Handbook of Experimental Pharmacology 253

Hans-Peter Landolt
Derk-Jan Dijk *Editors*

Sleep-Wake Neurobiology and Pharmacology

Springer

Handbook of Experimental Pharmacology

Volume 253

The *Handbook of Experimental Pharmacology* is one of the most authoritative and influential book series in pharmacology. It provides critical and comprehensive discussions of the most significant areas of pharmacological research, written by leading international authorities. Each volume in the series represents the most informative and contemporary account of its subject available, making it an unrivalled reference source.

HEP is indexed in PubMed and Scopus.

More information about this series at http://www.springer.com/series/164

Hans-Peter Landolt • Derk-Jan Dijk
Editors

Sleep-Wake Neurobiology and Pharmacology

Springer

Editors
Hans-Peter Landolt
Human Sleep Psychopharmacology Laboratory
Institute of Pharmacology and Toxicology
University of Zürich
Zürich, Switzerland

Derk-Jan Dijk
Surrey Sleep Research Centre
Faculty of Health and Medical Sciences
University of Surrey
Guildford, UK

ISSN 0171-2004 ISSN 1865-0325 (electronic)
Handbook of Experimental Pharmacology
ISBN 978-3-030-11270-7 ISBN 978-3-030-11272-1 (eBook)
https://doi.org/10.1007/978-3-030-11272-1

This Springer imprint is published by the registered company Springer Nature Switzerland AG.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

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."

Zürich, Switzerland — Hans-Peter Landolt
Guildford, UK — Derk-Jan Dijk

Contents

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

M. G. Frank
Department of Biomedical Sciences, Elson S. Floyd College of Medicine, Washington State University Spokane, Spokane, WA, USA

H. C. Heller (✉)
Department of Biology, Stanford University, Stanford, CA, USA
e-mail: hcheller@stanford.edu

H.-P. Landolt, D.-J. Dijk (eds.), *Sleep-Wake Neurobiology and Pharmacology*,
Handbook of Experimental Pharmacology 253, https://doi.org/10.1007/164_2018_140

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

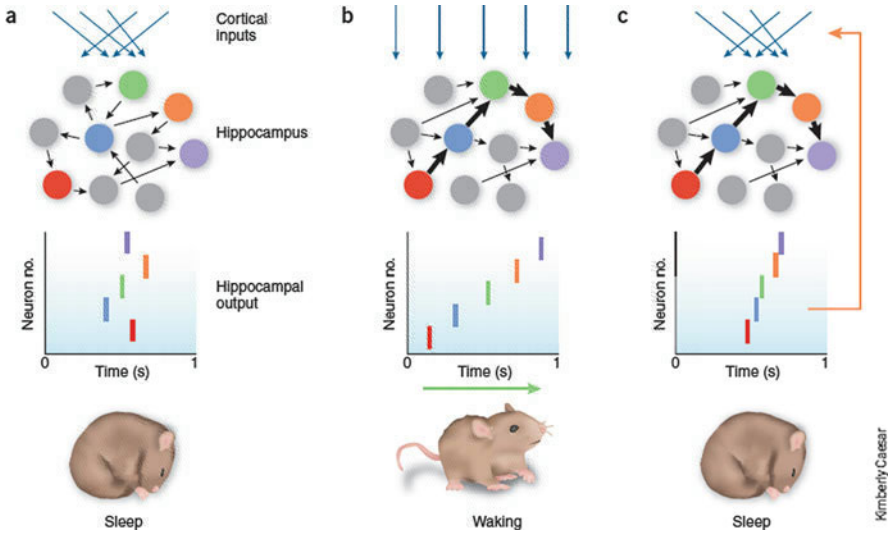

Fig. 1 Firing of "hippocampal place cells" corresponds to specific locations in space. (**a**) Recordings of a population of place cells during sleep in a naive animal show no obvious pattern of firing. (**b**) However, during wake activity running in a maze sequence patterns of place cell firing are 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

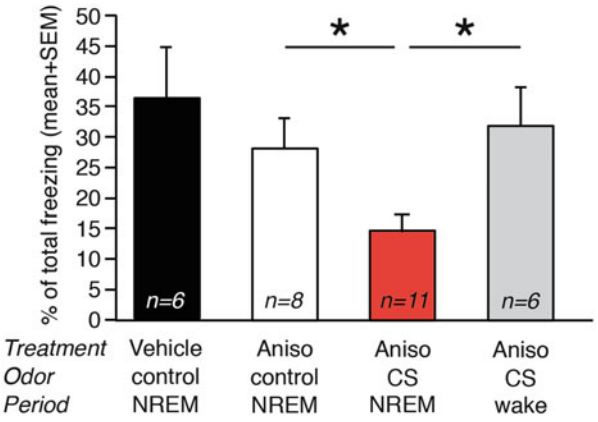

Fig. 2 Protein synthesis inhibitor (anisomycin) in combination with cued fear memory replay during sleep weakens the strength of that fear memory when cued during subsequent wake. In each case, the treatment (vehicle or anisomycin) was administered prior to the sleep phase, and during sleep the animal 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).

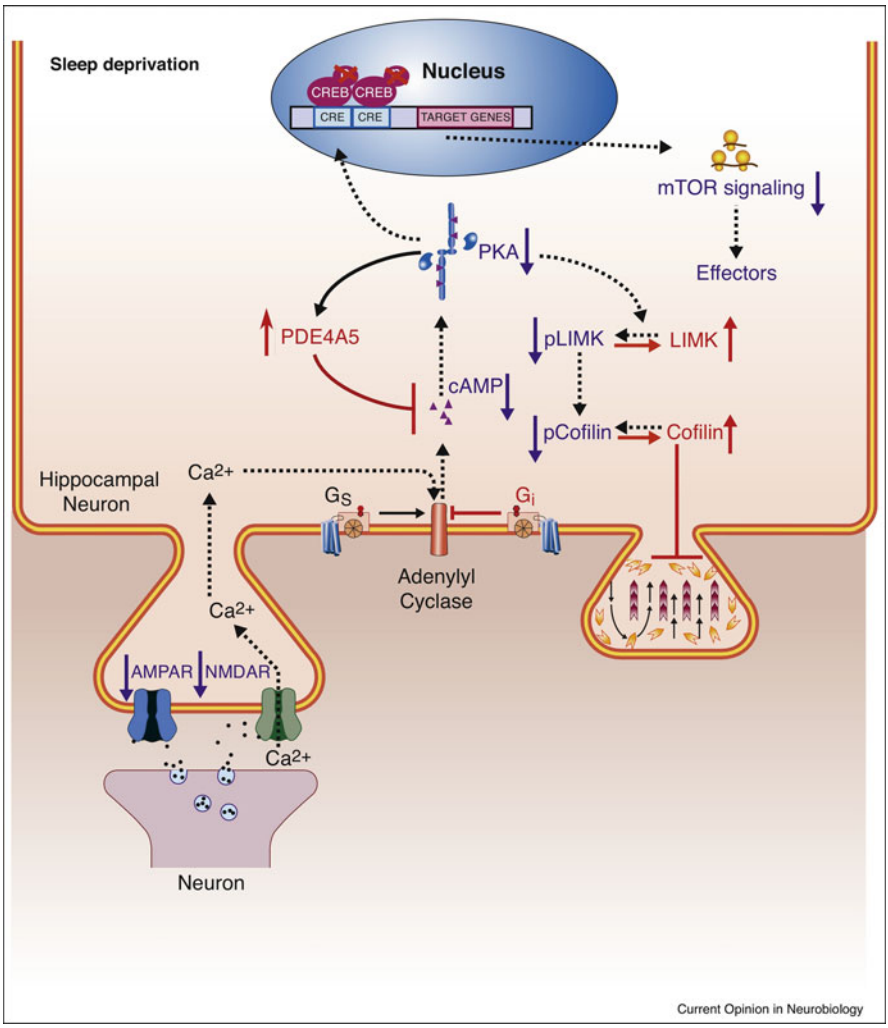

Fig. 3 The molecular impact of sleep deprivation. A schematic overview of hippocampal signaling pathways whose modulation by sleep deprivation may contribute to effects on memory formation. Sleep deprivation has been reported to reduce glutamatergic signaling while increasing adenosine levels. Sleep 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)

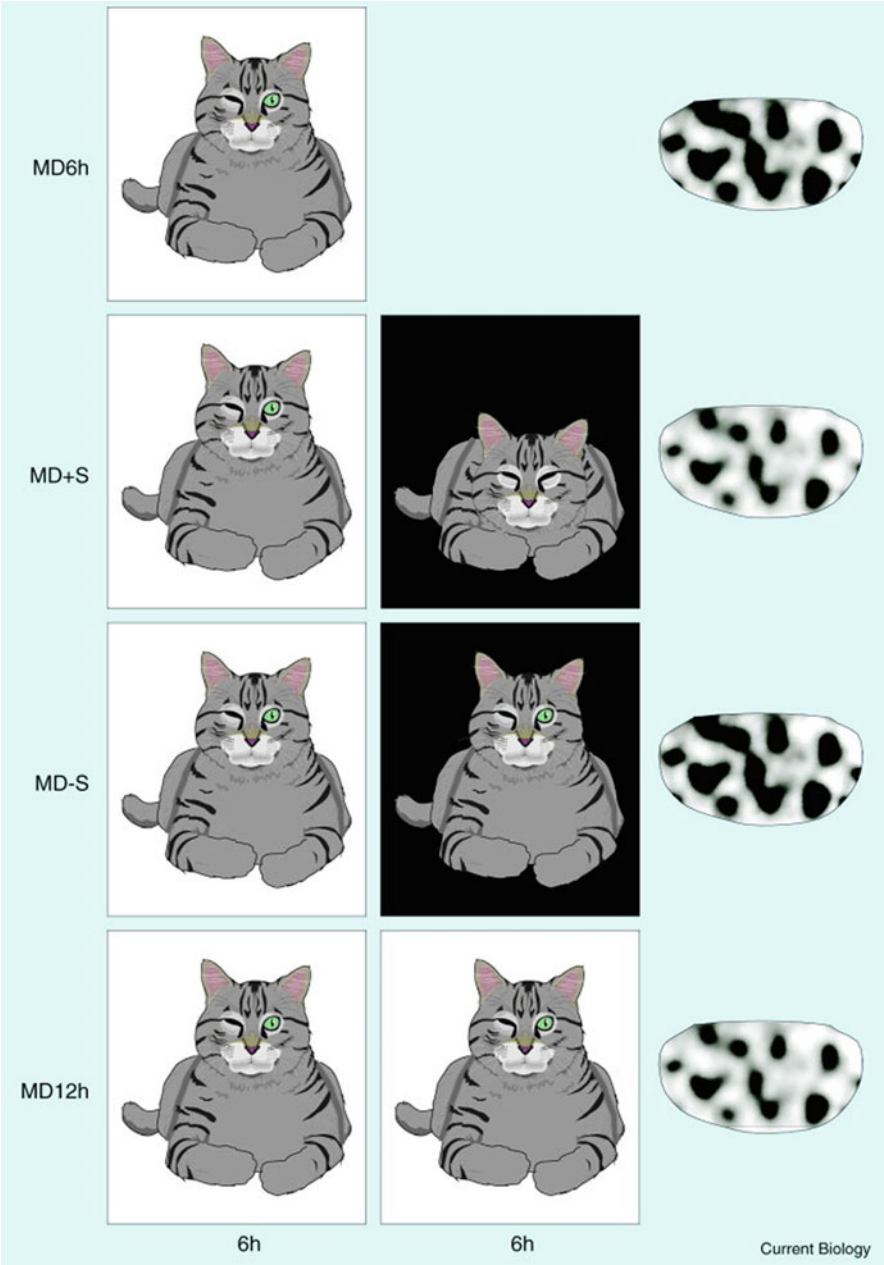

Fig. 4 Effect of sleep on the magnitude of the ocular dominance shift induced by monocular deprivation. The first two columns depict the rearing conditions of kittens employed by Frank et al. (2001). The right-most column schematically shows ocular dominance maps obtained from primary visual cortex under 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)

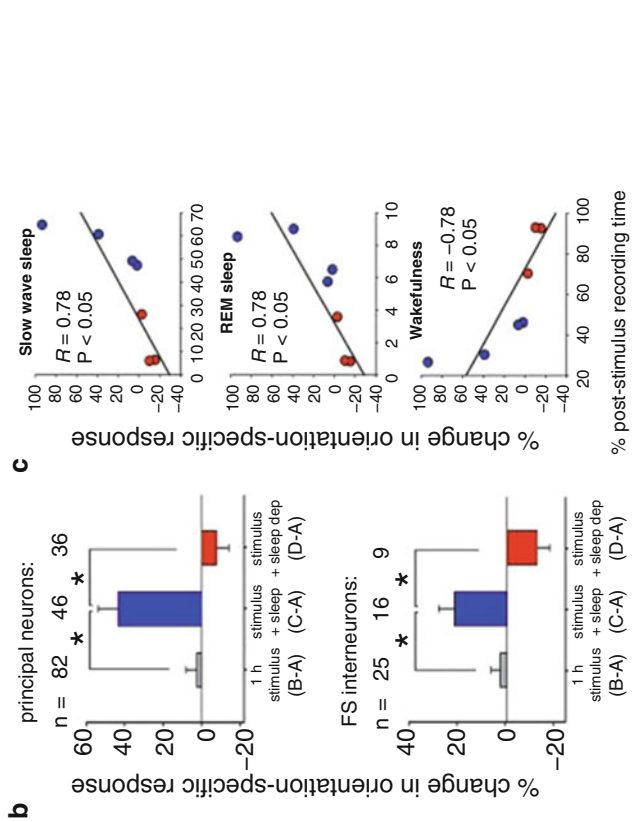

Fig. 5 Stimulus-specific response potentiation (SRP) is consolidated during poststimulus sleep. (**a**) V1 neurons were recorded across a baseline period of ad libitum sleep and wakefulness, a 1-h period of oriented grating stimulus presentation in the awake mouse (starting at lights-on), and a 6-h period 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).