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Sleep and the single neuron: the role of global slow oscillations in individual cell rest

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Abstract

Sleep is universal in animals, but its specific functions remain elusive. We propose that sleep's primary function is to allow individual neurons to perform prophylactic cellular maintenance. Just as muscle cells must rest after strenuous exercise to prevent long-term damage, brain cells must rest after intense synaptic activity. We suggest that periods of reduced synaptic input ('off periods' or 'down states') are necessary for such maintenance. This in turn requires a state of globally synchronized neuronal activity, reduced sensory input and behavioural immobility — the well-known manifestations of sleep.

Sleep has been found in all animal species carefully studied to date[1,2]. Multiple studies, as well as our own experience, clearly show that sleep is necessary for normal functioning during waking. The effects of sleep loss are especially apparent after total sleep deprivation, which has serious consequences on subjective alertness, psychomotor vigilance, sustained attention and many other cognitive functions[3–6]. However, the biological function of sleep remains unclear[1,7,8]. There are two types of sleep: rapid eye movement (REM) sleep, which occurs in relatively short episodes usually associated with dreaming, and non-REM (NREM) sleep, which makes up the bulk of sleeping time. This Opinion article proposes a hypothesis for the function of adult NREM sleep.

Sleep can be defined on at least two distinct levels: the behaviour of the whole organism and the spatiotemporal patterns of neuronal activity in the brain. At the behavioural level, sleep is associated with immobility and sensory disconnection from the environment. At the level of brain activity, cortical neuronal firing patterns in NREM sleep are characteristically different from those in active wakefulness[9–12]. Specifically, upon falling asleep, cortical networks alternate between periods of generalized population firing and depolarization, and periods of relative silence and hyperpolarization[9,12–17]. This pattern of neuronal activity gives rise to electroencephalogram (EEG) and local field potential (LFP) oscillations at frequencies approximately between 1–4 Hz, which are termed slow waves[12,18–20]. For the purposes of this article, we refer to periods of enhanced and reduced population spiking activity as 'on periods' and 'off periods', respectively (FIG. 1). On and off periods in a local population are strongly associated both with LFP waves and with depolarized and

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hyperpolarized membrane potentials of individual neurons ('up' and 'down' states, respectively)[21–24]. Cortical neurons receive most of their inputs from nearby cells and are thus hyperpolarized when their neighbours do not spike. In physiological conditions, the occurrence of global neuronal off periods and high-amplitude, spatially synchronous slow waves in the neocortex generally coincides with behavioural manifestations of sleep.

Sleep probably has several roles, allowing an organism or the brain to perform multiple functions that are necessary for continued survival and performance but which cannot be performed during active behaviour. The idea that sleep has a restorative role fits well with our subjective experience. In general, 'restorative' theories suggest that sleep could provide the opportunity for processes such as synthesis of macromolecules[25], recuperation from oxidative stress or toxins that accumulate during waking[26,27] or replenishment of energy stores such as glycogen[28,29]. Although there is substantial evidence for metabolic functions of sleep, how restorative changes occurring at the level of individual cells are related to the complex spatiotemporal patterns of brain activity characteristic of sleep and why global, continuous sleep is necessary to perform them is still unknown.

Another proposed role for sleep is in information processing and synaptic plasticity[30–38]. Some of these theories ascribe a particular role in memory consolidation to sleep and are supported by evidence of similarities in the fine structure of neuronal activity patterns in waking during learning and subsequent sleep[39,40]. In this view, the slow oscillation could serve as a 'carrier wave' that allows or drives the transfer of information between structures such as the hippocampus and neocortex, thereby serving consolidation of memory traces[41–44]. Another view holds that sleep is associated with a net decrease of synaptic strength that would lead to the elimination of weak connections while preserving strong ones, thereby renormalizing neuronal firing rates and network excitability[37]. Although there is substantial evidence that sleep may be involved in neuronal plasticity at the level of fine cortical microcircuits[33,45] and in the consolidation of certain memories[46], the question remains as to why sleep deprivation has such serious consequences, including impaired subjective alertness, reduced sustained attention, impaired performance in executive tasks, state instability and decreases in many other cognitive functions[4–6,47–49], rather than merely memory loss or a decreased ability to learn.

Thus, any conceptual framework that addresses the function of sleep should integrate the molecular, behavioural and electrophysiological correlates of sleep states and explain how these phenomena relate to sleep's restorative role. Here we propose one such framework. We propose that the most important role of NREM sleep is to provide individual neurons with a period of time in which to perform processes of cellular maintenance, which are required after periods of high synaptic activity. In this view, sleep allows neurons to rest and recuperate after intense activity in the same way that skeletal muscles must rest after a period of strenuous physical exercise. We propose that just as a complex machine cannot be easily repaired and maintained while it is running, such periods of rest are required for the maintenance of neurons and synapses. And, as with the maintenance of a complex machine, we propose that the maintenance of neurons is not simply a reactive process that fixes problems that already affect brain function, but a prophylactic or preventive process by which minor cellular wear and tear is repaired before it has a major impact on neuronal function. In other words, fixing minor cellular problems early can prevent the catastrophic build-up of problems that lead to permanent damage.

The main question, therefore, is why maintenance functions at a single-cell level require behavioural sleep and its associated synchronous, high-amplitude slow-wave activity (SWA) in the neocortex. We argue that although most cells in the body can presumably undergo rest relatively independently, neurons must rest together, owing to their extensive

interconnectivity[50]. Thus, the only strategy to provide rest for an individual neuron in the neocortex is to synchronously reduce activity of the entire network that directly or indirectly projects to that neuron. Last, we propose that although localized off periods can and indeed do occur during waking[51], they are much less efficient in promoting cellular maintenance because they are too rare and too short owing to both high levels of arousal-promoting neuromodulators and high network activity that prevents the occurrence of sustained down states[52–54]. We also propose that because off periods during waking have a deleterious effect on behavioural performance[49,51,55,56], the animal's survival would be endangered if the brain's need for rest was fulfilled entirely during waking. We thus posit that rest at the single-cell level requires both synchronized network activity and withdrawal from the environment — that is, the well-known manifestations of sleep.

Sleep homeostasis and slow waves

The idea that sleep has a restorative function is supported by its homeostatic regulation. The need for sleep ('sleep pressure') increases in proportion to the duration and quality of the preceding waking episode and dissipates during subsequent sleep in proportion to its duration and intensity[57,58]. The main feature of deep, intense sleep after prolonged waking is an increased arousal threshold, which is manifested in a reduced ability to waken upon stimulation[59], and the best characterized physiological indicator of sleep–wake history is the level of cortical EEG SWA — that is, EEG power between 0.5 and 4.0 Hz — during NREM sleep[57,58,60,61]. In mammals, sleep SWA is correlated with sleep pressure: it is high in early sleep and after sleep deprivation and decreases progressively to reach low levels in late sleep[62–64]. It has therefore been proposed that SWA is a direct signature of the restorative processes occurring during sleep[37,65]. Sleep homeostasis has been found in all animal species that have been carefully studied, including invertebrates, suggesting that sleep is required for an essential restorative process throughout the animal kingdom[2].

The idea that sleep has a restorative role is supported by the local regulation and homeostasis of SWA[66–68]. Although a prevailing view is that brain states are regulated in a global fashion, research over the past few decades has revealed that spontaneous brain activity during sleep can be locally modulated[19,55]. The most extreme example of this is the unihemispheric sleep of seals and dolphins[69,70]. In adults of terrestrial species, SWA is more intense in frontal areas compared with more posterior areas, especially in early sleep or after sleep deprivation[71–74], and shows interhemispheric asymmetries[75,76]. Regional differences are also apparent at the level of individual sleep slow waves: although the alternation of periods of increased neuronal activity and silence is usually correlated across cortical regions and individual neurons[19,77,78], an up state can sometimes be seen in one region of the cortex while another region is in a down state, with these states often spreading as travelling waves[18,19,51,79–82]. Importantly, topographic differences in brain activity during sleep are functional, as peripheral stimulation or the spontaneous use of circumscribed cortical areas leads to more intense local EEG SWA[65,83–87]. Such data demonstrate that not only waking duration per se but also strong activity in specific regions affects the 'intensity' of subsequent sleep. Notably, during sleep, increased SWA is associated with decreased regional cerebral blood flow, which suggests lower metabolic demand[88,89]. Together, these data indicate that SWA is associated with the restorative processes of sleep.

In fact, we suggest that SWA is necessary for the restorative process to occur. Specifically, we propose that restorative maintenance of individual neurons requires a reduction in the activity of its synaptic inputs and outputs. Our central argument is that because cortical cells receive extensive synaptic input from other neurons, this restorative maintenance can only be achieved by a coordinated reduction in the spiking of all neurons in a functionally interconnected network: that is, by an off period. (The types of maintenance that may be

performed and the reasons they require reduced synaptic activity are discussed later in the article.) As increased SWA reflects the synchronous occurrence of down states among large cortical populations[12,20], SWA can be seen both as a marker of the increased need for cellular rest and as a necessary condition for achieving rest most efficiently at a single-cell level. Indeed, sleep with high SWA, such as under high sleep pressure, is characterized by increased local and global synchronization of cortical activity[12,19,51,77,90,91]. The fact that cortical areas that were highly active during waking exhibit additional SWA during subsequent sleep is thus an indication of their increased need for compensatory cellular maintenance.

Cellular stress and neuronal inactivity

Neurons — like all cells — have a limited metabolic and biosynthetic capacity. Running close to these limits for extended periods of time leads to multiple forms of 'cellular stress'[92,93]. Intense metabolic rates lead to mitochondrial production of reactive oxygen species (ROS), which can cause damage to DNA, proteins, lipids and other biomolecules ('oxidative stress') owing to their high and relatively unspecific chemical reactivity[94]. Increased temperature or strong molecular motions can lead to unfolding, entanglement and aggregation of proteins ('heat shock')[95]. Prolonged and intense protein synthesis can in turn lead to an accumulation of misfolded proteins in the endoplasmic reticulum (ER), which causes 'ER stress'[96]. Cells mount coordinated responses to these stresses, which restore cellular homeostasis. For example, heat shock leads to the expression of cytoplasmic chaperones (heat shock proteins) that bind to exposed hydrophobic residues of unfolded proteins, preventing their aggregation and leading to either their refolding or degradation. ER stress leads to an analogous unfolded protein response (UPR) — which includes expression of ER chaperones (such as binding immunoglobulin protein) and a general reduction in protein translation — that reduces the load on the ER[96]. Cellular stress has been implicated in many pathological conditions, including cardiac diseases[97] and neurodegenerative disorders[98]. It is important to note, however, that cellular stress does not necessarily cause long-term damage. Although extreme, prolonged stress can lead to apoptosis, milder stress leads to upregulation of survival pathways that protect against future insults — a phenomenon known as stress hardening or hormesis[92,93].

The role of cellular rest in the recovery from cellular stress can be seen in skeletal muscle. Repeated, intense use of muscles leads to a temporary decline in performance known as muscle fatigue[99]. Although this arises in part from adaptation in spinal motor neurons[100], in large part it occurs in the muscle itself, with multiple factors such as decreased myofibrillar calcium sensitivity and calcium release from the sarcoplasmic reticulum playing a part[99,101–105]. These fatigue processes are triggered by stressors such as ROS and by the increased temperature of the muscle during exercise[106]. Importantly, however, muscle fatigue does not occur because cellular damage has compromised the ability of the muscles to contract. Rather, it is a prophylactic response by which the activity of the muscles is capped before the level of accumulated damage reaches a critical threshold that would cause irreversible dysfunction[107]. Recent research suggests that one of the key mediators of skeletal muscle repair is the UPR: it has been shown that acute exercise causes the expression of transcription factors involved in the UPR, and in transgenic mice in which the ATF6α UPR pathway is compromised, repetitive exercise causes long-lasting exercise intolerance and muscle damage[108].

The central tenet of our hypothesis is that the off periods of sleep provide neurons with an analogous opportunity to rest and repair minor cellular damage before it becomes irreversible. Indeed, prolonged wakefulness or sleep deprivation activates the UPR, as indicated by increased expression of mRNA for heat shock proteins and ER chaperones after

acute short-term sleep deprivation in the brains of rats[109], mice[110,111] and *Drosophila melanogaster*[112]. Notably, although wild-type flies survive sleep deprivation with no long-term effects, flies carrying a mutation for the heat shock protein *Hsp83* die after sleep deprivation[112]. It is likely that waking activity causes multiple types of cellular stress, which require multiple solutions at the cellular level (FIG. 2). For example, in rodents, waking is associated with increased brain temperature[113] and intensified mitochondrial metabolism[114], which could lead to thermal and oxidative stress, respectively. Furthermore, the high synaptic activity that occurs during waking probably results in a growing need for the biosynthesis of new vesicle components (both proteins and lipids) to replace those damaged by constant cycling[115–118]. However, the synthetic capacity of the ER is limited, and it is reduced still further when the cell is metabolically highly active, recharging ionic gradients dissipated by synaptic activity[119]. We propose that homeostasis can only be restored by a temporary reduction in synaptic activity, allowing the cell to clear its backlog of biosynthetic work, which is made easier without intense respiration and the associated oxidative stress. Consistent with this hypothesis, a recent study found that lysolipids — chemically compromised membrane components — are increased both *in vivo* in the cortex of sleep-deprived mice and *in vitro* in an organotypical cortical neuronal culture after stimulation that promotes intense wake-like activity[120]. Moreover, many of the approximately 100 known genes whose expression increases during sleep are involved in protein synthesis, membrane trafficking and cellular maintenance[109].

We emphasize that the proposed role of off periods is not to repair major cellular damage that has already occurred. Instead, the occurrence of down states is a prophylactic or preventive process in which cells ‘shut down’ before relatively minor and easily fixed problems become irreparable. Because neurons in most brain regions cannot be replaced, we suggest that this prophylactic process must be very strong (BOX 1). Consistent with this theory, even prolonged sleep deprivation for up to several hours or days does not cause obvious signs of neuronal degeneration[121] or oxidative damage[122] in the brains of rats. We suggest this is because sleep deprivation presents neurons with a difficult choice: either continue at high levels of synaptic activity and face long-term cellular damage; or shut down activity sufficiently to prevent permanent damage but at a cost to behavioural performance. For the long-term good of the organism, neurons almost always choose to reduce synaptic activity: even relatively short-term sleep deprivation is invariably associated with an occurrence of sleep-like activity at the level of the EEG and neuronal activity in behaviourally awake animals[1,51,123]. In other words, changes incurred at an individual cell level after a certain amount of activity are incompatible with a continuation of their activity; this ensures that the neuron is never damaged permanently.

Box 1

Does waking induce ‘wear and tear’ on neurons?

It is commonly held that sleep has a restorative function that serves to repair ‘wear and tear’ produced by waking[168]. In support of this view, several studies reported that wakefulness and sleep deprivation are associated with an upregulation of molecules, such as molecular chaperones, that are involved in the response to cellular stress[109–112,169]. Moreover, voluntary wheel running in mice, which is known to prolong spontaneous waking[170,171], results in increased levels of markers of the unfolded protein response in several brain regions[172]. Molecules that are released during the high synaptic activity of waking, such as tumour necrosis factor-α[173] and amyloid-β[152], can activate cellular stress responses[174,175]. Nevertheless, substantial neuronal damage has not been reported in wild-type animals after spontaneous physiological waking or sleep deprivation[121,122]. Although it is impossible to keep an animal permanently and fully awake for more than a few hours or days[1], these results suggest that even without extended sleep, protective

responses — perhaps including waking off periods — may be sufficient to prevent rapid and permanent damage to neural circuits. Studies in genetically compromised animals or pathological models, however, suggest that sleep deprivation or excessive synaptic activity can have major consequences. In a mouse model of early-onset Alzheimer's disease, chronic sleep restriction led to a marked increase in amyloid-β plaque deposition[152]. Sleep deprivation after experimental ischaemia led to increased neurodegeneration in rats[176]. In flies mutant for the chaperone protein Hsp83, prolonged sleep deprivation rapidly led to death of the whole organism[112]. Moreover, intense synaptic activity generated by experimental induction of seizures triggers neuronal degeneration in the cortex, hippocampus and parahippocampal regions[177–180]. Notably, prolonged seizure-induced neuronal loss is attenuated in transgenic mice overexpressing the molecular chaperone 14-3-3ζ[181], whereas its depletion increases kainic acid-induced damage to neurons[182]. Thus, existing evidence suggests that in non-pathological conditions, physiological defence mechanisms are probably sufficient to prevent immediate and permanent damage to neurons. Whether chronic sleep deprivation can lead to an increased probability of later developing neurodegenerative disorders (which are often associated with disturbed sleep[183]) remains to be firmly established.

Thus, we suggest that there are two key aspects to the function of off periods at the single-neuron level. First, the periods of neuronal inactivity are signs of neuronal fatigue and serve the prophylactic purpose of reducing activity in those neurons that are primarily affected before damage becomes permanent. Second, cessation of synaptic activity could contribute actively to the processes of cellular restoration by re-allocating energy substrates to protein synthesis and membrane repair and by placing less demand on synaptic vesicles, which in turn would lead to lower biosynthetic requirements (FIG. 2). Of course, the processes of cellular maintenance enabled by off periods do not have to be completed within a single off period, which has a duration of a few hundred milliseconds and therefore is far too short for processes such as protein synthesis. Rather, we suggest that regular brief pauses in neuronal spiking can reduce cellular energy consumption and synaptic activity sufficiently to allow cellular maintenance processes that occur over a much longer timescale. Now we can turn to the questions that we raised at the beginning: why can maintenance functions at a single-cell level not be fulfilled during waking, and why do they require global sleep?

Single-cell rest during waking

Why could periods of metabolic 'rest' not occur during waking, allowing an animal to continue performing in the world while its neurons take turns to rest? Indeed, the fact that animals do exhibit off periods during quiet waking[22,51,124] suggests that, in principle, they could get away without global sleep by doing this all the time. There are two main arguments against this possibility.

First, the occurrence of neuronal off periods or slow waves during waking compromises behavioural and cognitive performance[49,51,56]. Sleep deprivation is associated with increased low-frequency EEG activity during waking in both animals and humans[51,86,113,123,125–129]. Recordings in rats suggested that this EEG pattern reflects local neuronal off periods[51]. Importantly, the occurrence of these off periods in the primary motor cortex is associated with reduced performance in a fine-skilled motor task[51]. The mechanisms by which off periods affect cortical function have not yet been clarified but are probably related to the difference in neuronal responses to incoming inputs between the up and down state, as has been shown in experiments using peripheral sensory or cortical electrical stimulation[130–134]. These experiments suggest that networks undergoing spontaneous slow oscillations are less able to faithfully process sensory stimuli or inputs

from other brain areas[16,135–137], which could in turn compromise information processing that is necessary for successful behaviour.

Second, off periods occurring during waking may be less effective at restoration than those occurring during deep sleep. Indeed, owing to the extensive lateral connectivity of the cortex, prolonged periods of zero synaptic activity can occur only when large neuronal populations engage in down states at the same time. Although sleep deprivation produces slow waves during waking, they are typically faster (4–7 Hz) and less globally coherent than those found during deep NREM sleep early in a sleep session; they show a closer homology to the light NREM sleep seen towards the end of the night[51,123] (FIG. 3). We propose that this early deep sleep reflects a highly restorative state that is enabled by prolonged off periods encompassing large distributed cortical networks (hence the requirement for global sleep rather than merely local sleep). Conversely, the shorter and more localized off periods during waking[51,123,126,128,129,138,139] are likely to have less restorative power, at most serving a prophylactic function for smaller cortical networks.

A related question is why ‘deep’ sleep is not even deeper. To rest most efficiently, why would the brain not simply cease all electrical activity rather than continuing to exhibit sporadic on periods? We propose that this again reflects a compromise. As well as restorative functions, sleep has been suggested to serve information processing functions related to memory consolidation and synaptic plasticity, which presumably require electrical activity. In addition, even while asleep, an animal must maintain the ability to discriminate background sensory stimuli from those that indicate danger, which again presumably requires some degree of neural activity. We propose that the alternation of on and off periods balances these competing priorities and that the stronger and more coherent off periods of early deep sleep reflect a prioritization of essential cellular maintenance, whereas lighter NREM sleep and REM sleep seen later in the night reflects performance of less critical information processing tasks.

In summary, we propose that slow waves during waking, as seen after sleep deprivation, are sufficient to prevent long-term neuronal damage but come with a large cost. First, the impaired behavioural performance caused by this state has serious ethological drawbacks, such as a higher risk of predation. Second, the lower restorative efficiency of waking off periods means that in order to be an effective substitute for sleep, they would need to occur for a much longer period or even continuously. Between continuous 24-hour waking activity but with substantial cognitive impairment or a shorter active day followed by a relatively brief period of intense sleep, evolution has favoured the latter. Aquatic mammals provide an intriguing example of a compromise between neuronal rest and performance impairment. Because these animals must continue swimming in order to breathe, they cannot show global sleep accompanied by complete immobility but instead enter a state in which their cerebral hemispheres alternately exhibit a sleep-like slow-wave EEG[69,140]. Interestingly, seals exhibit prominent unihemispheric sleep when they are in the water[70], but on land global bihemispheric sleep is more abundant[141]. This suggests that unihemispheric sleep is a suboptimal solution — tolerated only when unavoidable.

From local to global sleep

As discussed above, sleep homeostasis is not just a global phenomenon but also a local phenomenon, with brain areas that were intensely active during waking exhibiting stronger SWA during subsequent sleep. We have hypothesized that this serves the increased restorative need of the local neurons. But what mechanisms could cause an increase in SWA specifically in those areas that need it most?

The mechanisms underpinning cortical slow oscillations are still uncertain. One of the leading theories is the 'excitable system' model. This model postulates that the on period is maintained by recurrent synaptic activity, but as the on period progresses, a build-up of processes such as synaptic depression and intrinsic hyperpolarizing conductances reduce network excitability or alter the excitation–inhibition balance, until activity can no longer be sustained[16,142,143]. This causes an off period during which synapses and cells recover from depression until a new on period can be triggered. A change in the propensity of the network to generate slow waves could therefore be caused by a change in the excitability or amount of adaptation of individual neurons. One can hypothesize that a build-up in the need for cellular maintenance could cause individual neurons to show lower excitability and stronger adaptation — analogous to how signalling pathways such as those involving ROS cause a decrease in myocyte excitability during muscle fatigue[99,144] or to increased potassium conductances in the suprachiasmatic nucleus[145]. In this way, down states would automatically occur where they are most needed, providing a potential explanation for local sleep patterns (FIG. 3). Similarly, it can be suggested that as soon as individual neurons and their neighbours have obtained necessary restoration from cellular stress, their excitability is restored, leading to a more wake-like pattern of activity.

Although the above mechanism in principle provides a sufficient explanation for how local sleep is regulated, considerable evidence also suggests a role for local paracrine signalling pathways and for global neuromodulation. For example, it is known that the level of sleep pressure correlates with the levels of adenosine, tumour necrosis factor-α, amyloid-β and other molecules, which are synthesized locally in those cells that were metabolically or synaptically active and are released in a local (that is, paracrine) manner[29,55,146–153]. Local neuromodulators could increase the propensity for local down states by reducing single-cell excitability or synaptic transmission[149,154]. The occurrence of isolated local off periods in waking could subsequently lead to global sleep through the involvement of neuromodulatory systems responsible for the generation of NREM sleep[153,155] and/or through 'behavioural feedback', whereby the impaired performance caused by local off periods triggers withdrawal from active behaviours and initiates consummatory behaviours directed towards satisfying sleep need.

In this article, we have taken a mostly 'corticocentric' view. However, it is reasonable to assume that the need to rest is shared by a great many neurons throughout the brain. Recordings in sleeping animals and during anaesthesia-induced sleep-like states revealed alternations between more active and more quiet periods of neuronal activity in many structures — including the hippocampus[156–158], cerebellum[159], thalamus[160] and basal ganglia[161] — that are at least partly correlated with cortical on and off periods. Notably, a generalized increase of SWA after sleep deprivation in cats was found in several subcortical structures, such as the hippocampus, amygdala, hypothalamus, nucleus centralis lateralis of the thalamus, septum, nucleus caudatus and substantia nigra[162]. This suggests that SWA may reflect a restorative process in both cortical and subcortical structures.

Conclusions

In this article, we proposed that a key function of NREM sleep is to allow individual neurons to experience a substantial lowering of synaptic and metabolic activity, preventing cellular damage and favouring processes of cellular maintenance after sustained synaptic activity during waking. Because of the extensive recurrent circuitry of the cortex, this requires off periods of coordinated silence in a local population. Although off periods can occur in waking as well as in sleep, we suggest that waking off periods are not only less efficient at cellular restoration but are also incompatible with efficient sensory, cognitive and behavioural function. Efficient and safe neuronal restoration therefore requires a state of

functional sensory disconnection from the environment and behavioural immobility. Thus, to allow individual neurons to obtain a sufficient amount of 'rest', the organism must be globally and behaviourally asleep. The electrographic manifestation of sleep — high-amplitude EEG slow waves — arises from such synchronized alternation between on and off periods across large cortical neuronal populations.

This hypothesis proposes a novel 'umbrella' framework that can reconcile the existing theories of sleep regulation and sleep function. It is fully compatible with 'metabolic' theories, inasmuch as we suggest that neuronal down states occur in response to the need for cellular restoration and contribute actively to the processes of prophylactic cellular maintenance. However, we propose here that the most crucial requirement for the cellular maintenance processes is that large distributed neuronal populations enter down states near-synchronously. Our view is also compatible with theories that link sleep and synaptic plasticity. It is reasonable to assume that synchronous recruitment of specific neuronal populations in up states that recur at about 1 Hz frequency may facilitate efficient information processing and transfer within a specific circuit, thereby providing ideal conditions for neural plasticity. Thus, although the slow oscillation ultimately serves single-cell rest, it could have other functions, such as selective strengthening or weakening of synaptic connections, thereby enabling memory consolidation and forgetting.

To test and strengthen our hypothesis, several specific questions have to be answered in future research. First, what specific stresses resulting from prolonged synaptic activity necessitate rest at a single-cell level? Second, what mechanisms translate the need for single- cell rest into the occurrence of an individual off period? Third, what specific functions do off periods allow for cellular maintenance at the molecular, structural and functional level? Fourth, how do local and globally distributed neuronal assemblies, which consist of thousands and millions of neurons, self-organize in order to allow the occurrence of uninterrupted off periods? Fifth, how is the restorative function of sleep at a single-cell level associated with the alternation of sleep stages? Sixth, can neurons in brain structures with different connectivity patterns (such as the cerebellum or the hippocampus) obtain rest relatively independently from each other and from the global behavioural state? Seventh, can this framework be expanded to include animal species with smaller and simpler brains, such as *Caenorhabditis elegans* or *D. melanogaster*? Last, can other brain states characterized by global slow-wave patterns — such as sleep in early perinatal age[163] (BOX 2), anaesthesia[164,165] and experimentally induced coma[166] — or artificially induced slow waves[167] fulfil the restorative function of physiological sleep? Different experimental models are required for addressing these specific questions: in some cases, simple preparations capable of generating spontaneous down states, such as acute slices or neuronal cultures suffice, whereas in other cases, a 'whole-brain' preparation with intact functional connectivity is the only option.

Box 2

Sleep during early development and in adulthood

Sleep shows pronounced changes across ontogeny, raising the question of whether it serves the same function in early perinatal age as in adulthood. As the definition of sleep and waking rely heavily on behavioural criteria and the electroencephalogram (EEG), studying sleep at early ages is a significant challenge, because behaviour is not yet well developed and it is often difficult to obtain continuous stable EEG recordings[184–186]. Nevertheless, studies have shown that sleep in early development has a different character to that in adults. A typical feature of sleep in neonate rodents (postnatal days 1–8) is the occurrence of prolonged periods of complete neuronal silence in the neocortex, interrupted regularly with bursts of spindle-like EEG activity[187]. This pattern of brain

activity remarkably resembles the ‘delta-brush’ pattern typical of human premature neonates[188,189]. Thus, sleep-like activity in early perinatal age seems to resemble the bursting pattern observed under certain anaesthetics or in coma more than the slow waves during adult sleep[187,190]. The function of this neonatal brain activity is unclear, although a role in organizing neuronal connections has been suggested[191]. After this early stage, sleep in children and adolescents is more similar to that seen in adults[192–196]. However, the total amount of sleep decreases continuously throughout life, with children and adolescents needing more sleep than adults[197]. The reasons for greater sleep need during development are still unclear. However, it is tempting to speculate that it is related to two proposed primary functions of sleep: synaptic plasticity and metabolic rest. Childhood and adolescence are times of intense learning, which could be expected to require greater sleep-related consolidation and synaptic plasticity[198]. In addition, several studies show that protein synthesis and turnover are substantially higher during early postnatal age as compared with adulthood[199–202]. This high rate of biosynthesis might place a heavy load on cellular machinery such as the endoplasmic reticulum, and the unfolded protein response is indeed expressed as early as during the embryonic development of the CNS[203]. This might in turn limit the amount of prolonged and intense synaptic activity that can be safely produced, accounting, at least in part, for the characteristic pattern of brain activity in early age.

Our hypothesis integrates multiple levels at which the function of sleep can be considered. Earlier theories have explored various possibilities, from individual cells or even subcellular components to global behaviour. According to our hypothesis, sleep can be defined as a global dynamic process that accommodates the need for rest at the level of individual cells that are embedded in highly interconnected complex networks. We propose that understanding the function of sleep requires one to bridge the gap between these different levels, because the most important defining characteristic of sleep — global cortical synchrony — appears to be a crucial prerequisite for the expression of down states at a single-neuron level, allowing them to fulfil their manifold restorative functions.

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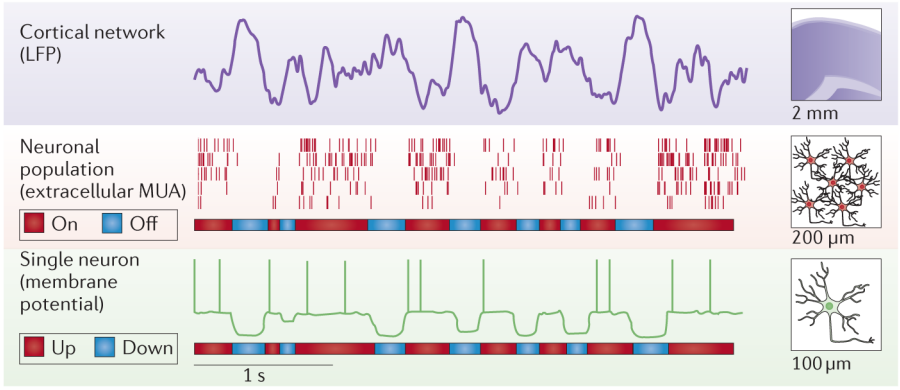

Figure 1. Active and inactive states at the network and neuronal level

A schematic depiction of the local field potential (LFP) recorded from deep cortical layers during spontaneous non-rapid eye movement sleep in a rat (top row), and a raster plot of the corresponding neuronal spiking activity of five individual neurons (middle row) are shown. Note that LFP slow waves are associated with generalized population silence (off periods), which alternates with periods of raised spiking activity (on periods), each of which lasts several hundred milliseconds. The bottom row shows a schematic representation of a membrane potential expected in one individual neuron within this network. Note that LFP slow waves and extracellular off periods are associated with a prominent membrane hyperpolarization (down state), which alternate regularly with periods of depolarization, when spiking propensity is increased (up state). MUA, multi-unit activity.

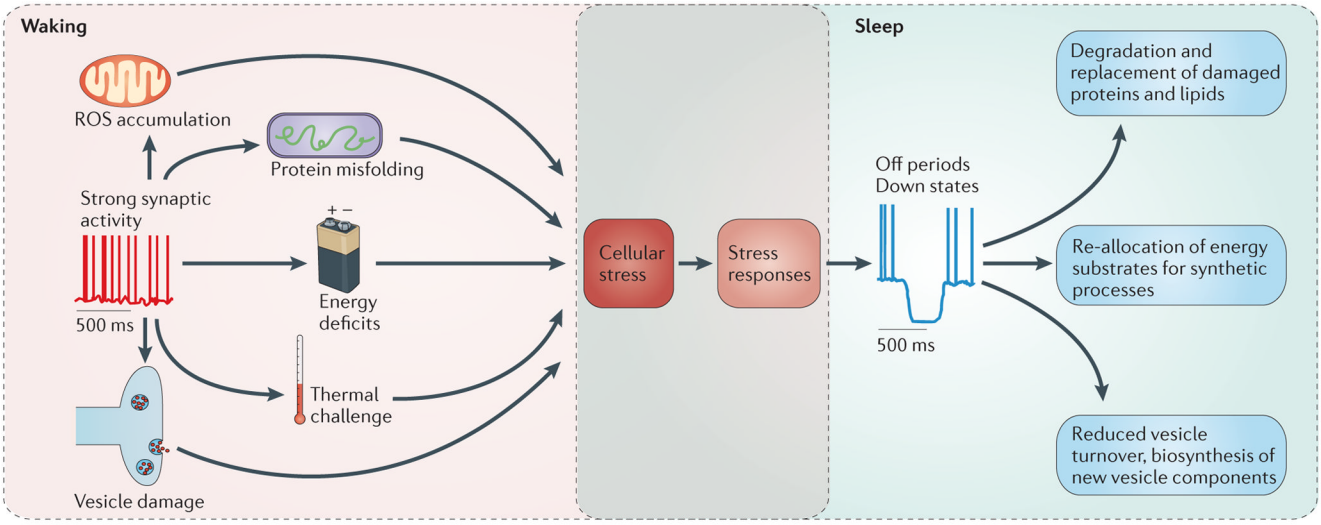

Figure 2. Transient network silences (off periods) allow cellular rest at a single-neuron level

We propose that the intense synaptic activity that is typical of active waking eventually leads to irreversible cellular damage if it is not compensated by intermittent periods of rest. This damage could occur through multiple pathways, including the accumulation of reactive oxygen species (ROS), repeated vesicle turnover, insufficient energy substrates for synthetic processes, increased brain temperature and accumulation of misfolded proteins in the endoplasmic reticulum lumen. These challenges trigger various stress responses that allow the cell to restore homeostasis. We suggest that a key effect of these stress responses is to produce ‘neuronal fatigue’ that reduces electrical excitability, promoting local or global off periods of generalized neuronal silence. These in turn reduce neuronal energy expenditure and lower the need to replace structures such as synaptic vesicles, allowing cells to perform essential maintenance before damage becomes irreversible.

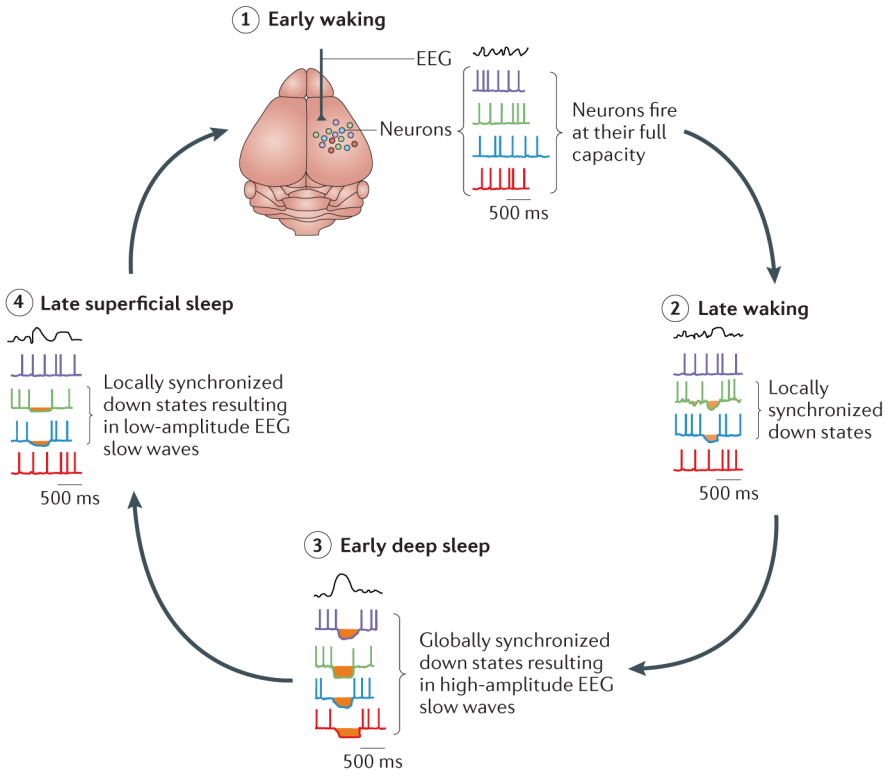

Figure 3. Global behavioural sleep provides conditions for single-cell rest by allowing sustained uninterrupted down states

In early waking (stage 1), neurons can fire at their full capacity. As waking time progresses (stage 2), the need for cellular maintenance builds up as a result of prolonged synaptic and spiking activity. The resulting down states are short, localized and easily interrupted by inputs arising from distal neurons during waking. Although they may fulfil the prophylactic function of preventing permanent damage, this comes at a cost of reduced cognitive performance. During early deep sleep (stage 3), sustained uninterrupted down states occur across large cortical neuronal populations, efficiently providing cellular rest to a large number of neurons. Synchronized occurrence of down states results in the high-amplitude electroencephalogram (EEG) slow waves that are typical of early non-rapid eye movement sleep. As individual neurons obtain the necessary amount of rest (stage 4) they resume firing, and progressively smaller neuronal populations engage in synchronized down states. This results in the low-amplitude EEG slow waves that are typical of later sleep.