Published in final edited form as:

Curr Opin Neurobiol. 2017 June ; 44: 72–79. doi:10.1016/j.conb.2017.03.016.

Sleep, synaptic homeostasis and neuronal firing rates

Chiara Cirelli

Department of Psychiatry, University of Wisconsin, Madison, WI 53719, USA

Abstract

The synaptic homeostasis hypothesis (SHY) states that wake brings about a net overall increase in synaptic strength in many brain circuits that needs to be renormalized by sleep. I will review recent studies that were either specifically designed to test SHY or were interpreted accordingly, including several experiments that focused on changes in neuronal firing rates. I will emphasize that central to SHY is the idea that what is being regulated across the sleep/wake cycle is synaptic strength, not firing rate, and firing rate taken in isolation is not necessarily an adequate proxy for synaptic strength.

Introduction

Changes in synaptic strength underlie learning and memory [1,2] and synaptic strength needs to be tightly regulated to avoid runaway potentiation and depression [3]. It is generally assumed that overall synaptic strength is balanced at all times. By contrast, the synaptic homeostasis hypothesis (SHY) suggests that due to energetic and informational constraints, learning should occur primarily through synaptic potentiation during wake, leading to a net increase in overall synaptic strength, while synaptic renormalization by net depression should occur during sleep, when animals are disconnected from the environment [4]. Previous results from electrophysiological, molecular, and anatomical experiments support the main claim of SHY. For instance, protein levels of GluA1-containing AMPA receptors are higher after wake than after sleep [5] across the entire rat cerebral cortex and hippocampus, and the number of immuno-labeled synaptic puncta increases with enriched wake and decreases with sleep in widespread regions of the fly brain [6]. Electrophysiological markers of synaptic efficacy, such as the slope of cortical evoked responses, also increase broadly after wake and decrease after sleep in both rodents [5] and humans [7].

In the past 2–3 years several other studies were either explicitly designed to test SHY's main idea [8–14], or interpreted their findings in light of SHY [15–17]. I will review these studies starting from those that used more direct measures of synaptic strength, such as number and size of synapses. I will also focus on experiments that assessed both cortical excitability,

Correspondence: Chiara Cirelli, Dept. of Psychiatry, University of Wisconsin-Madison, 6001 Research Park Blvd, Madison, WI 53719 USA, ccirelli@wisc.edu, (608) 263-9236.

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.

defined as the propensity to fire, as well as the potential of the cortex to undergo synaptic changes. Finally, I will summarize the studies that used changes in firing rate to infer the effects of sleep/wake history on synaptic strength. These distinctions are important because SHY's core idea is that what is being regulated across the 24-hour sleep/wake cycle is overall synaptic strength, *not* firing rate. Moreover, changes in firing rates may or may not follow changes in synaptic strength, as they heavily depend on the neuromodulatory tone and the balance between excitation and inhibition, which in turn can affect cortical excitability.

Sleep/wake history and number and size of synapses

One way to assess synaptic strength is by measuring the size and number of synapses. We recently used serial block-face scanning electron microscopy to reconstruct ~ 7,000 spines containing synapses in mouse motor and sensory cortices [10]. We measured the axon-spine interface (ASI), the direct area of apposition between the pre- and the post-synapse, which is a structural measure of synaptic strength [18]. Spine density did not change, and ASI decreased by ~18% after ~ 7 hours of sleep compared to 7 hours of either spontaneous wake at night or enforced wake during the day, indicating that ASI changes are driven by sleep and wake and not by circadian time. The ASI decrease during sleep was proportional to ASI size, indicative of multiplicative scaling. The extent to which this scaling applies to individual synapses could not be assessed, because we could not follow the same synapses across the sleep/wake cycle. At the population level, however, scaling was selective, occurring in ~ 80% of the synapses but sparing those (~ 20%) that were large and/or lacked recycling endosomes, suggesting a distinction between a majority of synapses that are weaker and more plastic, and a minority that are stronger and more stable. This distinction could help explain how sleep allows a widespread synaptic renormalization and at the same time favors memory consolidation, integration, and 'smart' forgetting [4]. These ultrastructural results, at least in the superficial layers of mouse cortex, support the main claim of SHY that a core function of sleep is to renormalize total synaptic strength increased by wake, including enforced wake associated with a novel experience. Crucially, "total" does not mean that all synapses need to be renormalized, and there are several molecular mechanisms by which some synapses could be targeted or protected from down selection during sleep [4]. For instance, a recent study confirmed in the mouse forebrain that sleep leads to an overall decrease in synaptic AMPA receptors, and characterized the crucial role of one molecule, Homer1a, in this process [11]. Interestingly, the same study repeatedly imaged several hundreds of cortical spines with a two-photon microscope over multiple days and nights, and found that downscaling during sleep affected mostly the largest synapses, those with the highest levels of AMPA receptors [11]. This finding contrasts with the ASI changes described above, which seem to spare the largest spines, but the two studies are difficult to compare directly: they both focused on superficial layers of motor cortex, but the ASI experiments were performed in young mice in which all synapses within the reconstructed dendritic branches were measured, while the two-photon experiment used mature mice and focused on stable spines of the apical dendrites of layer V neurons. Thus, the extent to which sleep-dependent downscaling varies across individual spines remains to be fully assessed. Similarly, we do not know how this scaling is modulated by a specific learning experience, such as training in a sleep-dependent motor task [19].

Repeated two-photon imaging studies in adolescent mouse cortex found that overall spine formation is higher after wake while spine elimination is higher after sleep [20,21]. A more recent study found that spine formation increases when motor learning is followed by sleep but is impaired by sleep deprivation, suggesting that spinogenesis triggered by learning is highly sensitive to sleep loss [22]. Of note, the study also showed that post-training spine formation occurs to a similar extent during the day, when mice are mostly asleep, and at night, when they are mostly awake [22]. Thus, it remains to be tested whether post-training sleep has any privileged role, relative to spontaneous wake, in promoting spinogenesis and/or in increasing or decreasing the size of existing spines. Recent findings suggest a crucial role of reduced inhibition in promoting post-learning spine stabilization [23]. Whether sleep and spontaneous wake differently affect inhibitory signaling after motor learning remains unknown.

A recent study in mice found that 5 hours of sleep deprivation led to a decrease in the number of spines and in the length of basal and apical dendrites of CA1 neurons [15]. These changes were due to enhanced pruning caused by increased cofilin activity, and 3 hours of sleep were sufficient for both measures to resume their pre-deprivation levels. The effects were region-specific, as spine density did not change in CA3 neurons and cofilin phosphorylation did not change in prefrontal cortex. The extent and speed of these changes, and the fact that they were associated with memory deficits, are remarkable and not easily explained by the “stress” of the sleep deprivation procedure, because acute stress is expected to increase, not decrease spine density. The study, based on Golgi staining, did not test whether sleep deprivation also affected the size of the remaining synapses, and thus overall synaptic strength. A previous study in rats used serial section transmission electron microscopy to show that number and size of CA1 synapses change in opposite direction after electrical induction of long-term potentiation: the small synapses disappeared and the remaining ones enlarged, so that the summed area occupied by the post-synaptic density, a structural measure of synaptic strength, was preserved [24]. Thus, future studies should assess the effects of spontaneous and forced wake on both number and size of CA1 synapses.

Finally, a recent two-photon imaging study in zebrafish found that the number of inhibitory synapses located on the dendrites of orexin neurons increases with sleep (size was not measured) [25], while the number of presynaptic puncta contacting the same neurons increases with wake [26]. A wake-related increase in excitatory synapses also occurs in other zebrafish neurons [27], suggesting that excitatory and inhibitory synapses may behave differently [25]. Thus, sleep/wake changes in number and size of cortical inhibitory synapses, which account for ~15–20 percent of all cortical synapses, should also be studied at the ultrastructural level.

Sleep/wake history and cortical excitability

Huber and colleagues were the first to test in humans how sleep/wake history affects cortical excitability, i.e. the propensity to respond to a fixed stimulus. They measured the EEG reaction to transcranial magnetic stimulation (TMS) and found that the slope of the early EEG response increased after a night of sleep deprivation and declined after recovery sleep

[7]. Two more recent studies also measured the TMS-induced cortical response as well as the potential of cortical neurons to undergo synaptic plasticity in humans. In one case [8] the authors found that the intensity of the TMS pulse required to induce a motor-evoked potential (MEP) of predetermined amplitude was smaller after a night of sleep deprivation as compared to a night of sleep, indicating increased cortical excitability after wake. Crucially, this study also used TMS to measure the ability of the cortex to undergo LTP-like plasticity with a paired associative stimulation protocol (PAS), in which peripheral electrical stimulation of the left median nerve was followed by TMS of the right primary motor cortex. The PAS protocol elicited an increase in the TMS-evoked MEP of the corresponding hand after sleep but not after sleep deprivation. This result was confirmed at the single-subject level, with 70% of the subjects showing an LTP-like increase after sleep and 75% of the subjects showing instead an LTD-like decrease after sleep deprivation. Testing after sleep and after sleep deprivation occurred at the same time of day, which is important because cortical excitability changes with time spent awake and with circadian time. Specifically, a recent study measured the amplitude of the TMS-evoked EEG response using a constant routine protocol, which helps unmasking circadian influences [28]. In line with previous sleep deprivation experiments that measured the EEG reaction to TMS [7] or intrinsic measures of cortical excitability [29], it was found that EEG responses were larger at the end as compared to the beginning of 29 hours of continuous wake [28]. The same TMS-evoked responses, however, covaried with cortisol levels and showed a significant “dip” in the evening around the so-called wake-maintenance zone, indicating a circadian effect [28].

In another study [9] subjects trained with the right dominant hand in a five-element motor sequence, a task known to induce cortical plasticity. Input-output (IO) curves were obtained before and after training by measuring the amplitude of MEPs elicited by TMS pulses of different intensity delivered to the left primary motor cortex. In the wake group the post-learning IO curve shifted to the left (stronger MEPs responses) relative to pre-training in the first session, which occurred in the morning, but not in the evening session, after 12 hours of wake. In the sleep group, vice versa, stronger MEPs responses after training relative to before training did not occur in the first session in the evening, but were present the next morning after sleep. The authors concluded that the lack of stronger post-training MEPs after 12 hours of wake reflected a reduced potential for plastic changes due to saturation, although their experimental design did not control for possible circadian effects. Overall, both studies reached similar conclusions: sustained (>24 hours) wake increases cortical excitability and, as in rats [5], impairs the potential of the cortex to undergo synaptic potentiation. The propensity to fire, like firing itself, may reflect not only synaptic strength but also the neuromodulatory tone and the balance between excitation and inhibition. Thus, changes in cortical excitability must be carefully evaluated in relation to SHY. The impaired potential to undergo plastic changes, on the other hand, can be seen as more directly supporting SHY's claim that wake-induced net synaptic strengthening brings synapses closer to saturation.

Sleep/wake history and firing rates

Brain activity changes in relation to sleep and wake and is modulated, within each behavioral state, by sleep/wake history. In both cases it is generally measured in terms of

mean population firing rates calculated over several hours of sleep or wake. Averaging across neuronal populations, however, ignore the fact that the firing of cortical and hippocampal cells approximates a log-normal distribution, with most neurons firing little and a few showing fast spiking [30]. Mean firing rates also do not take into account that neuronal activity changes within each behavioral state, especially in wake, due to specific behaviors [31]. Two recent studies have addressed the first issue by measuring how sleep/wake history affects not only mean firing rates, but also the firing of slow and fast neurons separately.

One study performed long recordings across the 24-hour cycle in the rat hippocampus. Using a set of > 1,000 putative CA1 pyramidal cells it found that wake bouts lasting > 15 min led to an increase in firing, while every hour of sleep led to a 11% decrease [16]. Specifically, across long (> 30 min) sleep episodes mean firing rates in NREM sleep declined during both the light and the dark period, and mean firing rates in REM sleep declined during the day (REM bouts at night were short and variable). The decline in firing across NREM sleep correlated with the occurrence of sharp wave ripples (SWRs) and spindles (but not that of hippocampal slow wave activity), while the decline during REM sleep correlated with theta activity. Moreover, theta activity in REM sleep was positively correlated with the incidence rate of spindles and SWRs in the preceding NREM sleep episode, suggesting that the mechanisms underlying the sleep-dependent decline in firing were triggered in NREM sleep and fully actuated during REM sleep [16]. The authors divided the neurons in five quintiles based on their firing rate, and implemented a strict shuffle correction to account for regression to the mean. They found that the decline in firing across sleep occurred in all CA1 neurons independent of their initial level of activity, but there were some differences: the slowest firing CA1 neurons showed the largest decline in firing across sleep, while moderately-firing neurons showed the largest increase in firing across wake [16]. The conclusion was that sleep has the same overall effect on all neurons – a decline in firing rate – but that the extent of these plastic changes differs, with low and medium firing cells showing the greatest firing “plasticity” across the sleep/wake cycle, and the fastest neurons being the least plastic [16,17]. Of note, another recent study found that relative to fast firing neurons, slow firing CA1 place cells promptly change their activity pattern in response to a novel environment, and show greater temporal coactivation and increased bursting during post-experience sleep [32]. Thus, it seems that the slowest spiking neurons in CA1 are both the most plastic during learning and those whose firing shows the largest decline in the course of sleep.

A second study reported that the per-cell mean firing rates of putative pyramidal neurons in the deep layers of the rat frontal cortex spanned 3 orders of magnitude, from 0.005 to 5 Hz, with a median firing rate below 1 Hz [12]. Using a dataset of ~ 1,000 neurons it was found that the mean of the population firing rate, calculated during uninterrupted episodes of NREM sleep, declined over the course of several hours of sleep, in line with previous findings in rat hippocampus (CA1; [33]) and barrel cortex [34]). As previously reported [34], this population effect persisted in the wake period that immediately followed the sleep episode, suggesting that it reflected long-term changes due to sleep/wake history. After dividing all neurons in six sextile groups based on their wake firing rates, the authors found that uninterrupted periods of NREM sleep significantly decreased the firing only of the most

active group, while REM bouts led to a decline in firing in all neurons. Moreover, short (< 40 sec) episodes of quiet wake within NREM bouts, defined as “microarousals”, increased the firing of the 3 slower firing groups [12]. The authors concluded that sleep as a whole (defined as NREM and REM bouts and microarousals) decreased the overall population firing because of its specific effects on the fastest spiking cells, while the least active neurons actually increased firing across episodes of NREM bouts and microarousals [12]. The authors suggested that sleep helps to “equalize” fast and slow spiking cells, boosting activity in the least active neurons and decreasing it in the most active ones, and interpreted these results as being inconsistent with SHY. However, firing rates cannot be used alone as proxy for synaptic strength, neither to support nor to refute SHY. Moreover, the finding of a sleep-related increased firing in the slowest cortical neurons relies on the questionable choice of counting the many microarousals (9% of total “sleep” time) as sleep. Finally, this study in cortex does not seem to have followed the same strict methods to correct for regression to the mean used in the hippocampal study [16] and in a further more detailed analysis of the same data [17].

Sleep/wake, firing rates in visual cortex and monocular deprivation

Another recent study performed long-term (9 days) recordings in the primary visual cortex (V1) of adolescent mice, to determine whether the recovery of neuronal activity occurring during 6 days of monocular deprivation (MD) is state-dependent. The 99 continuously recorded neurons showed a large range of baseline firing, from 0.02 to 20 Hz. During MD, neurons in the contralateral monocular part of V1 showed a decline in mean firing rate that reached a nadir after 2 days of MD, followed by a gradual recovery of firing over the following 3–4 days [14]. While the early loss and late recovery of firing were expected [35], the novel finding was that the deprived neurons reverted to their baseline activity in a state-dependent manner: firing increased across periods of active wake, showed a trend to increase during quiet wake, but did not change during NREM sleep and REM sleep [14]. V1 neurons in the control (no-MD) hemisphere showed no clear changes in mean population firing during 4 hours spent mainly (> 65% of total time) asleep or awake. The authors concluded that the homeostatic recovery of firing during visual deprivation is enabled by wake but inhibited by sleep, and surprisingly presented these findings as evidence against SHY. In fact, previous MD studies found that the recovery of function in the deprived eye is mediated by Hebbian and/or homeostatic mechanisms that lead to increases in synaptic strength, and in SHY’s view it is wake, not sleep, that biases the brain towards synaptic potentiation. Thus, when - and only when - taken together with more direct evidence for synaptic potentiation, these results seem at the very least consistent with SHY.

The recent results in mice are also notable in light of a series of previous experiments in cats using a much shorter (6 hours) MD paradigm. MD led to a shift in ocular dominance due to a small decline in the visual cortical responses to the deprived eye, and an increase in the response to the non-deprived eye [36,37]. Notably, this shift was promoted by post-MD sleep, probably both NREM sleep [36] and REM sleep [38], but not by sleep deprivation. Two underlying mechanisms have been proposed. One involves the activation of the ERK-MEK pathway during early sleep (but not wake) immediately after MD [39], and the other involves a rapid switch in the excitation/inhibition balance [40]. Indeed, likely because of its

short duration, MD in cats does not decrease the firing of pyramidal neurons responding to the deprived eye, but reduces those of fast spiking interneurons, prompting the authors to propose that reduced inhibition may account for the later increased firing during sleep of the pyramidal cells responding to the open eye [40]. Consistent with this idea, two-photon imaging in mice showed that long MD does not change the total number of spine synapses, but enhances the elimination of inhibitory synapses [41]. In summary, the recovery of function in mice, as assessed in the course of a long MD by measuring spontaneous firing of pyramidal cells, occurs during wake but not during sleep. In cats, a short MD does not affect the firing of pyramidal neurons but the early loss of inhibition seems to play a crucial role in boosting the activity of the spared eye. Of note, in cats sleep does not seem to be required for the recovery of function after reverse MD, a different paradigm in which the previously closed eye is reopened and the previously spared eye is closed [42]. Thus, the effects of sleep vary and the factors underlying these differences may include animal species, duration and the paradigm used for MD, whether recovery was measured during or after MD, and how recovery of function was measured.

Finally, independent of MD, another study assessed changes in firing rates in V1 after mice were exposed for one hour to a grating stimulus of a specific orientation [13,43]. Firing rates did not change immediately after the one-hour training, but sustained increase in firing of V1 neurons occurred for several hours after training in sleep, while firing decreased in wake. Visually evoked firing in response to the presented stimulus increased only several hours after training, and only if this time was spent asleep. These findings were interpreted as not supportive of SHY, but the authors acknowledge that what they measured was neuronal activity, not synaptic strength [43].

Sleep need and firing rates

While the experiments reviewed so far looked at the effects of sleep/wake history on neuronal activity, two recent studies asked how the need for sleep changes after neurons display a sleep-like firing pattern during wake, or are forced to maintain wake-like firing during sleep. In one experiment rats were mostly awake for more than 6 hours after receiving systemic repeated injections of atropine, a cholinergic antagonist [44]. The animals were moving and appeared to behave normally, although performance in learning and vigilance tasks was not assessed. Despite the wake-like behavior, the EEG showed sleep-like slow waves (unit activity was not recorded) and the expression of Fos in the cerebral cortex was low. When the effects of atropine abated, the rats showed an increase in NREM sleep duration relative to baseline, associated with an increase in slow wave activity (SWA), a reliable measure of sleep intensity [45]. During the atropine-induced state of wake with slow waves, Fos expression was high in many brainstem and hypothalamic arousal systems, prompting the authors to conclude that activation of these centers, in a seemingly normally behaving animal, was sufficient to trigger a sleep homeostatic response [44].

In another study the same group of mice underwent 6 hours of sleep deprivation with exposure to novel objects and, a few days apart and in counterbalanced order, 6 hours of optogenetic stimulation during NREM sleep [46]. In the latter condition laser stimulation was restricted to a local population of cortical neurons, with the goal of forcing these cells to

maintain high levels of activity during sleep, as high as those seen during sleep deprivation. Neurons in this area switched to wake-like tonic firing, but the laser pulses did not wake up the mice and the rest of the brain showed the expected sleep pattern of activity. Sleep deprivation led to an increase in SWA during subsequent sleep, together with an increase in cortical neuronal synchrony and in the number and duration of OFF periods. The increase in SWA was correlated with both neuronal synchrony and time spent OFF, confirming that in mouse cortex, as in rat cortex [34], the post-deprivation rebound in SWA is linked to changes in both cortical synchrony and OFF periods measures. Optogenetic stimulation during sleep produced very different results: SWA and cortical synchrony decreased, the number and duration of OFF periods did not change, and SWA was not correlated with either measure. The conclusion was that a sustained increase in population firing alone is unlikely to be the main reason why sleep pressure builds up during prolonged wake. A more likely candidate is synaptic potentiation, which can also account for the increase in neuronal coupling and cortical synchrony observed after prolonged wake [46]. A long-standing hypothesis is that neurons fire less during NREM sleep to recover from the “fatigue” accrued during wake, when overall synaptic activity is higher than in sleep [47,48]. These two studies, however, seem to suggest that the link between sleep need and cortical firing is not direct.

Conclusions

Several recent studies, mainly in rodents and humans, have tested SHY by measuring how number and/or size of synapses, neuronal excitability, and the potential to undergo plastic changes are affected by the sleep/wake history. An ultrastructural study in mouse cortex found that both spontaneous and forced wake led to an almost identical increase in the size of excitatory synapses without change in number, while sleep led to a corresponding decrease. Results consistent with SHY are also seen in human cortex after sleep deprivation, in terms of propensity to fire (increased) and potential to undergo plastic changes (decreased). Several other studies draw conclusions in favor or against SHY based solely on changes in firing rate although the latter, when taken in isolation, is not a good proxy for synaptic strength.

Acknowledgments

Supported by National Institute of Health grants 1R01MH099231 and 1P01NS083514. I thank Giulio Tononi for his comments.

References

*of special interest

**of outstanding interest

1. Feldman DE. Synaptic mechanisms for plasticity in neocortex. *Annu Rev Neurosci.* 2009;32, 33–55.
2. Huganir RL, Nicoll RA. Ampars and synaptic plasticity: The last 25 years. *Neuron.* 2013; 80(3): 704–717. [PubMed: 24183021]
3. von der Malsburg C. Self-organization of orientation sensitive cells in the striate cortex. *Kybernetik.* 1973; 14(2):85–100. [PubMed: 4786750]

4. Tononi G, Cirelli C. Sleep and the price of plasticity: From synaptic and cellular homeostasis to memory consolidation and integration. *Neuron*. 2014; 81(1):12–34. A recent comprehensive review of the synaptic homeostasis hypothesis. [PubMed: 24411729]
5. Vyazovskiy V, Cirelli C, Pfister-Genskow M, Faraguna U, Tononi G. Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. *Nature neuroscience*. 2008;11, 200–208. [PubMed: 18073776]
6. Bushey D, Tononi G, Cirelli C. Sleep and synaptic homeostasis: Structural evidence in drosophila. *Science*. 2011;332, 1576–1581. [PubMed: 21659569]
7. Huber R, Mäki H, Rosanova M, Casarotto S, Canali P, Casali AG, Tononi G, Massimini M. Human cortical excitability increases with time awake. *Cerebral cortex*. 2013; 23(2):1–7. [PubMed: 22275480]
8. Kuhn M, Wolf E, Maier JG, Mainberger F, Feige B, Schmid H, Burklin J, Maywald S, Mall V, Jung NH, Reis J, et al. Sleep recalibrates homeostatic and associative synaptic plasticity in the human cortex. *Nature communications*. 2016; 7(12455) A TMS study assessing cortical excitability and the potential of the cortex to undergo synaptic potentiation after sleep deprivation.
9. de Beukelaar TT, Van Soom J, Huber R, Wenderoth N. A day awake attenuates motor learning-induced increases in corticomotor excitability. *Front Hum Neurosci*. 2016; 10(138)
10. de Vivo L, Bellesi M, Marshall W, Bushong EA, Ellisman MH, Tononi G, Cirelli C. Ultrastructural evidence for synaptic scaling across the wake/sleep cycle. *Science*. 2017; 355(6324):507–510. The first 3-D electron microscopy study to assess changes in synaptic size across sleep and wake in mouse cortex. It shows broad but selective downscaling of synapses with sleep. [PubMed: 28154076]
11. Diering GH, Nirujogi RS, Roth RH, Worley PF, Pandey A, Huganir RL. Homer1a drives homeostatic scaling-down of excitatory synapses during sleep. *Science*. 2017; 355(6324):511–515. The first identification of a specific molecular mechanism underlying sleep-mediated synaptic renormalization. [PubMed: 28154077]
12. Watson BO, Levenstein D, Greene JP, Gelinas JN, Buzsaki G. Network homeostasis and state dynamics of neocortical sleep. *Neuron*. 2016; 90(4):839–852. A study showing the effects of sleep and wake on the firing of pyramidal neurons in frontal cortex. [PubMed: 27133462]
13. Aton SJ, Suresh S, Broussard C, Frank MG. Sleep promotes cortical response potentiation following visual experience. *Sleep*. 2014; 37(7):1163–1170. [PubMed: 25061244]
14. Hengen KB, Pacheco AT, McGregor JN, Van Hooser SD, Turrigiano GG. Neuronal firing rate homeostasis is inhibited by sleep and promoted by wake. *Cell*. 2016; 165(1):180–191. A study that by recording V1 neurons for many days shows how the recovery of firing during monocular deprivation is gated by behavioral states. [PubMed: 26997481]
15. Havekes R, Park AJ, Tudor JC, Luczak VG, Hansen RT, Ferri SL, Bruinenberg VM, Poplawski SG, Day JP, Aton SJ, Radwanska K, et al. Sleep deprivation causes memory deficits by negatively impacting neuronal connectivity in hippocampal area ca1. *Elife*. 2016;5. A study showing rapid effects of sleep deprivation on the number of CA1 spines, how they relate to memory deficits, and the underlying molecular mechanisms.
16. Miyawaki H, Diba K. Regulation of hippocampal firing by network oscillations during sleep. *Curr Biol*. 2016; 26(7):893–902. A well controlled study showing the effects of sleep and wake on hippocampal CA1 firing. [PubMed: 26972321]
17. Miyawaki H, Diba K. Differential state-dependence of low and high firing neurons in the hippocampus. *BioRxiv*. 2016 A detailed analysis showing that strict controls for regression to the mean are necessary when assessing the effects of sleep and wake on low and high firing neurons.
18. Cheetham CE, Barnes SJ, Albieri G, Knott GW, Finnerty GT. Pansynaptic enlargement at adult cortical connections strengthened by experience. *Cerebral cortex*. 2014; 24(2):521–531. [PubMed: 23118196]
19. Nagai H, de Vivo L, Bellesi M, Ghilardi M, Tononi G, Cirelli C. Sleep consolidates motor learning of complex movement sequences in mice. *Sleep*. 2016 In press.
20. Maret S, Faraguna U, Nelson AB, Cirelli C, Tononi G. Sleep and waking modulate spine turnover in the adolescent mouse cortex. *Nature neuroscience*. 2011; 14(11):1418–1420. [PubMed: 21983682]

21. Yang G, Gan WB. Sleep contributes to dendritic spine formation and elimination in the developing mouse somatosensory cortex. *Dev Neurobiol.* 2012; 72(11):1391–1398. [PubMed: 22058046]
22. Yang G, Lai CS, Cichon J, Ma L, Li W, Gan WB. Sleep promotes branch-specific formation of dendritic spines after learning. *Science.* 2014; 344(6188):1173–1178. A study showing the effects of sleep loss on spine formation and motor learning. [PubMed: 24904169]
23. Chen SX, Kim AN, Peters AJ, Komiyama T. Subtype-specific plasticity of inhibitory circuits in motor cortex during motor learning. *Nature neuroscience.* 2015; 18(8):1109–1115. A study showing that after motor learning changes in excitatory spines are preceded by structural changes in inhibitory axons. [PubMed: 26098758]
24. Bourne JN, Harris KM. Coordination of size and number of excitatory and inhibitory synapses results in a balanced structural plasticity along mature hippocampal cal dendrites during ltp. *Hippocampus.* 2011; 21(4):354–373. [PubMed: 20101601]
25. Elbaz I, Zada D, Tovin A, Braun T, Lerer-Goldshtein T, Wang G, Mourrain P, Appelbaum L. Sleep-dependent structural synaptic plasticity of inhibitory synapses in the dendrites of hypocretin/orexin neurons. *Mol Neurobiol.* 2016
26. Appelbaum L, Wang G, Yokogawa T, Skariah GM, Smith SJ, Mourrain P, Mignot E. Circadian and homeostatic regulation of structural synaptic plasticity in hypocretin neurons. *Neuron.* 2010; 68(1):87–98. [PubMed: 20920793]
27. Elbaz I, Lerer-Goldshtein T, Okamoto H, Appelbaum L. Reduced synaptic density and deficient locomotor response in neuronal activity-regulated pentraxin 2a mutant zebrafish. *FASEB J.* 2015; 29(4):1220–1234. [PubMed: 25466900]
28. Ly JQM, Gaggioni G, Chellappa SL, Papachilleos S, Brzozowski A, Borsu C, Rosanova M, Sarasso S, Middleton B, Luxen A, Archer SN, et al. Circadian regulation of human cortical excitability. *Nature communications.* 2016; 7 A TMS study showing that cortical excitability depends on wake duration as well as on circadian time.
29. Meisel C, Schulze-Bonhage A, Freestone D, Cook MJ, Achermann P, Plenz D. Intrinsic excitability measures track antiepileptic drug action and uncover increasing/decreasing excitability over the wake/sleep cycle. *Proceedings of the National Academy of Sciences of the United States of America.* 2015; 112(47):14694–14699. [PubMed: 26554021]
30. Buzsaki G, Mizuseki K. The log-dynamic brain: How skewed distributions affect network operations. *Nat Rev Neurosci.* 2014; 15(4):264–278. A comprehensive review of the evidence for log-normal distribution of several brain features, from firing rates to synaptic size. [PubMed: 24569488]
31. Fisher SP, Cui N, McKillop LE, Gemignani J, Bannerman DM, Oliver PL, Peirson SN, Vyazovskiy VV. Stereotypic wheel running decreases cortical activity in mice. *Nature communications.* 2016;7.
32. Grosmark AD, Buzsaki G. Diversity in neural firing dynamics supports both rigid and learned hippocampal sequences. *Science.* 2016; 351(6280):1440–1443. A study assessing the ability of CA1 neurons to change their pattern of activity in response to a learning experience, showing that slow and fast firing neurons behave differently. [PubMed: 27013730]
33. Grosmark AD, Mizuseki K, Pastalkova E, Diba K, Buzsaki G. Rem sleep reorganizes hippocampal excitability. *Neuron.* 2012; 75(6):1001–1007. [PubMed: 22998869]
34. Vyazovskiy VV, Olcese U, Lazimy YM, Faraguna U, Esser SK, Williams JC, Cirelli C, Tononi G. Cortical firing and sleep homeostasis. *Neuron.* 2009; 63(6):865–878. [PubMed: 19778514]
35. Hengen KB, Lambo ME, Van Hooser SD, Katz DB, Turrigiano GG. Firing rate homeostasis in visual cortex of freely behaving rodents. *Neuron.* 2013; 80(2):335–342. [PubMed: 24139038]
36. Frank MG, Issa NP, Stryker MP. Sleep enhances plasticity in the developing visual cortex. *Neuron.* 2001; 30(1):275–287. [PubMed: 11343661]
37. Aton SJ, Seibt J, Dumoulin M, Jha SK, Steinmetz N, Coleman T, Naidoo N, Frank MG. Mechanisms of sleep-dependent consolidation of cortical plasticity. *Neuron.* 2009; 61(3):454–466. [PubMed: 19217381]
38. Dumoulin B, Aton SJ, Seibt J, Renouard L, Coleman T, Frank MG. Rapid eye movement sleep promotes cortical plasticity in the developing brain. *Sci Adv.* 2015; 1(6):e1500105. [PubMed: 26601213]

39. Dumoulin MC, Aton SJ, Watson AJ, Renouard L, Coleman T, Frank MG. Extracellular signal-regulated kinase (erk) activity during sleep consolidates cortical plasticity in vivo. *Cerebral cortex*. 2015; 25(2):507–515. [PubMed: 24047601]
40. Aton SJ, Broussard C, Dumoulin M, Seibt J, Watson A, Coleman T, Frank MG. Visual experience and subsequent sleep induce sequential plastic changes in putative inhibitory and excitatory cortical neurons. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; 110(8):3101–3106. [PubMed: 23300282]
41. Chen JL, Villa KL, Cha JW, So PT, Kubota Y, Nedivi E. Clustered dynamics of inhibitory synapses and dendritic spines in the adult neocortex. *Neuron*. 2012;74, 361–373.
42. Dadvand L, Stryker MP, Frank MG. Sleep does not enhance the recovery of deprived eye responses in developing visual cortex. *Neuroscience*. 2006; 143(3):815–826. [PubMed: 17000056]
43. Durkin J, Aton SJ. Sleep-dependent potentiation in the visual system is at odds with the synaptic homeostasis hypothesis. *Sleep*. 2016; 39(1):155–159. [PubMed: 26285006]
44. Qiu MH, Chen MC, Lu J. Cortical neuronal activity does not regulate sleep homeostasis. *Neuroscience*. 2015:297, 211–218.
45. Borbely AA, Daan S, Wirz-Justice A, Deboer T. The two-process model of sleep regulation: A reappraisal. *J Sleep Res*. 2016; 25(2):131–143. [PubMed: 26762182]
46. Rodriguez AV, Funk CM, Vyazovskiy VV, Nir Y, Tononi G, Cirelli C. Why does sleep slow-wave activity increase after extended wake? Assessing the effects of increased cortical firing during wake and sleep *The Journal of Neuroscience*. 2016; 36(49):12436–12447. [PubMed: 27927960]
47. Rechtschaffen A. Current perspectives on the function of sleep. *Perspect Biol Med*. 1998; 41(3): 359–390. [PubMed: 9604368]
48. Vyazovskiy VV, Harris KD. Sleep and the single neuron: The role of global slow oscillations in individual cell rest. *Nat Rev Neurosci*. 2013; 14(6):443–451. [PubMed: 23635871]

Highlights

- In mouse cortex sleep is associated with broad but selective synaptic downscaling
- Acute sleep loss negatively impacts synapses in CA1 more than in cortex
- Cortical excitability in humans varies with circadian time and increases with sleep deprivation
- The potential of the cortex to undergo synaptic potentiation is reduced after sleep deprivation

Figure 1.

Top: schematic diagram describing the main claim of SHY: net synaptic increase occurs during wake (during the day in humans and diurnal animals), when many circuits in the brain get potentiated (red lines in the brain schematic), resulting in cellular and systems' costs, followed by synaptic renormalization during sleep, when most, if not all circuits undergo synaptic down-selection (green lines). Bottom: parameters used to test SHY. The color gradient in the column labeled "synaptic strength" indicates that while structural and molecular measures more directly reflect synaptic strength, electrophysiological measures such as firing rates and evoked responses can be strongly affected by other factors that modulate intrinsic neuronal excitability, including the levels of neuromodulators and the

balance between excitation and inhibition, and thus cannot be used alone to infer synaptic strength. In the upper panels (structural and molecular), the red line indicates the axon to spine interface (ASI), and the yellow area outlines the head of the spine. Excitatory glutamatergic receptors (AMPA) are shown as squared boxes close to the ASI. Minis = miniature excitatory synaptic currents.