human blood transcriptome. Proc Natl Acad Sci U S A 110(12):E1132–E1141

Monroe LJ (1967) Psychological and physiological differences between good and poor sleepers. J Abnorm Psychol 72(3):255–264

Morris CJ, Aeschbach D, Scheer FA (2012) Circadian system, sleep and endocrinology. Mol Cell Endocrinol 349(1):91–104

Murray JM, Sletten TL, Magee M, Gordon C, Lovato N, Bartlett DJ, Kennaway DJ, Lack LC, Grunstein RR, Lockley SW, Rajaratnam SM; Delayed Sleep on Melatonin Study Group (2017) Prevalence of circadian misalignment and its association with depressive symptoms in delayed sleep phase disorder. Sleep 40(1):zsw002

Muto V, Jaspar M, Meyer C, Kusse C, Chellappa SL, Degueldre C, Balteau E, Shaffii-Le Bourdiec A, Luxen A, Middleton B, Archer SN, Phillips C, Collette F, Vandewalle G, Dijk DJ, Maquet P (2016) Local modulation of human brain responses by circadian rhythmicity and sleep debt. Science 353(6300):687–690

Newman RA, Kamimori GH, Wesensten NJ, Picchioni D, Balkin TJ (2013) Caffeine gum minimizes sleep inertia. Percept Mot Skills 116(1):280–293

O'Callaghan EK, Green EW, Franken P, Mongrain V (2018) Omics approaches in sleep-wake regulation. Handb Exp Pharmacol. https://doi.org/10.1007/164_2018_125

Pachito DV, Eckeli AL, Desouky AS, Corbett MA, Partonen T, Rajaratnam SM, Riera R (2018) Workplace lighting for improving alertness and mood in daytime workers. Cochrane Database Syst Rev 3:CD012243

Peake JM, Kerr G, Sullivan JP (2018) A critical review of consumer wearables, mobile applications, and equipment for providing biofeedback, monitoring stress, and sleep in physically active populations. Front Physiol 9:743

Perrault AA, Khani A, Quairiaux C, Kompotis K, Franken P, Muhlethaler M, Schwartz S, Bayer L (2019) Whole-night continuous rocking entrains spontaneous neural oscillations with benefits for sleep and memory. Curr Biol 29(3):402–411.e3

Rajaratnam SM, Dijk DJ, Middleton B, Stone BM, Arendt J (2003) Melatonin phase-shifts human circadian rhythms with no evidence of changes in the duration of endogenous melatonin secretion or the 24-hour production of reproductive hormones. J Clin Endocrinol Metab 88 (9):4303–4309

Rajaratnam SM, Middleton B, Stone BM, Arendt J, Dijk DJ (2004) Melatonin advances the circadian timing of EEG sleep and directly facilitates sleep without altering its duration in extended sleep opportunities in humans. J Physiol 561(Pt 1):339–351

Rasch B, Born J (2013) About sleep's role in memory. Physiol Rev 93(2):681–766

Rhodes JA, Lane JM, Vlasac IM, Rutter MK, Czeisler C, Saxena R (2018) Association of DAT1 genetic variants with habitual sleep duration in the UK biobank. Sleep 42(1):zsy193

Richardson C, Cain N, Bartel K, Micic G, Maddock B, Gradisar M (2018) A randomised controlled trial of bright light therapy and morning activity for adolescents and young adults with Delayed Sleep-Wake Phase Disorder. Sleep Med 45:114–123

Riedner BA, Goldstein MR, Plante DT, Rumble ME, Ferrarelli F, Tononi G, Benca RM (2016) Regional patterns of elevated alpha and high-frequency electroencephalographic activity during nonrapid eye movement sleep in chronic insomnia: a pilot study. Sleep 39(4):801–812

Roenneberg T (2013) Chronobiology: the human sleep project. Nature 498(7455):427–428

Roth T, Lines C, Vandormael K, Ceesay P, Anderson D, Snavely D (2010) Effect of gaboxadol on patient-reported measures of sleep and waking function in patients with Primary Insomnia: results from two randomized, controlled, 3-month studies. J Clin Sleep Med 6(1):30–39

Rupp TL, Wesensten NJ, Balkin TJ (2012) Trait-like vulnerability to total and partial sleep loss. Sleep 35(8):1163–1172

Rusterholz T, Durr R, Achermann P (2010) Inter-individual differences in the dynamics of sleep homeostasis. Sleep 33(4):491–498

Santhi N, Thorne HC, van der Veen DR, Johnsen S, Mills SL, Hommes V, Schlangen LJ, Archer SN, Dijk DJ (2012) The spectral composition of evening light and individual differences in the suppression of melatonin and delay of sleep in humans. J Pineal Res 53(1):47–59

Santhi N, Groeger JA, Archer SN, Gimenez M, Schlangen LJ, Dijk DJ (2013) Morning sleep inertia in alertness and performance: effect of cognitive domain and white light conditions. PLoS One 8 (11):e79688

Santhi N, Lazar AS, McCabe PJ, Lo JC, Groeger JA, Dijk DJ (2016) Sex differences in the circadian regulation of sleep and waking cognition in humans. Proc Natl Acad Sci U S A 113 (19):E2730–E2739

Schwarz JFA, Akerstedt T, Lindberg E, Gruber G, Fischer H, Theorell-Haglow J (2017) Age affects sleep microstructure more than sleep macrostructure. J Sleep Res 26(3):277–287

Schwarz J, Axelsson J, Gerhardsson A, Tamm S, Fischer H, Kecklund G, Akerstedt T (2018) Mood impairment is stronger in young than in older adults after sleep deprivation. J Sleep Res 25: e12801. https://doi.org/10.1111/jsr.12801

Scullin MK, Bliwise DL (2015) Sleep, cognition, and normal aging: integrating a half century of multidisciplinary research. Perspect Psychol Sci 10(1):97–137

Shekari Soleimanloo S, White MJ, Garcia-Hansen V, Smith SS (2017) The effects of sleep loss on young drivers' performance: a systematic review. PLoS One 12(8):e0184002

Shi G, Wu D, Ptacek LJ, Fu YH (2017) Human genetics and sleep behavior. Curr Opin Neurobiol 44:43–49

Siclari F, Baird B, Perogamvros L, Bernardi G, LaRocque JJ, Riedner B, Boly M, Postle BR, Tononi G (2017) The neural correlates of dreaming. Nat Neurosci 20(6):872–878

Sivertsen B, Lallukka T, Salo P, Pallesen S, Hysing M, Krokstad S, Simon O (2014) Insomnia as a risk factor for ill health: results from the large population-based prospective HUNT Study in Norway. J Sleep Res 23(2):124–132

Skeldon AC, Derks G, Dijk DJ (2016) Modelling changes in sleep timing and duration across the lifespan: changes in circadian rhythmicity or sleep homeostasis? Sleep Med Rev 28:96–107

Skeldon AC, Phillips AJ, Dijk DJ (2017) The effects of self-selected light-dark cycles and social constraints on human sleep and circadian timing: a modeling approach. Sci Rep 7:45158

Skorucak J, Arbon EL, Dijk DJ, Achermann P (2018) Response to chronic sleep restriction, extension, and subsequent total sleep deprivation in humans: adaptation or preserved sleep homeostasis? Sleep 41(7):zsy078

Sletten TL, Magee M, Murray JM, Gordon CJ, Lovato N, Kennaway DJ, Gwini SM, Bartlett DJ, Lockley SW, Lack LC, Grunstein RR, Rajaratnam SMW, Delayed Sleep on Melatonin (DelSoM) Study Group (2018) Efficacy of melatonin with behavioural sleep-wake scheduling for delayed sleep-wake phase disorder: a double-blind, randomised clinical trial. PLoS Med 15 (6):e1002587

Strogatz SH, Kronauer RE, Czeisler CA (1987) Circadian pacemaker interferes with sleep onset at specific times each day: role in insomnia. Am J Phys 253(1 Pt 2):R172–R178

Sullivan SS, Kushida CA (2008) Multiple sleep latency test and maintenance of wakefulness test. Chest 134(4):854–861

Svetnik V, Snyder ES, Ma J, Tao P, Lines C, Herring WJ (2017) EEG spectral analysis of NREM sleep in a large sample of patients with insomnia and good sleepers: effects of age, sex and part of the night. J Sleep Res 26(1):92–104

Svetnik V, Snyder ES, Tao P, Scammell TE, Roth T, Lines C, Herring WJ (2018) Insight into reduction of wakefulness by suvorexant in patients with insomnia: analysis of wake bouts. Sleep 41(1):zsx178

Te Lindert BHW, Van Someren EJW (2018) Skin temperature, sleep, and vigilance. Handb Clin Neurol 156:353–365

Trachsel L, Dijk DJ, Brunner DP, Klene C, Borbély AA (1990) Effect of zopiclone and midazolam on sleep and EEG spectra in a phase-advanced sleep schedule. Neuropsychopharmacology 3 (1):11–18

Urade Y (2017) Neurobiological basis of hypersomnia. Sleep Med Clin 12(3):265–277

Van Cauter E, Plat L, Scharf MB, Leproult R, Cespedes S, L'Hermite-Baleriaux M, Copinschi G (1997) Simultaneous stimulation of slow-wave sleep and growth hormone secretion by gamma-hydroxybutyrate in normal young men. J Clin Invest 100(3):745–753

Van der Maren S, Moderie C, Duclos C, Paquet J, Daneault V, Dumont M (2018) Daily profiles of light exposure and evening use of light-emitting devices in young adults complaining of a delayed sleep schedule. J Biol Rhythm 33(2):192–202

van Diepen HC, Lucassen EA, Yasenkov R, Groenen I, Ijzerman AP, Meijer JH, Deboer T (2014) Caffeine increases light responsiveness of the mouse circadian pacemaker. Eur J Neurosci 40 (10):3504–3511

Van Dongen HP, Maislin G, Mullington JM, Dinges DF (2003) The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. Sleep 26(2):117–126

Van Dongen HP, Baynard MD, Maislin G, Dinges DF (2004) Systematic interindividual differences in neurobehavioral impairment from sleep loss: evidence of trait-like differential vulnerability. Sleep 27(3):423–433

Vgontzas AN, Fernandez-Mendoza J, Liao D, Bixler EO (2013) Insomnia with objective short sleep duration: the most biologically severe phenotype of the disorder. Sleep Med Rev 17(4):241–254
Vienne J, Lecciso G, Constantinescu I, Schwartz S, Franken P, Heinzer R, Tafti M (2012) Differential effects of sodium oxybate and baclofen on EEG, sleep, neurobehavioral performance, and memory. Sleep 35(8):1071–1083
Viola AU, Archer SN, James LM, Groeger JA, Lo JC, Skene DJ, von Schantz M, Dijk DJ (2007) PER3 polymorphism predicts sleep structure and waking performance. Curr Biol 17 (7):613–618
Viola AU, James LM, Archer SN, Dijk DJ (2008a) PER3 polymorphism and cardiac autonomic control: effects of sleep debt and circadian phase. Am J Physiol Heart Circ Physiol 295(5): H2156–H2163
Viola AU, James LM, Schlangen LJ, Dijk DJ (2008b) Blue-enriched white light in the workplace improves self-reported alertness, performance and sleep quality. Scand J Work Environ Health 34(4):297–306
Walch OJ, Cochran A, Forger DB (2016) A global quantification of "normal" sleep schedules using smartphone data. Sci Adv 2(5):e1501705
Walsh JK, Deacon S, Dijk DJ, Lundahl J (2007) The selective extrasynaptic GABAA agonist, gaboxadol, improves traditional hypnotic efficacy measures and enhances slow wave activity in a model of transient insomnia. Sleep 30(5):593–602
Walsh JK, Snyder E, Hall J, Randazzo AC, Griffin K, Groeger J, Eisenstein R, Feren SD, Dickey P, Schweitzer PK (2008) Slow wave sleep enhancement with gaboxadol reduces daytime sleepiness during sleep restriction. Sleep 31(5):659–672
Walsh JK, Hall-Porter JM, Griffin KS, Dodson ER, Forst EH, Curry DT, Eisenstein RD, Schweitzer PK (2010) Enhancing slow wave sleep with sodium oxybate reduces the behavioral and physiological impact of sleep loss. Sleep 33(9):1217–1225
Watson LA, Phillips AJK, Hosken IT, McGlashan EM, Anderson C, Lack LC, Lockley SW, Rajaratnam SMW, Cain SW (2018) Increased sensitivity of the circadian system to light in delayed sleep-wake phase disorder. J Physiol 596(24):6249–6261
Wei HG, Riel E, Czeisler CA, Dijk DJ (1999) Attenuated amplitude of circadian and sleep-dependent modulation of electroencephalographic sleep spindle characteristics in elderly human subjects. Neurosci Lett 260(1):29–32
Werth E, Dijk DJ, Achermann P, Borbély AA (1996) Dynamics of the sleep EEG after an early evening nap: experimental data and simulations. Am J Phys 271(3 Pt 2):R501–R510
Wilckens KA, Ferrarelli F, Walker MP, Buysse DJ (2018) Slow-wave activity enhancement to improve cognition. Trends Neurosci 41(7):470–482
Wild CJ, Nichols ES, Battista ME, Stojanoski B, Owen AM (2018) Dissociable effects of self-reported daily sleep duration on high-level cognitive abilities. Sleep 41(12):zsy182
Wilson J t, Reid KJ, Braun RI, Abbott SM, Zee PC (2018) Habitual light exposure relative to circadian timing in delayed sleep-wake phase disorder. Sleep 41(11):zsy166
Winsky-Sommerer R, de Oliveira P, Loomis S, Wafford K, Dijk DJ, Gilmour G (2018) Disturbances of sleep quality, timing and structure and their relationship with other neuropsychiatric symptoms in Alzheimer's disease and schizophrenia: insights from studies in patient populations and animal models. Neurosci Biobehav Rev 97:112–137
Wisden W, Yu X, Franks NP (2017) GABA receptors and the pharmacology of sleep. Handb Exp Pharmacol. https://doi.org/10.1007/164_2017_56
Wittenbrink N, Ananthasubramaniam B, Munch M, Koller B, Maier B, Weschke C, Bes F, de Zeeuw J, Nowozin C, Wahnschaffe A, Wisniewski S, Zaleska M, Bartok O, Ashwal-Fluss R, Lammert H, Herzel H, Hummel M, Kadener S, Kunz D, Kramer A (2018) High-accuracy determination of internal circadian time from a single blood sample. J Clin Invest 128 (9):3826–3839
Woelders T, Beersma DGM, Gordijn MCM, Hut RA, Wams EJ (2017) Daily light exposure patterns reveal phase and period of the human circadian clock. J Biol Rhythm 32(3):274–286
Wright KP Jr, Hughes RJ, Kronauer RE, Dijk DJ, Czeisler CA (2001) Intrinsic near-24-h pacemaker period determines limits of circadian entrainment to a weak synchronizer in humans. Proc Natl Acad Sci U S A 98(24):14027–14032

Wright KP Jr, Gronfier C, Duffy JF, Czeisler CA (2005) Intrinsic period and light intensity determine the phase relationship between melatonin and sleep in humans. J Biol Rhythm 20 (2):168–177

Wyatt JK, Ritz-De Cecco A, Czeisler CA, Dijk DJ (1999) Circadian temperature and melatonin rhythms, sleep, and neurobehavioral function in humans living on a 20-h day. Am J Phys 277 (4 Pt 2):R1152–R1163

Wyatt JK, Cajochen C, Ritz-De Cecco A, Czeisler CA, Dijk DJ (2004) Low-dose repeated caffeine administration for circadian-phase-dependent performance degradation during extended wakefulness. Sleep 27(3):374–381

Wyatt JK, Dijk DJ, Ritz-de Cecco A, Ronda JM, Czeisler CA (2006) Sleep-facilitating effect of exogenous melatonin in healthy young men and women is circadian-phase dependent. Sleep 29 (5):609–618

Yang H, Haack M, Dang R, Gautam S, Simpson NS, Mullington JM (2018) Heart rate variability rebound following exposure to persistent and repetitive sleep restriction. Sleep 42(2):zsy226

Yu X, Zecharia A, Zhang Z, Yang Q, Yustos R, Jager P, Vyssotski AL, Maywood ES, Chesham JE, Ma Y, Brickley SG, Hastings MH, Franks NP, Wisden W (2014) Circadian factor BMAL1 in histaminergic neurons regulates sleep architecture. Curr Biol 24(23):2838–2844

Yu X, Franks NP, Wisden W (2018) Sleep and sedative states induced by targeting the histamine and noradrenergic systems. Front Neural Circuits 12:4

Zant JC, Kim T, Prokai L, Szarka S, McNally J, McKenna JT, Shukla C, Yang C, Kalinchuk AV, McCarley RW, Brown RE, Basheer R (2016) Cholinergic neurons in the basal forebrain promote wakefulness by actions on neighboring non-cholinergic neurons: an opto-dialysis study. J Neurosci 36(6):2057–2067

Zeitzer JM, Dijk DJ, Kronauer R, Brown E, Czeisler C (2000) Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. J Physiol 526 (Pt 3):695–702

Zerbini G, Kantermann T, Merrow M (2018) Strategies to decrease social jetlag: reducing evening blue light advances sleep and melatonin. Eur J Neurosci. https://doi.org/10.1111/ejn.14293

Zitting KM, Munch MY, Cain SW, Wang W, Wong A, Ronda JM, Aeschbach D, Czeisler CA, Duffy JF (2018) Young adults are more vulnerable to chronic sleep deficiency and recurrent circadian disruption than older adults. Sci Rep 8(1):11052