Sleep loss and structural plasticity
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Wakefulness and sleep are dynamic states during which brain functioning is modified and shaped. Sleep loss is detrimental to many brain functions and results in structural changes localized at synapses in the nervous system. In this review, we present and discuss some of the latest observations of structural changes following sleep loss in some vertebrates and insects. We also emphasize that these changes are region-specific and cell type-specific and that, most importantly, these structural modifications have functional roles in sleep regulation and brain functions. Selected mechanisms driving structural modifications occurring with sleep loss are also discussed. Overall, recent research highlights that extending wakefulness impacts synapse number and shape, which in turn regulate sleep need and sleep-dependent learning/memory.

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Introduction
Sleep is required for life in mammals as well as many non-mammalian species. Accordingly, sleep loss triggers numerous consequences for the nervous system and also importantly impacts other physiological functions [1]. In the nervous tissue specifically, current research supports that preventing sleep for only few hours can modify the structure of cellular compartments, especially for compartments serving neuronal communication such as dendritic spines [2**,3**]. Similar structural changes have also been reported to occur under normal/undisturbed alternations of wakefulness and sleep states. For instance, a higher number of synaptic puncta was observed in the day compared to the night in orexin neurons in zebrafish [4*], and higher synapse density was shown in the mouse cerebral cortex or hippocampus during the early night compared to early day [5,6]. However, these last observations may also be the results of simultaneous changes in internal circadian time [7], and we will thus here focus on research featuring sleep deprivation data. Importantly, the field has now entered an era that identifies the functional roles of sleep loss-driven structural changes in neuronal functions including in sleep regulation itself, which renders latest discoveries even more exciting in terms of their contribution to neuroscience.

We will first emphasize in this review some of the latest findings regarding the impact of sleep loss on the structure of specific components of the central nervous system, and then discuss their functional implications in sleep regulation and in sleep-dependent brain functions. Lastly, we will present some key mechanisms likely underlying these sleep loss-driven structural changes, and conclude with future steps to further understanding of origins and roles of structural changes occurring with sleep loss.

Puncta, spines and glia
The structural features that have received the most attention in sleep deprivation studies are the number of synaptic puncta or of dendritic spines [2**,3**,4*,8,9,10**,11], which is generally considered an index of synapse number. Other structural aspects shown to be impacted by sleep loss concern the size of synaptic puncta [10**], the length of dendritic branches [3**,9], and the astrocytic coverage of (apposition to) synaptic contacts [12].

Synaptic puncta
Earlier findings have highlighted that sleep deprivation results in more synaptic puncta and spines in comparison to sleep in specific cell populations in zebrafish and Drosophila [4*,9]. These findings have recently been extended to a subset of neurons (R2 neurons) of the ellipsoid body (EB) of the fruit fly [10**]. The EB is part of the central complex of the fly head that comprises other sleep regulatory regions such as the dorsal fan-shaped body [13,14**]. In the study of Liu et al. [10**], the presynaptic marker Bruchpilot (BRP) was used as an index of active zones. Sleep deprivation (12-hours) increased both the number and size of BRP puncta, which was observed to be restored to baseline values 24-hours after sleep deprivation. This observation was specific to R2 neurons as authors observed no increase in BRP staining in R3 cells, another neuronal subset of the EB,
and in local neurons of antennal lobes. However, it remains to be determined if this observation is reflecting an increase in the number of synaptic contacts for a given neuronal branch size (i.e., density), or if sleep loss rather increases the length and arborisation of neurites, which results in increased puncta number but not necessarily density — two situations that can affect the magnitude of cell excitability differently. More spines after sleep deprivation have indeed been shown to be fully explained by increased dendrite length [9], and assessment of puncta density normalized per µm of neuronal branch would eventually clarify the scenario for R2 neurons. Importantly, for a given cell, structural changes following sleep deprivation seem to be synapse-specific. In fact, for orexin neurons of zebrafish larvae, the number of synaptic puncta of axons (i.e., presynaptic puncta) is increased by sleep deprivation [4*], but inhibitory synapses on dendrites of the same neurons are decreased [15]. The exact relationship between these changes remains to be identified, but decreased inhibition to these cells could induce a state of hyperexcitability.

**Dendritic spines**

In the cerebral cortex, two-photon sleep studies imaging the same neuronal branch of layer V pyramidal neurons across different sleep conditions indicated that spines are formed and eliminated during both wakefulness and sleep, and that sleep loss seems to reduce spine elimination compared to sleep in young mice [11,16] but not in adult mice [11]. Similarly, a more recent observation indicates that a 24-hours sleep deprivation increases spine density of pyramidal neurons of the layer III of the prefrontal cortex in old rats only, and not in young adult animals [8]. This could suggest that the effect of sleep deprivation on spine number in the cerebral cortex of adult rodent are subtle, at least for the regions and cell types examined (e.g., pyramidal neurons of sensorimotor, barrel and prefrontal cortices). Alternatively, sleep loss-driven spine changes could depend on previous neuronal activity (e.g., learning task-dependent) or occur at only specific locations for a given cell. Indeed, a compelling study showed that sleep loss impairs the formation and survival of new spines on the apical tuft of layer V pyramidal neurons in the motor cortex after motor training [3**]. This observation was enabled by a detailed characterization of different dendritic branches, and the resulting finding that motor training was driving spine formation preferably on specific branches [3**]. In this case, learning-associated spine formation was impaired by sleep deprivation on very specific neuronal branches of a precise cell type (Figure 1), which is reminiscent of the recent observations made in the hippocampus detailed below.

The hippocampus has repeatedly been shown to be impacted by sleep loss, which leads to impaired memory [Havekes and Abel, this issue of *Curr Opin Neurobiol*], and recent studies have also quantified structural changes in the hippocampus in response to sleep deprivation. A decrease in spine density was observed after 5 hours or 24 hours of sleep deprivation in the CA1 region [2**,8], but not in CA3 [2**]. In addition to a sleep loss-dependent reduction in spine density, Havekes *et al.* observed that sleep deprivation reduced dendrite length and that both spine density and dendrite length were restored to non-sleep deprived values after recovery sleep [2**]. Again, this observation seems to apply to specific dendritic branches of pyramidal cells, because it was not the case for branches very close or far from the soma. In sum, pyramidal cells of both the cerebral cortex and the

**Figure 1**

Schematic view of the impact of sleep and sleep deprivation on spine formation and density. (a) The effect of sleep on dendritic spine formation in pyramidal neurons of the motor cortex after motor training is depicted (schematic representation of findings in Ref. [3**]). Sleep promotes spine formation on specific branches of dendrites of the apical tuft of layer V pyramidal neurons (spines newly formed represented in red), which was impaired by sleep deprivation. (b) The effect of acute sleep deprivation and sleep on dendritic spine density and dendritic branch length of hippocampal CA1 pyramidal neurons in mice (schematic representation of findings in Ref. [2**]). Sleep restores spine density and dendritic length of hippocampal neurons (spines removed by acute sleep deprivation represented in blue).
hippocampus respond to sleep deprivation by a decrease in spine number in comparison to a sleep condition (Figure 1). Although spines of pyramidal cells are defined postsynaptic structures allowing an easier quantification, future structural studies should also consider imaging the presynaptic compartment as well as synaptic contact in other cell types such as in cortical interneurons shown to respond to elevated sleep need [17].

Thus, in general, sleep loss appears to increase synaptic puncta and spine number compared to sleep in neuronal populations of the fruit fly head, whereas in rodents, sleep loss leads to a lower spine density and shorter dendrite length. The reason for this difference is unclear but may reside in the fact that sleep-regulatory neurons have been studied in the fly, whereas neuronal populations regulating sleep-dependent functions but not tightly involved in sleep regulation were targeted in rodents. In addition, investigations of pre or postsynaptic structural markers are likely not directly comparable, and, for the dendritic tree only, structural changes should originate as a function of inputs received that will differ according to the location within the tree.

Astrocytes

Although the vast majority of structural changes characterized after sleep loss have been described for neurons, modifications are also to be expected for astrocytes in particular, given their required role in basal synaptic transmission and neuronal plasticity [18,19]. Astrocytes have a privileged access to synapses with their ‘morphologically active’ perisynaptic astrocytic processes (PAPs), whose motility is increased by synaptic activity [20]. PAPs were indeed shown to move closer to the synaptic cleft of larger spines after a 4-hours sleep deprivation and after 4 days of sleep restriction in the mouse prefrontal cortex [12]. Recent observation of reversible changes in cellular localization of glial glutamate transporter-1 as a function of sleep history in the mouse lateral hypothalamus also support structural changes in astrocytes associated with sleep loss in subcortical neuronal structures [Briggs et al., submitted]. Nevertheless, how acute sleep loss impacts the morphology of glial cells in different nervous tissues mostly remains to be established for both non-mammalian and mammalian species.

Functional roles

Of great importance is the fact that structural changes observed in response to sleep loss have now been shown to have functional implications, and not solely a non-functional consequence of prolonged wake. This was to be expected as structural changes shape synaptic functioning. For instance, increased length of spine neck reduces synaptic strength as indexed by the amplitude of excitatory postsynaptic potential [21]. Two types of functional roles of sleep loss-dependent structural plasticity will be addressed in a nutshell below: their role in sleep regulation and in sleep-regulated functions.

Regulation of sleep

First, research in non-mammals supports that changes in synaptic puncta and spines are regulating sleep, because altering these structural features changes sleep amount. For example, social enrichment in young flies, which was shown to increase synaptic puncta in ventral lateral neurons, results in increased sleep [22]. In older flies, social enrichment neither increase puncta number nor sleep, but increased sleep after social enrichment was restored by rescuing changes in synaptic puncta using different approaches [22]. In a zebrafish model of neurological disorder, decreased synapse density and number of dendritic branches associated with increased total sleep and sleep fragmentation [23]. Importantly, in the fly study mentioned above [10**], structural changes in the R2 neurons after sleep deprivation contributed to homeostatic sleep need as preventing sleep loss-driven changes in number and size of BRP puncta attenuated the sleep rebound. Therefore, structural synaptic changes appear causally linked to sleep regulation.

Sleep-regulated functions

Second, impairments in spine formation or density under sleep loss were directly shown to be detrimental to spatial memory and motor learning [2**,**3**]. Specifically, in the article by Yang et al. [3**], sleep-dependent spine formation and survival in the motor cortex contributes to motor performance improvement in the rotarod task as quantified by increased average running speed, because sleep deprivation-induced attenuation of spine formation was detrimental to performance. Moreover, in the hippocampus, blocking structural changes occurring with sleep deprivation using two different genetic strategies prevented performance decrement in object recognition as well as the change of its electrophysiological correlate (i.e., long-term potentiation) [2**]. Recently, hippocampal sleep-dependent memory consolidation was shown to be regulated by rhythmic oscillations during sleep [24**]. Therefore, although sleep loss-driven structural changes in the hippocampus may be anticipated to mostly shape functions regulated by sleep and not necessarily sleep itself, the co-existing relationships between structural plasticity and memory and between memory and sleep oscillations may suggest a role for structural changes in sleep regulation more directly.

Underlying mechanisms

The identification of mechanisms underlying sleep loss-dependent structural plasticity will increase understanding of the roles of these changes in neuronal systems. Among the multiple molecular modifications that can impact neurons and glia morphology, we will briefly emphasize some of the most recent and relevant in the context of sleep deprivation.
Neurotransmission and intracellular signaling

One main pathway triggering structural changes with sleep loss applying to excitatory synapses concerns the cascade by which glutamate transmission changes calcium signaling (Figure 2). The modulation of N-methyl-D-aspartate glutamate receptors (NMDAR) activity is a well-documented way to modify the structure of dendritic spines [25], and sleep deprivation impacts NMDAR in different neuronal structures [10**,26,27]. As such, blocking NMDAR or intracellular Ca\(^{2+}\) modifications prevents sleep deprivation-driven changes in the number and size of synaptic puncta [10**], while NMDAR inhibition attenuated sleep-dependent spine formation [3**]. In flies, sleep loss appears to increase NMDAR activity and intracellular Ca\(^{2+}\) in specific circuits, which not only increases presynaptic puncta number and size, but could also explain increased neuronal excitability [10**,28]. In rodents, acute sleep loss changes NMDAR in a manner that decreases their Ca\(^{2+}\) permeability (i.e., predominance of NR2A over NR2B) in both the hippocampus and

Figure 2

Schematic view of effects of acute sleep loss on synaptic features that can mediate structural reorganization. (a) Sleep deprivation was show to increase intracellular [Ca\(^{2+}\)] via an N-methyl-D-aspartate glutamate receptor (NMDAR)-dependent mechanism in R2 neurons of the fruit fly, which functionally associates with increased size and number of active zones, with higher neuronal excitability (indicated by the upper traces), and also with enhanced sleep rebound during recovery (schematic representation of findings in Ref. [10**]). NMDAR could be increased either in a presynaptic or postsynaptic manner as depicted by a hypothetical terminal (?), and the role of glial cells in this context remains to be defined. (b) As indicated in the text, sleep deprivation was shown to impact NMDAR-mediated currents, NMDAR subunit content, synaptic adhesion proteins (e.g., Neuroligin-1), and to decrease cofilin and Ca\(^{2+}\)/calmodulin-dependent kinase II (CamKII) phosphorylation, which are modifications shown to negatively impact dendritic spines. Increase in astrocyte intracellular [Ca\(^{2+}\)] with sleep deprivation can induce gliotransmitter release (e.g., ATP depicted) and persynaptic astrocytic process motility. GluN1/2A: NMDAR containing subunits 1 and 2A, GluN1/2B: NMDAR containing subunits 1 and 2B (more permeable to Ca\(^{2+}\)), ATP: adenosine triphosphate.
cerebral cortex [26,27], which likely contributes to decreased phosphorylation of Ca\(^{2+}\)/calmodulin-dependent kinase II (CamKII) [29] (Figure 2), a mechanism that can negatively regulate spine size [30]. In parallel, structural synaptic changes implicate signaling that impact the protein synthesis machinery [31], and sleep loss was shown to affect mammalian target of rapamycin (mTOR)-dependent protein synthesis [32,33], and to consequently impair sleep-regulated functions [34]. Sleep loss-dependent changes in the translation of structural components such as cytoskeleton and cell adhesion proteins are expected to underlie structural plasticity as discussed hereafter.

**Cytoskeleton and cell adhesion**

Presynaptic and postsynaptic structural changes need to involve modifications of the cytoskeleton. This has been elegantly shown by Havekes *et al.* [2**], who observed that decreased spine density and dendritic branch length after sleep deprivation in the hippocampus resulted in the activation, by de-phosphorylation, of the protein coflin, which degrades actin filaments of the synapse (Figure 2). Interestingly, coflin was shown to be regulated by the postsynaptic adhesion molecule Neuroligin-1 (NLGN1) [35], which itself regulates spine formation [36], and was observed to be decreased at the synapse by sleep deprivation in the mouse anterior forebrain [37]. The gene expression of *Nlgn1* and other cell adhesion molecules as well as the protein level of cell adhesion elements was also shown to be modified by sleep deprivation in a brain area-dependent manner [37–41], providing a mechanism by which sleep loss can modify synaptic structures. In addition, considerable support for a role of structural plasticity in sleep regulation comes from findings that sleep and neuronal activity during sleep are disrupted when proteins shaping the cytoskeleton and cell adhesion, such as Neurexin-1, Neuroligin-1, and the Ephrin receptor A4, are genetically modified [37–39,42–44].

**Glia functions**

As indicated above, astrocytes themselves are submitted to sleep loss-driven morphological changes, and a number of findings support a role for astrocytes in sleep regulation [45], notably involving intracellular [Ca\(^{2+}\)] increase and adenosine acting on neurons via A1 receptors [46–48]. Also, extracellular ion concentration has recently been shown to be causally linked to sleep-wake regulation [49], and astrocytes are predicted to be master regulators of extracellular ion concentration. These mechanisms are also potentially underlying a role for astrocytes in the regulation of neuronal morphology (Figure 2). In fact, astrocytes regulate synapse elimination during development likely via changes in intracellular [Ca\(^{2+}\)] and ATP release [50]. In both the hippocampus and somatosensory cortex, increased neuronal activity enhances the synaptic coverage by PAPs, through intracellular Ca\(^{2+}\) and metabotropic glutamate receptors (mGluR), which impacts spine stability [20]. Furthermore, besides astrocytes, changes in spine number with sleep deprivation could also be mediated by microglia, which have been shown to shape spine density [5]. Unfortunately, little is known about the role of non-astrocyte glia in sleep loss-induced structural neuronal changes.

**Conclusion**

Overall, recent discoveries highlight that plastic structural changes occurring with sleep loss are various, depend on the structural element, and are functionally shaping neuronal networks and sleep. Structural plasticity is expected to contribute to stable cell-wide changes in neuronal activity, which takes place as a function of wakefulness [10**,51]. Of importance is the fact that acute sleep loss does not have a uniform impact on neuronal structures and systems. During prolonged wakefulness, sleep drive-related neuronal regions in *Drosophila* have the structure of their synaptic contacts enlarged [10**], whereas in mice, learning/memory-associated spine enhancement is reduced [2**,5**].

To further our understanding of sleep loss-induced structural plasticity, future research should: 1) simultaneously investigate pre and postsynaptic structures, as made possible by novel methodologies [52]; 2) examine intermediate states of dendritic spines, such as filopodia, which might correlate with changes in vigilance states [53]; 3) study changes in the length of spine neck that modifies excitatory postsynaptic potential [21]; and 4) consider interactions of cytoskeleton and cell adhesion components. In mammals, specific roles of the different sleep states also need to be more systematically investigated to grasp the origin of structural modifications, because rapid eye movement (REM) and non-REM sleep were shown to be distinctly involved in different types of structural changes [3**,54]. Finally, an area that has received minor attention in the context of sleep loss in *Drosophila* and zebrafish is the role of the crosstalk between glia and neurons in shaping synaptic contact. Given the relative ease to image structural elements in these model organisms, such research will certainly help to expose the functions of glial cells in sleep regulation. A better understanding of the role of glial cells in sleep loss-driven structural neuronal changes will also clarify their roles in additional sleep-regulated functions like mood regulation [55,56].

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Conflict of interest

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


This study showed that 5 hours of sleep deprivation decreases dendritic spine density selectively on specific dendritic branches of neurons of the hippocampal CA1 area. This change was reversible with sleep, associated with an increased activity of the filamentous actin severing protein cofilin, and was functionally linked to object recognition memory.


This article used an innovative and robust approach and a simple and thorough protocol. It showed that sleep after motor learning promotes the formation and maintenance of postsynaptic dendritic spines on a subset of branches of layer V pyramidal neurons in the mouse motor cortex. Sleep deprivation, independent of corticosterone, importantly decreased spine formation following motor learning.


The authors have shown, using a presynaptic marker, that synapse number in orexin/hypocretin neurons in zebrafish is modulated by the circadian clock and increased by sleep deprivation. They also reported that a protein implicated in glutamate receptor clustering, NPTX2, regulated circadian changes in synapse number.


This paper used sleep deprivation to increase sleep pressure combined with functional imaging and translational profiling. It showed that a subset of Ellipsoid body (EB) neurons of the fruit fly respond to sleep deprivation by increases in size of active zones indexed with bruchpilot puncta, in cytosolic Ca²⁺ levels, in NMDAR expression, and in cell excitability. These changes were causally linked to sleep rebound after sleep deprivation.


This article revealed that, in the fruit fly, dorsal fan-shaped body neurons change their activity pattern as a function of prolonged wakefulness and sleep, which depend on dopamine neuronomisation and potassium conductance. Importantly, these changes were linked to modifications in sleep drive.


This work defines the response of specific neurons, nNOS/NK1 neurons of the rat cerebral cortex, to sleep and sleep loss. Using sleep deprivation and pharmacological manipulation of non-REM sleep duration to dissociate sleep time from sleep need, it reports a lower proportion of activated nNOS/NK1 neurons (i.e., Fos+) when animals are under lower sleep need.


These convincing data showed that optogenetic silencing of γ-aminobutyric acid (GABA) neurons from the medial septum in mice, during REM sleep specifically, attenuates REM sleep theta activity without changing sleep duration. More importantly, this silencing drastically impaired both object recognition and contextual memory.


43. Profit MF, Deurreuiller S, Robertson GS, Rusak B, Semba K: Disruptions of sleep/wake patterns in the stable tubule only polypeptide (STOP) null mouse model of schizophrenia. Schizophr Bull 2016, 42:1207-1215.


This article used mice lacking inositol 1,4,5-trisphosphate receptor type 2 to disrupt Ca2+ signaling in astrocytes. It showed that this disturbance impaired the elimination of synapse of the ventral postero medial nucleus that takes place during development. In addition, it revealed that injection of ATP or of the purinergic agonist P2Y1 rescued synapse elimination.


