Published in final edited form as:

Trends Neurosci. 2011 September ; 34(9): 452–463. doi:10.1016/j.tins.2011.07.005.

Synaptic plasticity in sleep: learning, homeostasis, and disease

Gordon Wang^{1,2}, Brian Grone¹, Damien Colas³, Lior Appelbaum⁴, and Philippe Murrain^{1,5}

¹Department of Psychiatry and Behavioral Sciences, Center for Sleep Sciences, Beckman Center, Stanford University, Palo Alto, California, 94305, USA.

²Department of Molecular and Cellular Physiology, Beckman Center, Stanford University, Palo Alto, California 94305, USA

³Department of Biology, Stanford University, Palo Alto, California, 94305 USA.

⁴Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, 52900, Israel.

⁵INSERM 1024, Ecole Normale Supérieure, Paris, 75005, France.

Abstract

Sleep is a fundamental and evolutionarily conserved aspect of animal life. Recent studies have shed light on the role of sleep in synaptic plasticity. Demonstrations of memory replay and synapse homeostasis suggest that one essential role of sleep is in the consolidation and optimization of synaptic circuits to retain salient memory traces despite the noise of daily experience. Here, we review this recent evidence, and suggest that sleep creates a heightened state of plasticity, which may be essential for this optimization. Furthermore, we discuss how sleep deficits seen in diseases such as Alzheimer's disease and autism spectrum disorders might not just reflect underlying circuit malfunction, but could also play a direct role in the progression of those disorders.

Introduction

While we all experience sleep, and so believe we know what it is, sleep remains a scientific enigma. A conclusive definition of sleep has eluded researchers and probably will continue to do so until the function of sleep is fully elucidated. Nevertheless, a working description of sleep as an electrophysiologically and behaviorally defined state has been established since the middle of the 20th century [1, 2]. In animals with a developed neocortex, including mammals and birds, sleep states are defined by specific patterns of whole-brain activity detected by an electroencephalograph (EEG), along with eye movement electrooculogram (EOG), and muscle tone electromyogram (EMG) patterns. Non-rapid eye movement sleep (NREM) is characterized by high-voltage synchronized slow waves of electrical activity throughout the cortex, and is referred to as slow-wave sleep (SWS) in its most synchronized form. Rapid eye movement (REM) sleep is characterized by rapid eye movement, muscle paralysis, and low-voltage irregular EEG waves similar to waves observed during wakefulness [3].

© 2011 Elsevier Ltd. All rights reserved.

Corresponding authors : Wang, G (drwonder@stanford.edu) and Murrain, P (murrain@stanford.edu)..

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

In the early 1980s, Irene Tobler extended this definition of sleep using additional behavioral criteria [4-6]: [i] decreased behavioral activity (immobility), [ii] site preference (e.g. bed), [iii] specific posture (e.g. lying), [iv] rapid reversibility (unlike coma), and most importantly [v] increased arousal threshold (offline state, no perception of the environment) and [vi] homeostatic control (sleep rebound after sleep deprivation). As of today, using the above criteria, sleep has been documented and studied in a wide range of vertebrates and invertebrates [7] and there is currently no clear evidence of an animal species that does not sleep [8]. The existence of an ancestral sleep state, combined with evidence that prolonged sleep deprivation leads to death in rats [9], fruit flies [10], and humans with fatal familial insomnia [11], strongly supports the hypothesis that sleep function serves a universal physiological need.

Using the above electrophysiological and behavioral criteria, major progress has been made in deciphering the mechanisms regulating sleep and wake states. Brain nuclei, circuits, neurotransmitters, and genes involved in sleep-wake regulation and state-switch have been identified [12, 13], but the most fundamental question remains: why do we sleep? Diverse theories have been postulated to account for the restorative effect of sleep and the importance of sleep for cognitive performance [14-19]. Sleep likely has multiple functions, but the strongest experimental evidence supports a primary role for sleep in the regulation of brain plasticity and cognition. Sleep deprivation impairs performance on motor and cognitive tasks [20] and sleep strengthens cognitive functions including visual discrimination [21], motor learning [22], and insight (gaining explicit understanding of an implicit rule) [23]. Evidence has been gathered at the behavioral, neuronal, synaptic, and molecular levels indicating that sleep promotes neural plasticity. Recent work in mammalian and non-mammalian models highlights the importance of sleep for synaptic remodeling and homeostasis (see Table 1). In this review, we will focus on the evidence for the role of sleep in synapse plasticity, a function conserved across animal phyla and critical for learning and memory as well as synaptic function and homeostasis.

Learning, Memory, and Plasticity Consolidation

The facilitation of memory retention is the most widely accepted and experimentally supported hypothesis explaining the neuronal need for sleep. Though learning mostly occurs during wake, sleep is of critical importance for memory processes. Sleep greatly enhances both the encoding and consolidation of memory [18, 19, 24]. Adequate sleep is necessary, both before and after an event, for that event to be properly encoded and stored in long-term memory [18, 19, 25]. Sleep-deprived humans have significantly impaired memory retention and degraded performance in memory encoding [26-28]. Long periods of sleep are clearly beneficial but gains in memorization performance have also been reported after short sleep periods. Recall of events is stronger and more accurate after daytime nap as brief as a few minutes, as compared to a similar wake period [29-31].

Quality of memory consolidation is not only a function of time spent asleep but can also vary depending on the type of memories, the relevance of the memorized event, and the motivation to remember. Following sleep, procedural memories (i.e. memorization of cognitive and motor skills) have been shown to benefit more than declarative memories (i.e. recollection of experiences and information) [19, 32]. Further, sleep had a stronger stabilizing effect on memories of tasks or events when there was a conscious effort or an incentive to memorize those. Simply put, conscious learning of a motor task associated with a potential reward generates memories that profit the most from sleep-dependent consolidation in contrast to unconscious and/or unmotivated learning of the same task [19, 32]. This uneven and contextual influence of sleep on different classes of memories suggests an intriguing possibility that sleep-dependent and sleep-independent plasticity coexist and

interact in the circuits and brain regions responsible for encoding and storing the different memory types.

While behavioral observations have shown that sleep as a whole is clearly important for memory consolidation, the roles of the different sleep phases are still being deciphered. Because of its relationship with dreams, REM sleep was first suspected to be critical for memory formation, but most of the EEG studies performed so far have reported that NREM, especially SWS, sleep is critical for memory retention. SWS/NREM sleep deprivation after learning prevents subsequent consolidation and enhancement of memories [19, 24]. Consistent with this observation, stimulation of slow wave oscillations during sleep enhances the retention of same-day memory traces for next-day retrieval [33]. Although SWS seems to have a primary role in memory formation, it is still unclear how other sleep phases participate in memory encoding and consolidation. NREM sleep spindles for example have been shown to be important for consolidation [34] and more recently encoding/learning capabilities [25]. REM sleep has also been associated with emotion-related memories [18]. Finally, in opposition to a dichotomous view associating a specific sleep stage with a specific type of memory, it has also been postulated that the sequence in which phases appear in normal sleep, i.e. NREMPREM succession, could be more important for optimal consolidation, whatever the memory type, than duration of each stage [35]. A better understanding of the molecular and physiological mechanisms generating the different sleep stages should shed light on their roles in hippocampal/cortical circuit plasticity and the different types of memory.

An intriguing and important mechanism proposed for sleep's role in the facilitation of memory consolidation is the replay of memory traces in hippocampal and cortical circuits during sleep (reviewed in [19, 36, 37]). Firing patterns recorded during wakefulness can be replayed during the following SWS/NREM sleep period [19, 37] and sometimes REM [38]. In neurons of the zebra finch song system, replay of patterns of bursts corresponding to singing sequence was observed during sleep [39, 40]. In rats, neuronal activation patterns recorded during maze learning are recreated during SWS [41, 42]. The human hippocampus is similarly reactivated during SWS following learning of a spatial task and the strength of this reactivation is associated with fidelity of learning [43]. Importantly, the reactivation of memories in humans by presenting, during SWS, odor or noise cues that were also present during learning leads to enhanced memory consolidation [44-46] and increased resistance of that memory to interference [46]. During a NREM nap, mental activity related to a spatial memory task is associated with enhanced memory consolidation [38]. Consistently, reactivation in SWS was correlated to activations of hippocampal and neocortical regions critical to learning and memory [46]. Interestingly, replay happens during the first 15-30min of sleep, when mammals are in SWS. During this SWS period, reactivated circuits undergo synaptic consolidation according to replay hypothesis, while others could be pruned according to the synaptic homeostasis hypothesis (see below). One could speculate that both hypotheses are not exclusive and that replay mechanisms could be important to protect fragile circuits against global synaptic downscaling.

While these recent reinstatement data are compelling, replay as a sleep-dependent mechanism for memory consolidation still remains to be fully established. Replay has mostly been studied in extensively trained rodents, except in a few cases [47], and thus, it may also reflect the firing of well-entrained circuitry. Moreover, replay is extremely transient and labile, and only a few studies have successfully investigated its function in memory transfer from the hippocampus to the neocortex (e.g. [48]). It is important to mention here that replay also occurs during wake, when it can similarly affect learning and memory consolidation [49, 50]. Reactivation of memories by odorants during sleep and during wake, however, activate different brain regions and elicit very different memory

responses. Odor cues that were present during learning activate hippocampal and posterior cortical regions and strengthen object-location memories when presented during sleep, but weaken those memories and activate mainly prefrontal cortical regions when presented during wakefulness [46]. Clearly, more work needs to be done to uncover the molecular and circuit properties of sleep/wake gating of brain activity and effects of memory reactivation on consolidation.

Consistent with the replay/reactivation studies, sleep is believed to consolidate synaptic connections required for encoding and retention of memories. Currently, the mechanisms underpinning synaptic consolidation during sleep in these hippocampal and cortical memory storage circuits remain unknown. Sleep has, however, already proven to be critical for consolidation of ocular dominance plasticity (ODP), a type of cortical plasticity widespread in mammals and particularly well-studied in cats. In ODP, deprivation of vision in one eye (monocular deprivation, MD) leads to increased rewiring of visual cortex by the non-deprived eye [51-53]. Interestingly, when MD is followed by just a few hours of sleep, cortical responses to non-deprived eye stimulation are strengthened [51]. Further, cortical consolidation was found correlated with the amount of NREM [51]. This finding suggests that sleep, especially NREM, has a critical function in cortical synaptic remodeling.

More recently, sleep-dependent consolidation in ODP was disrupted when major molecular actors of synaptic potentiation and plasticity such as NMDA receptors (NMDARs) and protein kinase A (PKA) were antagonized [53]. Increased phosphorylation and activation of downstream targets of these pathways [e.g. extracellular signal-regulated kinase (ERK), Ca^{2+} -calmodulin-dependent protein kinase (CaMKII) and the AMPA receptor (AMPA) GluR1 subunit] were observed only after post-MD sleep [53]. These data show that sleep can change the strength of neuronal connections and that pathways involved in synaptic plasticity are activated. Furthermore, all these data suggest that synchronous reactivation of behaviorally relevant neural circuits during sleep can mediate meaningful and functionally relevant changes in the brain, and further dissection of the molecular mechanisms underlying these activity states is critical to the understanding of sleep in the consolidation and optimization of brain circuit function.

Homeostatic control of synaptic plasticity

The synaptic homeostasis hypothesis

The memory consolidation hypothesis proposes a specific mechanism of synapse modification by which the encoding of memory traces is rendered more efficient through modification of relevant synapses. Recently a new hypothesis, the Synaptic Homeostasis Hypothesis (SHH), has emerged postulating that sleep globally downscale all synapses to compensate for the net increase in synapse formation and strength during wake [14, 54] (Figure 1a). The SHH assumes that wakefulness causes net cortical synaptic potentiation throughout the brain, and this potentiation drives slow wave activity (SWA) during NREM sleep. This SWA-mediated downscaling of synaptic strength provides a beneficial effect on neuronal efficiency and function. Indeed, mathematical modeling suggests that changes in synaptic strength can explain such changes in SWS intensity [55]. The formulation of the SHH is based on the observation that synaptic density and amplitude of long term potentiation (LTP) increase during exploration of enriched environments [56, 57] and following extended mechanical stimulation of sensory modalities [58]. This daily type of anatomical and physiological increase in synaptic function is also associated with the modification of gene expression and neural chemical systems that are critical to the expression and maintenance of synaptic potentiation. Changes in cAMP responsive element binding protein (CREB), activity-regulated cytoskeleton-associated protein (Arc), brain-derived neurotrophic factor (BDNF), AMPAR subunits [59], Homer, neuronal activity

regulated pentraxin (NARP) [60-62], acetylcholine [63], and norepinephrine [64] have been observed, although it should be noted that Arc and Homer are also required for long-term depression (LTD) and/or homeostatic downscaling. Thus, the SHH provides a compelling set of testable hypotheses for describing the synaptic changes in sleep.

Waking is not associated only with increases in synaptic strength; the duration of wake time also controls the amplitude and duration of SWA. Moreover, sensory or mechanical stimulation that extends wakefulness leads to higher amplitude slow waves in NREM sleep with steeper slopes and fewer multi-peak waves, a feature that is more characteristic of late phase SWS, during which the sleep homeostatic pressure is low [65]. Furthermore, this increase in SWA appears to be locally regulated as behavioral tasks designed to activate a single cortical region elevated the level of SWA specifically in that region during SWS [66]. Thus, there is support for the first two tenets of the SHH: synaptic strength is likely increased during wake, and this increase does regulate the amount of SWA during SWS. The final conjecture that SWA is associated with synaptic downscaling is yet to be directly observed in mammalian species. Still, there is suggestive evidence in rodents from cortical evoked responses and local field potential recording during sleep [59] and recent analysis of spontaneous synaptic events [67, 68] showing that synaptic downscaling may occur. Nonetheless, the most convincing demonstration of this sleep-dependent decrease in synaptic strength and connectivity appears in non-mammalian and non-cortical neural circuits in fruit flies and zebrafish (Figure 1). These recent data show that homeostasis of synaptic strength during sleep is neither a novel invention of mammals nor the sole purview of the cortex, but a more ancestral function, inherent in synaptic circuits, that is essential for the proper maintenance and efficient operation of networks of connected neurons.

The idea that synapses can be homeostatically regulated is not unique to SHH; it is a documented phenomenon in the field of synaptic scaling. In synaptic scaling, bidirectional changes of strength of individual synapses induce compensatory changes in synaptic strength that are proportional across multiple synapses of a given postsynaptic neuron [69-73]. Thus, modulation of network activity leads to the uniform scaling of synaptic strength across groups of synapses or entire neurons [74, 75]. This allows neurons to normalize their output without changing the relative signaling strength of individual synapses, thus presumably maintaining the information fidelity of the system. Synaptic scaling may occur presynaptically or postsynaptically, and can involve changes in intrinsic excitability, inhibitory and/or excitatory synaptic strength and number, or metaplasticity (adjusting the extent of other forms of plasticity). Molecular mechanisms mediating synaptic scaling include soluble factors [e.g. BDNF and tumor necrosis factor (TNF)], transsynaptic signaling and cell adhesion molecules [e.g. $\beta 3$ integrin, major histocompatibility complex (MHC1)], and intracellular signaling molecules [e.g. CaMKs, Arc, polio-like kinase 2 (PLK2), and cyclin-dependent kinase 5 (CDK5)]. According to the synaptic scaling model, lower activity levels or quiescent network states should increase synaptic strength rather than downscale them, which appears to be counterintuitive. One must, however, note that sleep is not a quiescent state: the energy use of the brain during sleep is not significantly lower than during wake and appears to even increase during the onset of SWS [76]. Furthermore, the apparent synchrony and slow EEG oscillations of SWS do not indicate that neurons are firing less, in fact extracellular recordings in the cortex during NREM sleep show that there is an increase in high frequency firing (>50 Hz with a peak at ~ 100 Hz) and low frequency firing (<15 Hz with a peak at 3-5 Hz) with a decrease in medium firing rate (15-50 Hz) [59]. Interestingly, the 100 Hz and 5 Hz firing rates that are exaggerated in sleep are exactly the stimulation frequency for inducing LTP and LTD, respectively, in a broad range of neuronal preparations both *in vivo* and *in vitro*. Thus, we suggest that synapses may be pruned, retuned, and even added during sleep to optimize the information stored in the

nervous system by mechanisms that may include synaptic scaling as well as Hebbian plasticity.

Circadian and homeostatic control of synaptic plasticity in fly and zebrafish

While the SHH was originally postulated based on mammalian electrophysiological observations, its first demonstration at the molecular and neuronal levels came from studies in two non-mammalian species, namely, the fruit fly *Drosophila melanogaster* and the zebrafish *Danio rerio*. Several studies in *Drosophila* had previously shown the existence of a day-night rhythm of neuronal structural plasticity at the cellular level (Table 2). In the pigment dispersing factor (PDF) circuit, rhythmic remodeling in axonal terminals was reported [77]. Similarly, the morphology of flight neuromuscular terminals changes between day and night [78], with a rhythm in synaptic bouton size [79]. Finally, in the fly visual system, the size and morphology of monopolar cell arborization also varies rhythmically over a 24h cycle [80]. In all these studies, the structural plasticity rhythm was found to be regulated by the circadian rhythm, because rhythmicity was maintained in constant darkness [78, 79], or controlled by clock genes [77, 80]. These data supported clock-controlled plasticity, but did not directly investigate the influence of sleep on synapses.

Recent studies in fruit fly demonstrate a sleep-dependent homeostatic process responsible for downregulation of synaptic components [81], downscaling of synapse number, and decrease in synapse volume and dendritic complexity (Figure 1 and Table 2) [82, 83]. Quantification in the fly central nervous system (CNS) of presynaptic and postsynaptic proteins including Bruchpilot (BRP) and Discs-large (DLG), the *Drosophila* homolog of the vertebrate postsynaptic density protein PSD-95, showed that synaptic protein levels increase throughout wake but decrease during sleep independently of the circadian time [81]. Sleep-deprived animals had up to 40% higher synaptic component levels than animals allowed to sleep, strongly suggesting that sleep has a role throughout the CNS in renormalizing synapses for the day to come (Figure 1b) [81]. This global sleep-dependent regulation of synaptic markers was confirmed and strengthened by circuit-specific studies investigating the precise influence of wake and sleep on synaptic terminals in the PDF circuit (Figure 1c) [83], as well as the γ lobe of the mushroom bodies and the dendrites of a unique neuron giant tangential neuron of the lobula plate vertical system (VS1) (Figure 1d) [82, 83]. Synaptic clusters were imaged and counted in transgenic flies expressing enhanced green fluorescent protein (EGFP)-tagged constructs of the presynaptic proteins synaptobrevin and synaptotagmin, the postsynaptic protein DLG, or actin to reveal the dendritic spines. Interestingly, synaptic terminal number and volume increased in flies maintained in an enriched environment and the number and volume of synaptic terminals were reduced during sleep [82, 83]. This decline in synaptic terminals was prevented by sleep deprivation [82, 83]. This finding, consistent with the SHH, revealed that sleep can downscale structural synaptic connections that are potentiated during waking experience.

A similar structural synaptic plasticity in a zebrafish neural circuit is regulated by both circadian and homeostatic processes. Transgenic fish expressing the pre-synaptic protein synaptophysin (SYP) fused to EGFP are a useful means of following synaptic structures in transparent zebrafish [84]. This fusion protein was targeted to the hypothalamic hypocretin (HCRT) neurons (Figure 1e, f), a well-known circuit involved in sleep/wake regulation [85-87]. Results from array tomography [88], a new proteomic imaging technique (see Box 1), showed that the majority (>85%) of EGFP-labeled SYP presynaptic boutons were in juxtaposition with postsynaptic PSD 95, confirming that SYP puncta represent good markers of structural synapses [84]. The optical clarity of larval zebrafish and infrared light-based two-photon imaging allowed longitudinal analysis in living zebrafish of the synaptic density in different regions of the HCRT circuits (see Box 1 and Figure 1 e, f). HCRT synapse density waxed and waned according to a circadian rhythm [84]. Critically, synapse number

was also homeostatically regulated by sleep [84]. Thus, sleep-deprived animals were deficient in nighttime synaptic downscaling. Overall, validation of the SHH in such phylogenetically distant species as zebrafish and *Drosophila* strongly suggests that the cellular processes demonstrated in synaptic potentiation and homeostasis have been conserved across evolution.

The aforementioned experiments demonstrate that synapses are dynamic during sleep and wake, and support a sleep-dependent synaptic homeostasis. However, supplementary evidence needs to be gathered to fully validate the SHH. First, none of the studies mentioned above proved a functional change in synaptic transmission or showed whether the changes in synaptic density actually affected the function of the circuit or the neurons within that circuit. This type of functional analysis will be critical for extending our understanding of synapse modification during sleep to explain the physiological role of sleep. It will be important to demonstrate that synapses are lost or gained by a selective mechanism that effectively reduces the physical footprint of memories without losing the details of that memory. Second, in the first studies that directly showed changes in synapse density, the synapses in question are in circuits (i.e. PDF [83] and HCRT [84]) known to be involved in circadian and sleep rhythm regulation. Although the recent evidence in mushroom bodies and visual system of flies is a great step forward [82], it will be important to extend studies of sleep regulation of synapse density throughout the nervous system. Finally, the SHH was primarily formulated on observations made in mammalian cortex. Thus, it is critical that sleep-mediated synapse density changes in mammalian neocortex be convincingly demonstrated, and that this change is mediated by sleep homeostatic pressure and is positively correlated with the amount of SWS. Recent advances in molecular and live imaging techniques (see Box 1) should enable unprecedented access to the fundamental mechanisms involved in synaptic changes during sleep.

What could be the primary ancestral function of sleep?

The accumulation of evidence linking sleep to synaptic and circuit plasticity in vertebrates, and more recently invertebrates (Table 1), allows informed speculations about what could be the ancestral and primary role of sleep. Across distantly related animal models, sleep has been shown to have a critical role in at least three main manifestations of circuit plasticity: brain and nervous system development, learning and memory, and synaptic homeostasis. Based on this observation, one convergent hypothesis is that sleep is primarily a plastic state for the development and remodeling of neural circuits. In view of these commonalities, sleep might be compared to a neurodevelopmental state: a functional state that has been evolutionary preserved from simple circuits to neocortical complex networks. In this hypothesis, the sleep state allows critical plasticity mechanisms to be brought on-line to facilitate the making and breaking of connections within neural circuits that during the desynchronized and unpredictable synaptic environment of wake could disrupt behavior or learning.

In mammals, the amount of sleep is highest early in life when maximal amounts of neural development are occurring [89, 90]. Newborns spend a majority of their time in a sleep state, and sleep has been shown to be critical for nervous system maturation [89, 90]. Sleep deprivation studies in young rodents lead to a loss of brain plasticity associated with reduced learning performance and negative long-term cognitive and behavioral effects [91]. NREM seems particularly important as human neonates respond to sleep deprivation with compensatory increases only in NREM sleep time but not REM [92, 93]. The critical role of sleep during mammalian nervous system development may reflect a highly evolutionary conserved process. Indeed, at the other extremity of the animal evolution ladder, sleep and development could not only be associated, but essentially identical. In the worm *C. elegans*,

a developmental stage called lethargus has also been characterized as a sleep-like state [94]. This developmental stage occurs before each of the four larval molts. Interestingly, lethargus can be induced by the epidermal growth factor (EGF) signaling pathway [95], known for its involvement in neuronal differentiation and synaptic plasticity in mammals [96, 97]. While synaptic remodeling of the worm GABAergic system is known to occur during the first molt before the larval L1-L2 transition [98, 99], no demonstration for a direct function of lethargus in this remodeling has been shown in the worm yet. It is noteworthy that lethargus/sleep, like any developmental process, is precisely timed. The timing of the molts has been correlated with the oscillation of the *C. elegans* ortholog of the well-known circadian factor Period [100]. It is tempting to speculate based on these correlations that sleep as a behavioral state and its circadian regulation could originate from an ancestral developmental state and its developmental timing program.

The mammalian and worm studies, coupled with the demonstration of conserved synaptic homeostasis and rhythmic plasticity during sleep in both zebrafish larvae [84] and adult flies [81, 83] also support the idea that the ancestral sleep function could be the same during development and adulthood. Furthermore, sleep as a recurrent state in normal brain function can be considered as an abridged version of brain development that recapitulates, on a limited scale, the activity-dependent global pruning and refining of connectivity following the increase in synapse density and strength during the earliest part of brain development. Each day, sleep provides the same function as provided during development by this early window of pruning, rewiring synaptic networks guided by salient neurological activity and thus selectively potentiating certain important synapses while simultaneously downscaling non-essential synaptic connections.

So, with the experimental knowledge gathered to date from memory consolidation, visual cortex wiring, and synaptic homeostasis studies, it is safe to acknowledge that sleep, on a synaptic level, is a specific type of plastic state likely conserved across circuits, developmental stages, and evolution. This critical state is not only important for the proper function of the nervous system, but is itself dependent on the prior activity and connectivity of the nervous system. While the effects of sleep on synaptic plasticity during normal physiological conditions will require extensive studies for many years to come, pathological conditions such as observed in neurodegenerative and neurodevelopmental disorders should also shed light on the association of abnormal sleep and cognition impairment.

Sleep abnormalities in cognitive disorders and related animal models

Our discussion thus far has focused on the role of sleep as a major organizer of synapse and circuit plasticity in the brain. In this role, sleep acts in synchrony with the circadian rhythm to normalize, modulate, and optimize the synaptic function and circuit connectivity of cortical and subcortical neural networks. The dark side of sleep's importance for synapse and circuit function is that sleep dysfunction is also connected to numerous neurological and neurodevelopment disorders, (Table 3), as discussed below.

Alzheimer disease (AD), a neurodegenerative disease, is characterized by progressive cognitive decline associated with synaptic and neuronal loss [101]. In particular, synaptic failure in AD has been linked to abnormal processing of the amyloid precursor protein (APP), abnormal intracellular organization of Tau proteins, and the development of cortical amyloid plaques [102]. Besides behavioral abnormalities, distinct sleep problems appear in AD. Clinicians report abnormal excitement at bedtime (sun-downing), increased awakenings and sleep fragmentation, reduced SWS, and slower EEG frequencies [103]. Additional abnormal features distinguish AD sleep problems compared to normal aging: REM sleep and abnormal rapid eye movements density [104], abnormal respiratory patterns and sleep

apnea [105, 106], abnormal EEG spectral component and synchrony, such as K-complex [107]. Of note, sleep disturbances are an early component of AD and are present in early onset AD [108, 109], and insomnia in adults represents a significant risk of AD [102]. These characteristics raise the possibility that early molecular mechanisms of AD could result in or at least accompany sleep disturbances. The use of mouse models of AD suggests a relationship between abnormal APP processing and sleep disturbances in AD patients. Mice with abnormal APP dosage or metabolism show sleep fragmentation, decreased SWS, and abnormal EEG synchrony at early stages and independently from plaque formation [110–112]. The beta-amyloid (A β) content of the cortex is under the influence of the sleep-wake cycle independently from plaque formation [113]. Moreover, imposing sleep reduces the A β burden and the associated APP-dependent synaptic abnormalities [113]. These preclinical data illustrate how closely related sleep and synaptic machineries can be. Therefore the possibility of restoring synaptic mechanisms through the management of sleep in AD is currently sought as an avenue of therapy [114].

Features of abnormal synaptic plasticity have also been shown to occur in a number of neurodevelopmental disorders, including Angelman syndrome (AS) and in autism spectrum disorder (ASD)-associated diseases Fragile X syndrome (FXS) and Rett syndrome (RS).. Specifically, AS, FXS and RS are caused by altered functional expression of key synaptic protein, including the E3 ubiquitin ligase, UBE3a [115, 116], fragile X mental retardation protein (FMR1P, encoded by the gene *FMR1*) and methyl CpG binding protein 2 (MeCP2), respectively. A mouse model for AS that specifically lacks *Ube3a* on the maternal allele (ie. *Ube3a*^{m-P+}) was observed to have impaired sleep homeostasis and insomnia [117]. *FMR1* loss in mice is associated with circadian dysfunction and perturbed rhythmic activity [118], and FMRP appears to be important for synaptic plasticity [119] and the sleep-dependent renormalization of synapses [82]. Sleep disturbances have been reported in patients with these disorders, even though quantitative EEG analysis is still scarce. Most problems relate to insomnia: difficulty in initiating sleep, sleep fragmentation, or maintaining sleep with longer sleep latency and less sleep efficiency [120, 121]. Qualitative analyses of sleep in children diagnosed with ASD and/or developmental delays have shown that undifferentiated sleep is increased, whereas NREM spindles, SWS, and REM are decreased [122, 123]. Optimizing sleep could be beneficial for some of the most detrimental behavioral abnormalities associated with these conditions. Accordingly, recent clinical data suggest a beneficial effect of melatonin supplementation on behavioral abnormalities in children with ASD [124]. Further studies will be necessary to understand the relationship between sleep quality and synaptic plasticity in ASD and other neurological disorders. It is hoped that, studying sleep in the context of these disorders may not only improve treatment and the early diagnosis of such disorders, but might also shed light on mechanisms and functions fundamental to sleep.

Concluding remarks

Although many questions remain to be answered (see Box 2), the scientific enigma as to why we sleep is beginning to be unraveled. In the brain, sleep is essential, and this need appears to require a level of synaptic plasticity that is unavailable during wake. This state of plasticity allows for homeostatic optimization of neural networks as well as the replay-based consolidation of specific circuits. Indeed, sleep plasticity appears to be focused not on acquiring new information, but on prioritizing and compressing known information to maintain optimal network function. Based on available data, we postulate that this optimization requires a state of structural and molecular plasticity that would be detrimental to sensory processing or long-term stability of memory in the asynchronous and unpredictable neural environment of wake. Thus, this optimization is facilitated in sleep during periods of highly synchronous activity.

Sleep resembles critical plastic periods during development and is an essential, recurring state of the brain that is required to maintain an optimal set point of connectivity that is sensitive to both environmental enrichment and genetic background. So, it is no surprise that when the underlying structure of the brain is perturbed by neuronal degeneration, or as occurs during aberrant neuronal development, sleep dysfunction arises as an early indication of such problems. Thus, sleep is universal because it is a critical plastic state that consolidates prior information and prioritizes network activity so that the brain functions efficiently in whatever new world we wake up in.

Acknowledgments

Our work is supported by the National Institutes of Health (NS062798, DK090065).

References

1. Aserinsky E, Kleitman N. Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. *Science*. 1953; 118:273–274. [PubMed: 13089671]
2. Jouvet M, et al. On a stage of rapid cerebral electrical activity in the course of physiological sleep. *C R Seances Soc Biol Fil*. 1959; 153:1024–1028. [PubMed: 14408003]
3. Carskadon MA, Dement WC. Normal Human Sleep: An Overview. *Principles and Practice of Sleep Medicine*. 2005:13–23.
4. Tobler I. Effect of forced locomotion on the rest-activity cycle of the cockroach. *Behav Brain Res*. 1983; 8:351–360. [PubMed: 6871020]
5. Campbell SS, Tobler I. Animal sleep: a review of sleep duration across phylogeny. *Neurosci Biobehav Rev*. 1984; 8:269–300. [PubMed: 6504414]
6. Tobler I, Borbely AA. Effect of rest deprivation on motor activity of fish. *J Comp Physiol [A]*. 1985; 157:817–822.
7. Hartse KM. The phylogeny of sleep. *Handb Clin Neurol*. 2011; 98:97–109. [PubMed: 21056182]
8. Cirelli C, Tononi G. Is sleep essential? *PLoS Biol*. 2008; 6:e216. [PubMed: 18752355]
9. Rechtschaffen A, et al. Physiological correlates of prolonged sleep deprivation in rats. *Science*. 1983; 221:182–184. [PubMed: 6857280]
10. Shaw PJ, et al. Stress response genes protect against lethal effects of sleep deprivation in *Drosophila*. *Nature*. 2002; 417:287–291. [PubMed: 12015603]
11. Fiorino AS. Sleep, genes and death: fatal familial insomnia. *Brain Res Brain Res Rev*. 1996; 22:258–264. [PubMed: 8957563]
12. Lu J, et al. A putative flip-flop switch for control of REM sleep. *Nature*. 2006; 441:589–594. [PubMed: 16688184]
13. Saper CB, et al. Sleep state switching. *Neuron*. 2010; 68:1023–1042. [PubMed: 21172606]
14. Tononi G, Cirelli C. Sleep function and synaptic homeostasis. *Sleep Med Rev*. 2006; 10:49–62. [PubMed: 16376591]
15. Frank MG. The mystery of sleep function: current perspectives and future directions. *Rev Neurosci*. 2006; 17:375–392. [PubMed: 17139839]
16. Mignot E. Why we sleep: the temporal organization of recovery. *PLoS Biol*. 2008; 6:e106. [PubMed: 18447584]
17. Krueger JM, et al. Sleep as a fundamental property of neuronal assemblies. *Nat Rev Neurosci*. 2008; 9:910–919. [PubMed: 18985047]
18. Walker MP. The role of sleep in cognition and emotion. *Ann N Y Acad Sci*. 2009; 1156:168–197. [PubMed: 19338508]
19. Diekelmann S, Born J. The memory function of sleep. *Nat Rev Neurosci*. 2010; 11:114–126. [PubMed: 20046194]
20. Pilcher JJ, Huffcutt AI. Effects of sleep deprivation on performance: a meta-analysis. *Sleep*. 1996; 19:318–326. [PubMed: 8776790]

21. Stickgold R, et al. Visual discrimination learning requires sleep after training. *Nat Neurosci.* 2000; 3:1237–1238. [PubMed: 11100141]
22. Walker MP, et al. Practice with sleep makes perfect: sleep-dependent motor skill learning. *Neuron.* 2002; 35:205–211. [PubMed: 12123620]
23. Wagner U, et al. Sleep inspires insight. *Nature.* 2004; 427:352–355. [PubMed: 14737168]
24. Walker MP, Stickgold R. Sleep, memory, and plasticity. *Annual Review of Psychology.* 2006; 57:139–166.
25. Mander BA, et al. Wake deterioration and sleep restoration of human learning. *Current Biology.* 2011; 21:R183–R184. [PubMed: 21377092]
26. Harrison Y, Horne JA. Sleep loss and temporal memory. *Q J Exp Psychol A.* 2000; 53:271–279. [PubMed: 10718074]
27. Drummond SP, et al. Altered brain response to verbal learning following sleep deprivation. *Nature.* 2000; 403:655–657. [PubMed: 10688201]
28. Drummond SP, Brown GG. The effects of total sleep deprivation on cerebral responses to cognitive performance. *Neuropsychopharmacology.* 2001; 25:S68–73. [PubMed: 11682277]
29. Lahl O, et al. An ultra short episode of sleep is sufficient to promote declarative memory performance. *J Sleep Res.* 2008; 17:3–10. [PubMed: 18275549]
30. Mednick S, et al. Sleep-dependent learning: a nap is as good as a night. *Nature Neuroscience.* 2003; 6:697–698.
31. Tucker MA, et al. A daytime nap containing solely non-REM sleep enhances declarative but not procedural memory. *Neurobiol Learn Mem.* 2006; 86:241–247. [PubMed: 16647282]
32. Stickgold R, Walker MP. Sleep-dependent memory consolidation and reconsolidation. *Sleep Med.* 2007; 8:331–343. [PubMed: 17470412]
33. Marshall L, et al. Boosting slow oscillations during sleep potentiates memory. *Nature.* 2006; 444:610–613. [PubMed: 17086200]
34. Nader, R.; Smith, C. A Role For Stage 2 Sleep in Memory Processing. In: Maquet, P., et al., editors. *Sleep and Brain Plasticity.* Oxford University Press; 2003. p. 87-99.
35. Giuditta A, et al. The sequential hypothesis of the function of sleep. *Behav Brain Res.* 1995; 69:157–166. [PubMed: 7546307]
36. Girardeau G, Zugaro M. Hippocampal ripples and memory consolidation. *Curr Opin Neurobiol.* 2011 doi:10.1016/j.conb.2011.02.005.
37. O'Neill J, et al. Play it again: reactivation of waking experience and memory. *Trends Neurosci.* 2010; 33:220–229. [PubMed: 20207025]
38. Wamsley EJ, et al. Dreaming of a learning task is associated with enhanced sleep-dependent memory consolidation. *Curr Biol.* 2010; 20:850–855. [PubMed: 20417102]
39. Dave AS, Margoliash D. Song replay during sleep and computational rules for sensorimotor vocal learning. *Science.* 2000; 290:812–816. [PubMed: 11052946]
40. Margoliash D. Sleep, learning, and birdsong. *ILAR J.* 2010; 51:378–386. [PubMed: 21131714]
41. Wilson MA, Ji DY. Coordinated memory replay in the visual cortex and hippocampus during sleep. *Nature Neuroscience.* 2007; 10:100–107.
42. Wilson MA, McNaughton BL. Reactivation of Hippocampal Ensemble Memories during Sleep. *Science.* 1994; 265:676–679. [PubMed: 8036517]
43. Peigneux P, et al. Are spatial memories strengthened in the human hippocampus during slow wave sleep? *Neuron.* 2004; 44:535–545. [PubMed: 15504332]
44. Rasch B, et al. Odor cues during slow-wave sleep prompt declarative memory consolidation. *Science.* 2007; 315:1426–1429. [PubMed: 17347444]
45. Rudoy JD, et al. Strengthening individual memories by reactivating them during sleep. *Science.* 2009; 326:1079. [PubMed: 19965421]
46. Diekelmann S, et al. Labile or stable: opposing consequences for memory when reactivated during waking and sleep. *Nature Neuroscience.* 2011; 14:381–386.
47. Peyrache A, et al. Replay of rule-learning related neural patterns in the prefrontal cortex during sleep. *Nat Neurosci.* 2009; 12:919–926. [PubMed: 19483687]

48. Girardeau G, et al. Selective suppression of hippocampal ripples impairs spatial memory. *Nat Neurosci.* 2009; 12:1222–1223. [PubMed: 19749750]
49. Foster DJ, Wilson MA. Reverse replay of behavioural sequences in hippocampal place cells during the awake state. *Nature.* 2006; 440:680–683. [PubMed: 16474382]
50. Carr MF, et al. Hippocampal replay in the awake state: a potential substrate for memory consolidation and retrieval. *Nat Neurosci.* 2011; 14:147–153. [PubMed: 21270783]
51. Frank MG, et al. Sleep enhances plasticity in the developing visual cortex. *Neuron.* 2001; 30:275–287. [PubMed: 11343661]
52. Frank MG, et al. Blockade of postsynaptic activity in sleep inhibits developmental plasticity in visual cortex. *Neuroreport.* 2006; 17:1459–1463. [PubMed: 16932158]
53. Aton SJ, et al. Mechanisms of Sleep-Dependent Consolidation of Cortical Plasticity. *Neuron.* 2009; 61:454–466. [PubMed: 19217381]
54. Tononi G, Cirelli C. Sleep and synaptic homeostasis: a hypothesis. *Brain Research Bulletin.* 2003; 62:143–150. [PubMed: 14638388]
55. Esser SK, et al. Sleep homeostasis and cortical synchronization: I. Modeling the effects of synaptic strength on sleep slow waves. *Sleep.* 2007; 30:1617–1630.
56. Sale A, et al. Enrich the environment to empower the brain. *Trends Neurosci.* 2009; 32:233–239. [PubMed: 19268375]
57. van Praag H, et al. Neural consequences of environmental enrichment. *Nat Rev Neurosci.* 2000; 1:191–198. [PubMed: 11257907]
58. Zito K, Svoboda K. Activity-dependent synaptogenesis in the adult mammalian cortex. *Neuron.* 2002; 35:1015–1017. [PubMed: 12354392]
59. Vyazovskiy VV, et al. Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. *Nat Neurosci.* 2008; 11:200–208. [PubMed: 18204445]
60. Silva AJ. Molecular and cellular cognitive studies of the role of synaptic plasticity in memory. *Journal of Neurobiology.* 2003; 54:224–237. [PubMed: 12486706]
61. Wallace CS, et al. Correspondence between Sites of Ngfi-a Induction and Sites of Morphological Plasticity Following Exposure to Environmental Complexity. *Mol Brain Res.* 1995; 32:211–220. [PubMed: 7500832]
62. Ying SW, et al. Brain-derived neurotrophic factor induces long-term potentiation in intact adult hippocampus: Requirement for ERK activation coupled to CREB and upregulation of Arc synthesis. *Journal of Neuroscience.* 2002; 22:1532–1540. [PubMed: 11880483]
63. Rasch B, et al. Impaired Off-Line Consolidation of Motor Memories After Combined Blockade of Cholinergic Receptors During REM Sleep-Rich Sleep. *Neuropsychopharmacology.* 2009; 34:1843–1853. [PubMed: 19194375]
64. Sara SJ. Reactivation, retrieval, replay and reconsolidation in and out of sleep: connecting the dots. *Front Behav Neurosci.* 2010; 4:185. [PubMed: 21179586]
65. Huber R, et al. Topography of EEG dynamics after sleep deprivation in mice. *Journal of Neurophysiology.* 2000; 84:1888–1893. [PubMed: 11024081]
66. Ghilardi MF, et al. Patterns of regional brain activation associated with different forms of motor learning. *Brain Research.* 2000; 871:127–145. [PubMed: 10882792]
67. Liu ZW, et al. Direct evidence for wake-related increases and sleep-related decreases in synaptic strength in rodent cortex. *J Neurosci.* 2010; 30:8671–8675. [PubMed: 20573912]
68. Lante F, et al. Removal of synaptic Ca(2+)-permeable AMPA receptors during sleep. *J Neurosci.* 2011; 31:3953–3961. [PubMed: 21411638]
69. O'Brien RJ, et al. Activity-dependent modulation of synaptic AMPA receptor accumulation. *Neuron.* 1998; 21:1067–1078. [PubMed: 9856462]
70. Turrigiano GG, et al. Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature.* 1998; 391:892–896. [PubMed: 9495341]
71. Wierenga CJ, et al. Postsynaptic expression of homeostatic plasticity at neocortical synapses. *J Neurosci.* 2005; 25:2895–2905. [PubMed: 15772349]

72. Kilman V, et al. Activity deprivation reduces miniature IPSC amplitude by decreasing the number of postsynaptic GABA(A) receptors clustered at neocortical synapses. *J Neurosci.* 2002; 22:1328–1337. [PubMed: 11850460]
73. Stellwagen D, Malenka RC. Synaptic scaling mediated by glial TNF- α . *Nature.* 2006; 440:1054–1059. [PubMed: 16547515]
74. Turrigiano GG, Nelson SB. Homeostatic plasticity in the developing nervous system. *Nat Rev Neurosci.* 2004; 5:97–107. [PubMed: 14735113]
75. Turrigiano GG. The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell.* 2008; 135:422–435. [PubMed: 18984155]
76. Dworak M, et al. Sleep and brain energy levels: ATP changes during sleep. *J Neurosci.* 2010; 30:9007–9016. [PubMed: 20592221]
77. Fernandez MP, et al. Circadian remodeling of neuronal circuits involved in rhythmic behavior. *PLoS Biol.* 2008; 6:e69. [PubMed: 18366255]
78. Gorska-Andrzejak J, et al. Structural daily rhythms in GFP-labelled neurons in the visual system of *Drosophila melanogaster*. *Photochem Photobiol Sci.* 2005; 4:721–726. [PubMed: 16121283]
79. Mehnert KI, et al. Circadian changes in *Drosophila* motor terminals. *Dev Neurobiol.* 2007; 67:415–421. [PubMed: 17443798]
80. Weber P, et al. Circadian control of dendrite morphology in the visual system of *Drosophila melanogaster*. *PLoS One.* 2009; 4:e4290. [PubMed: 19173003]
81. Gilestro GF, et al. Widespread changes in synaptic markers as a function of sleep and wakefulness in *Drosophila*. *Science.* 2009; 324:109–112. [PubMed: 19342593]
82. Bushey D, et al. Sleep and Synaptic Homeostasis: Structural Evidence in *Drosophila*. *Science.* 2011; 332:1576–1581. [PubMed: 21700878]
83. Donlea JM, et al. Use-dependent plasticity in clock neurons regulates sleep need in *Drosophila*. *Science.* 2009; 324:105–108. [PubMed: 19342592]
84. Appelbaum L, et al. Circadian and homeostatic regulation of structural synaptic plasticity in hypocretin neurons. *Neuron.* 2010; 68:87–98. [PubMed: 20920793]
85. Faraco JH, et al. Regulation of hypocretin (orexin) expression in embryonic zebrafish. *J Biol Chem.* 2006; 281:29753–29761. [PubMed: 16867991]
86. Yokogawa T, et al. Characterization of sleep in zebrafish and insomnia in hypocretin receptor mutants. *PLoS Biol.* 2007; 5:2379–2397.
87. Appelbaum L, et al. Sleep-wake regulation and hypocretin-melatonin interaction in zebrafish. *Proc Natl Acad Sci U S A.* 2009; 106:21942–21947. [PubMed: 19966231]
88. Micheva KD, Smith SJ. Array tomography: a new tool for imaging the molecular architecture and ultrastructure of neural circuits. *Neuron.* 2007; 55:25–36. [PubMed: 17610815]
89. Graven S. Sleep and brain development. *Clin Perinatol.* 2006; 33:693–706. vii. [PubMed: 16950320]
90. Ednick M, et al. A review of the effects of sleep during the first year of life on cognitive, psychomotor, and temperament development. *Sleep.* 2009; 32:1449–1458. [PubMed: 19928384]
91. Maquet P., et al. Sleep and brain plasticity. Oxford University Press; New York: 2003. p. 1-13.
92. Anders TF, Roffwarg HP. The effects of selective interruption and deprivation of sleep in the human newborn. *Dev Psychobiol.* 1973; 6:77–89. [PubMed: 4353450]
93. Thomas DA, et al. The effect of sleep deprivation on sleep states, breathing events, peripheral chemoresponsiveness and arousal propensity in healthy 3 month old infants. *Eur Respir J.* 1996; 9:932–938. [PubMed: 8793454]
94. Raizen DM, et al. Lethargus is a *Caenorhabditis elegans* sleep-like state. *Nature.* 2008; 451:569–572. [PubMed: 18185515]
95. Van Buskirk C, Sternberg PW. Epidermal growth factor signaling induces behavioral quiescence in *Caenorhabditis elegans*. *Nat Neurosci.* 2007; 10:1300–1307. [PubMed: 17891142]
96. Huang YZ, et al. Regulation of neuregulin signaling by PSD-95 interacting with ErbB4 at CNS synapses. *Neuron.* 2000; 26:443–455. [PubMed: 10839362]
97. Kwon OB, et al. Neuregulin-1 reverses long-term potentiation at CA1 hippocampal synapses. *J Neurosci.* 2005; 25:9378–9383. [PubMed: 16221846]

98. White JG, et al. Connectivity changes in a class of motoneurone during the development of a nematode. *Nature*. 1978; 271:764–766. [PubMed: 625347]
99. Hallam SJ, Jin Y. lin-14 regulates the timing of synaptic remodelling in *Caenorhabditis elegans*. *Nature*. 1998; 395:78–82. [PubMed: 9738501]
100. Jeon M, et al. Similarity of the *C. elegans* developmental timing protein LIN-42 to circadian rhythm proteins. *Science*. 1999; 286:1141–1146. [PubMed: 10550049]
101. Wilson RS, et al. Cognitive decline in prodromal Alzheimer disease and mild cognitive impairment. *Arch Neurol*. 2011; 68:351–356. [PubMed: 21403020]
102. Osorio RS, et al. Greater Risk Of Alzheimer's Disease In Older Adults With Insomnia. *J Am Geriatr Soc*. 2011; 59:559–562. [PubMed: 21391952]
103. Petit D, et al. Sleep and quantitative EEG in neurodegenerative disorders. *J Psychosom Res*. 2004; 56:487–496. [PubMed: 15172204]
104. Dykierk P, et al. The value of REM sleep parameters in differentiating Alzheimer's disease from old-age depression and normal aging. *J Psychiatr Res*. 1998; 32:1–9. [PubMed: 9693995]
105. Daulatzai MA. Early stages of pathogenesis in memory impairment during normal senescence and Alzheimer's disease. *J Alzheimers Dis*. 2010; 20:355–367. [PubMed: 20164576]
106. Cooke JR, et al. Sustained use of CPAP slows deterioration of cognition, sleep, and mood in patients with Alzheimer's disease and obstructive sleep apnea: a preliminary study. *J Clin Sleep Med*. 2009; 5:305–309. [PubMed: 19968005]
107. Hoch CC, Reynolds CF 3rd. Electroencephalographic sleep in late-life neuropsychiatric disorders. *Int Psychogeriatr*. 1989; 1:51–62. [PubMed: 2490904]
108. Bird, TD. Early-Onset Familial Alzheimer Disease. 1993.
109. Yesavage JA, et al. Sleep/wake disruption in Alzheimer's disease: APOE status and longitudinal course. *J Geriatr Psychiatry Neurol*. 2004; 17:20–24. [PubMed: 15018693]
110. Colas D, et al. Sleep-wake architecture in mouse models for Down syndrome. *Neurobiol Dis*. 2004; 16:291–299. [PubMed: 15193286]
111. Colas D, et al. Sleep and EEG features in genetic models of Down syndrome. *Neurobiol Dis*. 2008; 30:1–7. [PubMed: 18282758]
112. Jyoti A, et al. EEG, activity, and sleep architecture in a transgenic AbetaPPswe/PSEN1A246E Alzheimer's disease mouse. *J Alzheimers Dis*. 2010; 22:873–887. [PubMed: 20858963]
113. Kang JE, et al. Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. *Science*. 2009; 326:1005–1007. [PubMed: 19779148]
114. Cardinali DP, et al. Clinical aspects of melatonin intervention in Alzheimer's disease progression. *Curr Neuropharmacol*. 2010; 8:218–227. [PubMed: 21358972]
115. Bourgeron T. A synaptic trek to autism. *Curr Opin Neurobiol*. 2009; 19:231–234. [PubMed: 19545994]
116. Horsthemke B, Wagstaff J. Mechanisms of imprinting of the Prader-Willi/Angelman region. *Am J Med Genet A*. 2008; 146A:2041–2052. [PubMed: 18627066]
117. Colas D, et al. Sleep disturbances in Ube3a maternal-deficient mice modeling Angelman syndrome. *Neurobiol Dis*. 2005; 20:471–478. [PubMed: 15921919]
118. Zhang J, et al. Fragile X-related proteins regulate mammalian circadian behavioral rhythms. *Am J Hum Genet*. 2008; 83:43–52. [PubMed: 18589395]
119. Soden ME, Chen L. Fragile X protein FMRP is required for homeostatic plasticity and regulation of synaptic strength by retinoic acid. *J Neurosci*. 2010; 30:16910–16921. [PubMed: 21159962]
120. Malow BA, et al. Characterizing sleep in children with autism spectrum disorders: a multidimensional approach. *Sleep*. 2006; 29:1563–1571. [PubMed: 17252887]
121. Miano S, Ferri R. Epidemiology and management of insomnia in children with autistic spectrum disorders. *Paediatr Drugs*. 2010; 12:75–84. [PubMed: 20218744]
122. Buckley AW, et al. Rapid eye movement sleep percentage in children with autism compared with children with developmental delay and typical development. *Arch Pediatr Adolesc Med*. 2010; 164:1032–1037. [PubMed: 21041596]
123. Limoges E, et al. Atypical sleep architecture and the autism phenotype. *Brain*. 2005; 128:1049–1061. [PubMed: 15705609]

124. Doyen C, et al. Melatonin in children with autistic spectrum disorders: recent and practical data. *Eur Child Adolesc Psychiatry*. 2011; 20(5):231–9. [PubMed: 21359552]
125. Loomis AL, et al. Potential Rhythms of the Cerebral Cortex during Sleep. *Science*. 1935; 81:597–598. [PubMed: 17739875]
126. Derbyshire AJ, et al. The effects of anesthetics on action potentials in the cerebral cortex of the cat. *American Journal of Physiology*. 1936; 116:577–596.
127. Michel F, et al. Etude Polygraphique Du Sommeil Chez Le Rat. *Cr Soc Biol*. 1961; 155:2389–2392.
128. Cirelli C, Tononi G. Gene expression in the brain across the sleep-waking cycle. *Brain Res*. 2000; 885:303–321. [PubMed: 11102586]
129. Weiss T, Fifkova E. Sleep Cycles in Mice. *Physiol Bohemoslov*. 1964; 13:242–245. [PubMed: 14167886]
130. Gao XB, et al. Prolonged wakefulness induces experience-dependent synaptic plasticity in mouse hypocretin/orexin neurons. *Journal of Clinical Investigation*. 2007; 117:4022–4033. [PubMed: 18060037]
131. Hendricks JC, et al. Rest in *Drosophila* is a sleep-like state. *Neuron*. 2000; 25:129–138. [PubMed: 10707978]
132. Zhdanova IV, et al. Melatonin promotes sleep-like state in zebrafish. *Brain Res*. 2001; 903:263–268. [PubMed: 11382414]
133. Raizen DM, et al. Lethargus is a *Caenorhabditis elegans* sleep-like state. *Nature*. 2008; 451:569–U566. [PubMed: 18185515]
134. Sternberg PW, Van Buskirk C. Epidermal growth factor signaling induces behavioral quiescence in *Caenorhabditis elegans*. *Nature Neuroscience*. 2007; 10:1300–1307.
135. Gonzales ML, LaSalle JM. The role of MeCP2 in brain development and neurodevelopmental disorders. *Curr Psychiatry Rep*. 2010; 12:127–134. [PubMed: 20425298]
136. Bertram L, et al. The genetics of Alzheimer disease: back to the future. *Neuron*. 2010; 68:270–281. [PubMed: 20955934]
137. Yashiro K, et al. Ube3a is required for experience-dependent maturation of the neocortex. *Nat Neurosci*. 2009; 12:777–783. [PubMed: 19430469]
138. Micheva KD, et al. Single-synapse analysis of a diverse synapse population: proteomic imaging methods and markers. *Neuron*. 2010; 68:639–653. [PubMed: 21092855]
139. Betzig E, et al. Imaging intracellular fluorescent proteins at nanometer resolution. *Science*. 2006; 313:1642–1645. [PubMed: 16902090]
140. Hell SW, Wichmann J. Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy. *Opt Lett*. 1994; 19:780–782. [PubMed: 19844443]
141. Hess ST, et al. Ultra-high resolution imaging by fluorescence photoactivation localization microscopy. *Biophys J*. 2006; 91:4258–4272. [PubMed: 16980368]
142. Huang B, et al. Three-dimensional super-resolution imaging by stochastic optical reconstruction microscopy. *Science*. 2008; 319:810–813. [PubMed: 18174397]
143. Willig KI, et al. STED microscopy reveals that synaptotagmin remains clustered after synaptic vesicle exocytosis. *Nature*. 2006; 440:935–939. [PubMed: 16612384]

Box 1**Current and Future Contributions of Imaging Modalities to studies of Sleep and Synapse Modulation****Confocal and 2-Photon imaging**

In vivo live imaging of *Drosophila* and zebrafish have already significantly contributed to the study of synapse modulation by sleep and circadian rhythms [82-84] (Figure I). However, live imaging of mice and rat cortex is required to further extend these findings into mammals where the majority of sleep physiology has been done in the past. Furthermore, network imaging using Ca²⁺ indicators in sleeping and awake animals should provide an added level of detail on the network firing patterns of the brain during the different periods of sleep and wake.

Array tomography

The synaptic plasticity in sleep is most likely mediated by changes in protein expression on a global level, and the quantification of such changes will be essential for furthering our understanding of sleep. Array tomography is a recent proteomic imaging technique [88, 138](Figure I). Its advantages include the ability to visualize dozens of proteins across entire cortical columns at the synaptic level of resolution, and thus, should be a valuable tool for performing quantitative comparisons of synaptic proteomic changes between tissue collected at different time points during the day/night cycle.

Stochastic Optical Reconstruction Microscopy (STORM)/ Photo-Activated Localization Microscopy (PALM) and Stimulated Emission Depletion (STED)

Proteins that are regulated during sleep (e.g. kinases, channels and receptors) are shuttled and modulated on a subsynaptic level. STORM/PALM and STED are super-resolution imaging technologies that enable single molecule resolution [139-143](Figure I). These technologies will provide the level of resolution needed to decipher the actual molecular modifications occurring at synapses during sleep.

Box 2**Outstanding Questions Box**

- Is sleep-dependent synaptic plasticity in the mammalian brain highly governed by the circadian clock, as has been observed in *Drosophila* and zebrafish? Or is the mammalian cortex different in terms of sleep plasticity, being more sleep-dependent and less clock-dependent?
- Are there specific epochs of synaptic plasticity in the brain? Are there quantitative differences between synaptic and structural plasticity during sleep versus wake?
- Does sleep plasticity occur similarly throughout the brain? More specifically, is there one cycle of synaptic strengthening and elimination, or are there multiple rhythms spread across different brain regions?
- Is sleep-dependent plasticity in the neocortex different from deeper brain regions? How is plasticity correlated with EEG measurements, and is the type of synchrony in the cortex measured via EEG a widespread phenomenon or specific to the cortex?
- How is sleep plasticity behaviorally adaptive? For instance, does sleep optimize function based on prior environmental experience?

Figure 1. Summary of recent data in support of the Synaptic Homeostasis Hypothesis (SHH)
 (a). Synapses, like learning and memories, are known to be affected by circadian rhythms and homeostatic regulation. The SHH posits that synapse accumulation during the day drives a need for synaptic downscaling, which preferentially occurs during sleep. (b-f) Recent studies from diurnal *Drosophila melanogaster* and *Danio rerio* have demonstrated increased synapse components or synapse numbers following wake, sleep, or sleep deprivation. Images are not all to the same scale. (b) Bruchpilot (BRP; an essential constituent of the active zone of all synapses) levels were measured in antennal lobes (AL), β lobes of the mushroom bodies (MB), and ellipsoid body of the central complex (CC) in *Drosophila* [81]. BRP immunofluorescence was found to be increased in animals sleep-deprived for 16 hrs compared to rested controls, shown false-colored on a quantitative scale, with yellow indicating highest levels. (c) Following social enrichment, sleep deprivation was found to lead to the retention of more synaptic terminals in *Drosophila* olfactory lobes (OL). Discs-large (DLG), a postsynaptic protein, was fused to GFP expressed in PDF neurons via a GAL4;UAS approach (i.e. pdf-GAL4/+::UAS-dlgWT-gfp/+). Social enrichment led to increased numbers of GFP-positive terminals that recovered to baseline levels following sleep but not following sleep deprivation [83]. (d) Synaptotagmin (a presynaptic protein) was fused to EGFP and expressed in the gamma lobe of the MB. Right panels show a higher magnification of the area indicated by the yellow square in the left panel. Sleep-deprived flies were found to contain larger GFP-positive puncta in the MB compared to sleeping controls [82]. Scale bar = 10 μ m. (e) Sleep deprivation also interferes with homeostatic downscaling of synapse number in larval zebrafish (7 day old) [84]. Live transgenic fish expressing synaptophysin fused to EGFP in hypocretin neurons (i.e. HCRT:SYPEGFP) displayed significantly more EGFP puncta in axons projecting to the pineal gland (PG) during diurnal wakefulness compared to the nocturnal sleep period. Red arrows depict additional synapses that were not observed during the sleep period in the same fish. (f) Live larval zebrafish expressing HCRT:SYP-EGFP also have more EGFP puncta in hindbrain

(HB) projections from the hypocretin neurons [84]. Red arrows indicate additional synapses that were not observed during the sleep period in the same fish. Abbreviations: HB, hindbrain; Hyp, hypothalamus; OT, optic tectum; PG, pineal gland. Reproduced, with permission, from [81] (b), [83] (c), [82] (d).

	Today	Tomorrow
Two-Photon Confocal	Synaptic puncta in <i>Drosophila</i> and zebrafish varies in accordance to sleep and the circadian rhythm.	Live imaging of spines and synaptic markers in mammals. Single neuron and network analysis of neuronal function during sleep and wake using Ca^{2+} imaging.
Array Tomography	N/A	Characterization of sleep modulation across specific synapse sub-populations. Region specific changes in synapse density and composition.
STORM/ PALM, STED	N/A	Sub-synaptic analysis of changes in channel and protein kinases in sleep and wake. Causal molecular mechanism for synaptic modification in sleep, and whether it is strictly different from wake.

Figure I.

Representative images to illustrate the type of images obtained using the different imaging modalities. Top panel: 2-Photon image of transgenic zebrafish expressing EGFP in all HCRT neurons. Middle panel: Array tomography reconstruction of mouse cortical dendrite labeled with yellow fluorescent protein (YFP; green) and synaptotagmin (red) to label synapses. Bottom panel: Segmented microtubule bundles in mouse cortical white matter as imaged using STORM. The colors are pseudocolors to separate out each individual microtubule. Image Credits: Gordon Wang (top and middle panels), Nicholas C. Weiler and Xiaowei Zhuang (bottom panel). Abbreviations: not available (N/A).

Table 1
Species commonly used in the study of neural plasticity during sleep

Over the past 75 years, studies of sleep have been extended from EEG-based definitions in mammals to behaviorally based sleep criteria encompassing animals with simpler nervous systems. Studies in each species have contributed valuable new information about sleep's effects on neural plasticity and learning.

Species ^a	Sleep state first described & criteria	Evidence for sleep's role in neural plasticity	Experimental approaches used ^b
<i>Homo sapiens</i>	1935 [125] EEG	<ul style="list-style-type: none"> -Learning leads to reactivation of hippocampus during SWS [43]. -Odors can enhance hippocampal activation and memory retention [43]. -Dreaming of a task is associated with enhanced memory [22, 38] 	<ul style="list-style-type: none"> -fMRI and EEG data in subjects following learning trials. -Increased spatial memory performance.
<i>Felis catus</i>	1936 [126] EEG	Ocular dominance column plasticity following monocular deprivation is enhanced by sleep [51].	Microelectrode recordings and optical imaging of visual cortex
<i>Rattus norvegicus</i>	1961 [127] EEG	<ul style="list-style-type: none"> - Specific microcircuits activated during wake are reactivated during sleep [42] -Circuit activity is synchronized between hippocampus and cortex [41]. -Sleep regulates neurogenesis and genes associated with neural function and plasticity [128]. 	<ul style="list-style-type: none"> -Multielectrode array recordings from hippocampus and cortex of mice during sleep following spatial learning tasks. -Microarray gene expression profiling -BrDU labeling for neurogenesis.
<i>Mus musculus</i>	1964 [129] EEG	Wakefulness shown to induce synaptic plasticity in HCRT neurons [130]	Extracellular and whole-cell voltage clamp recording of mouse hypothalamic slices
<i>Drosophila melanogaster</i>	2000 [131] Behavioral criteria -immobility -prone position -arousal threshold -homeostatic regulation	Sleep reduces synapse number throughout the CNS and in specific sets of neurons [81-83]	Measurement of synaptic components throughout the CNS and quantification of synapses in specific neurons using EGF-labeled synaptic proteins
<i>Danio rerio</i>	2001 [132] Behavioral criteria -immobility -floating head-down or horizontal -decreased arousal in response to gentle tapping -homeostatic regulation	Homeostatic and circadian plasticity demonstrated in the hypocretin circuit connecting the hypothalamus to pineal and hindbrain [84]	Two-photon fluorescence microscopy of EGFP-labeled synapses in living larvae

Species ^a	Sleep state first described & criteria	Evidence for sleep's role in neural plasticity	Experimental approaches used ^b
<i>Caenorhabditis elegans</i>	2008 [133] Behavioral criteria - immobility - arousal threshold - homeostatic regulation	Conserved EGF signaling induces Lethargus [134], a sleep-like state correlated with synapse remodeling [98, 99]	Currently correlational only

^a Image credits (from top to bottom): Katharine Mach, emptysound, Eddy Van 3000, bundu, Max Westby, Brian Grone, AJ Cann.

^b Abbreviations: Functional magnetic resonance imaging (fMRI); 5-Bromodeoxyuridine (BrdU)

Table2
Current evidence for circadian and sleep regulation of structural synaptic and circuit changes in non-mammalian animal models

Currently, the primary demonstration of rhythmic changes in synaptic circuits comes from studies in diurnal *Drosophila* and zebrafish. In those systems, dendritic morphology and/or synaptic bouton density and structure have shown to vary according to a daily rhythm, and the magnitude and direction of these changes support the idea that during sleep overall synapse number decreases.

Morphological and structural changes and regulatory process	Circuit and animal model	Experimental technique/ Synaptic markers ^a	Refs
Circadian changes in the size of nuclei, caliber of the axon and morphology and length of the dendritic tree	Monopolar L2 cells in the visual system in <i>Drosophila</i>	IHC on fixed tissue using membrane mCD8-GFP and nuclear S65T-GFP fusion proteins	[78, 80]
Circadian changes in synaptic bouton size	Flight motor neurons in <i>Drosophila</i>	IHC using neuronal markers and the pre-synaptic protein synaptotagmin	[79]
Circadian remodeling in axonal terminals	Pacemaker PDF neurons in <i>Drosophila</i>	IHC using membrane mCD8-GFP and the pre-synaptic fusion protein synaptobrevin-GFP	[77]
Homeostatic sleep-dependent control of synapse number	PDF neurons in <i>Drosophila</i>	IHC using the post synaptic localized fusion protein DLG-GFP and the presynaptic synaptobrevin-GFP fusion protein	[83]
Homeostatic sleep-dependent control of synapse size or number	PDF, visual system VS1 and mushroom body gamma neurons in <i>Drosophila</i>	IHC using the presynaptic synaptotagmin-EGFP marker and an actin-GFP fusion protein to reveal the dendritic spines	[82]
Circadian and homeostatic control of synapse number	Hypocretin/orexin neurons in larval zebrafish	Time-lapse two-photon live imaging using the presynaptic synaptophysin-EGFP fusion protein	[84]

^a Abbreviations: mCD8, mouse lymphocyte marker CD8; IHC, immunohistochemistry

Summary of molecular, synaptic and sleep deficits in various neurodevelopmental and neurological disorders^a.

Table 3

Disease family	Candidate genes	Pathway	Plasticity mechanism	Animal model	Sleep abnormalities	Refs
Alzheimer' s disease	<i>APP</i>	A β accumulation	LTP/LTD, synapse maintenance	TgAPP	Sleep fragmentation, reduced SWS, and slower EEG frequencies	[103, 110, 112, 135, 136]
Angelman syndrome	<i>Ube3a</i>	Ubiquitination	Activity-dependent synaptic proteolysis, LTP	<i>Ube3a</i> ^{m-/p+}	Insomnia, reduced SWS and REMS %, abnormal homeostasis	[117, 137]
Fragile X	<i>FMR1</i>	RNA-binding	Activity-dependent synaptogenesis	<i>FMR1</i> KO	Loss of circadian rhythms	[118, 119]
Rett syndrome	<i>MeCP2</i>	Epigenetic regulator	Activity-dependent synaptic homeostasis	N/A		[135]

^a Abbreviations: not available (N/A); knockout (KO); transgenic mouse model expressing the human APP variant that contains the Swedish mutation known to be associated with familial AD, or wild-type human APP (TgAPP). Mouse model lacking the *Ube3a* gene specifically on the maternal allele (*Ube3a*^{m-/p+}).