

A role for sleep in brain plasticity

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Abstract

The idea that sleep might be involved in brain plasticity has been investigated for many years through a large number of animal and human studies, but evidence remains fragmentary. Large amounts of sleep in early life suggest that sleep may play a role in brain maturation. In particular, the influence of sleep in developing the visual system has been highlighted. The current data suggest that both Rapid Eye Movement (REM) and non-REM sleep states would be important for brain development. Such findings stress the need for optimal paediatric sleep management. In the adult brain, the role of sleep in learning and memory is emphasized by studies at behavioural, systems, cellular and molecular levels. First, sleep amounts are reported to increase following a learning task and sleep deprivation impairs task acquisition and consolidation. At the systems level, neurophysiological studies suggest possible mechanisms for the consolidation of memory traces. These imply both thalamocortical and hippocampo-neocortical networks. Similarly, neuroimaging techniques demonstrated the experience-dependent changes in cerebral activity during sleep. Finally, recent works show the modulation during sleep of cerebral protein synthesis and expression of genes involved in neuronal plasticity.

Keywords: *Sleep, plasticity, brain development, memory, functional neuroimaging*

La idea de que dormir pudiera estar involucrado en la plasticidad cerebral ha sido investigada por muchos años a través de un gran número de estudios en animales y humanos, pero la evidencia permanece fragmentada. Grandes cantidades de sueño en los inicios de la vida sugiere que el sueño puede tener un papel en la maduración cerebral. En particular ha sido remarcada la influencia del sueño en el desarrollo del sistema visual. Los datos actuales sugieren que las etapas de sueño REM y no-REM podrían ser importantes para el desarrollo cerebral. Tales hallazgos demandan la necesidad de un manejo óptimo del sueño pediátrico. En el cerebro adulto, el papel del sueño en el aprendizaje y la memoria es enfatizado por estudios a niveles conductual, sistémico, celular y molecular. Primero, se reporta que las cantidades de sueño aumentan después de una tarea de aprendizaje y la falta del sueño limita la adquisición y consolidación de la tarea. A nivel sistémico, los estudios neurofisiológicos sugieren posibles mecanismos para la consolidación de los rastros de memoria. Esto implica a las redes tálamo-cortical y la hipocampo-neocortical. De igual manera, las técnicas de neuroimagen demostraron los cambios dependientes de la experiencia, en la actividad cerebral durante el sueño. Finalmente, trabajos recientes muestran durante el sueño la modulación de la síntesis proteica cerebral y la expresión de los genes involucrados en la plasticidad neuronal.

Introduction

Sleep appears to be essential for the survival and integrity of most living organisms. However, its exact functions remain speculative despite growing understanding of the processes initiating and maintaining sleep. Many non-mutually exclusive roles have been attributed to sleep: brain thermoregulation [1], neuronal detoxification [2], energy conservation [3], tissue restoration [4] and immune defence [5]. Another hypothesis has focused much attention for the last years and proposes that sleep might participate in brain plasticity [6]. The latter refers to the ability of the brain to persistently modify its structure and function according to genetic

information and environmental changes or to comply with the interaction between these two factors [7]. Links between sleep and brain plasticity have been considered during early life as well as in the adult organism. It is known that sleep amounts are greater during neonatal periods of rapid brain development than at any other time of life [8–10]. This suggests that sleep should be important for brain development and synaptic plasticity during early life. This study will first review experimental data testing this relationship, after a brief description of sleep organization and basic physiology. The second part of this article will provide a general description of sleep implication in learning and memory, in adult subjects.

Sleep organization and stages

In homeotherms, sleep is composed of two main stages. Rapid eye movement (REM) sleep, also known as paradoxical sleep (PS), is characterized by ocular saccades, muscular atonia and high-frequency, low-amplitude rhythms on electroencephalographic (EEG) recordings. Non-REM sleep is characterized by specific EEG oscillations: spindles, delta and slow rhythms. In humans, non-REM sleep is further categorized in light (stage 2) and deep (stages 3 and 4) stages. Spindles, a prominent feature of light non-REM sleep, are defined as waxing-and-waning oscillations within the 12–15 Hz (sigma band) frequency range, lasting at least 0.5 seconds [11]. During deep non-REM sleep, also referred to as slow wave sleep (SWS), the EEG is mainly characterized by a slower oscillation in the delta range (1–4 Hz). A slow rhythm (<1 Hz) occurs both during light and deep non-REM sleep and manifests itself respectively as the regular recurrence of spindles every 3–10 seconds or as slow waves below 1 Hz [12,13]. Refinement in the arbitrary categorization of non-REM sleep stages varies amongst species. Subdivided into light and deep SWS in carnivores such as cats or dogs, only one non-REM stage is usually defined in rats or mice. The distribution of stages is unequal throughout the night: SWS is most abundant during the first half of the night in humans, up to 80% of the sleep time in this period, whereas the proportion of REM sleep dramatically increases in the second half of the night. The duration and intensity of sleep is thought to be regulated by the interaction of homeostatic processes, in which the requirement for sleep builds during waking and is relieved by sleep, and circadian rhythms, which determine the timing of the sleep/wake cycle according to internal (e.g. the suprachiasmatic biological clock) and external (e.g. the light–dark cycle) signalling systems [14].

Sleep and brain plasticity during neural development

REM sleep

Most of the work concerning sleep and brain development has studied the behavioural, morphological and electrophysiological consequences of REM sleep deprivation during critical periods of brain maturation in animals, because REM sleep occupies a large proportion of time during early brain development [8]. Indeed, REM sleep time seems to correlate with maturity at birth [15,16]. In an altricial mammal, that is an animal born in a relatively immature state, such as the rat, the high amount of REM sleep at birth declines to a low level during the first month of life. On the other hand, in a precocial

mammal such as the guinea pig, prenatally high levels of REM sleep decrease to low levels at birth. Thus, it appears that the amount of REM sleep declines to a low adult level when the rapid period brain maturation is completed [9]. Indeed, in human newborns at birth, more than half of the daily 16–18 hours of sleep are occupied by REM sleep [17]. Then the decline in REM sleep is much slower in humans and reaches low levels only at the pre-school years period [8,17]. Globally, the time course of REM sleep development in humans and other mammals corresponds well with the brain maturation period.

During a period in which environmental experiences are very limited, the development of precise neuronal connections in the mammalian brain requires a high level of endogenous neuronal activation. REM sleep is characterized by a high endogenous phasic neuronal activity and a particular neuromodulatory context that favourably influences early neural development [16]. This phasic activity is characterized in the visual system by pontogeniculo-occipital (PGO) waves [18,19]. REM sleep is also associated with acetylcholine (ACh) release [20], a neurotransmitter that influences neural development [21] and synaptic remodelling [22]. The most popular experimental approach to test the role of REM sleep during brain maturation is to selectively deprive the developing animal of REM sleep.

REM sleep deprivation during brain maturation. One possibility is to deprive the mammal of its normal quota of REM sleep during the critical period of brain development and then to study the consequences of this deprivation on later brain function and plasticity in adulthood. Investigators have used a pharmacological approach, since long-term instrumental deprivation or lesion studies are not feasible [23–28]. REM sleep was, therefore, suppressed using anti-depressant drugs such as clomipramine or antihypertensive molecules like clonidine during the second and third weeks of post-natal development in rats.

Neonatal REM sleep-deprived animals showed long-lasting changes such as anxiety, disturbed sleep, reduced sexual activity, despair behaviour, reduced pleasure seeking and increased alcohol preference [26,29–34]. It has been proposed that these behavioural changes could reflect symptoms of endogenous depression [25]. There are several other arguments for this hypothesis, provided by other studies [24,29,30]. For example, adult rats neonatally treated with clomipramine displayed reduced shock-induced aggression and enhanced defensive responses. Administration of anti-depressant drugs

to these animals in adulthood improved some of these behavioural changes. These findings suggest that neonatal REM sleep deprivation induces adult depression [16].

Regional brain measurements in these neonatally REM sleep-deprived rats displayed a significant size reduction of the cerebral cortex and brainstem. A proportional reduction of tissue protein was also found in the affected brain areas [33]. Neurotransmitter circuitry was also modified: in the cerebral cortex, the level of the gamma-aminobutyric-acidergic (GABAergic) depression of the glutamate-induced single cortical neurons responses was greater in the neonatally REM sleep-deprived rats as compared to controls [35,36], while there was a hyper-sensitivity of the pyramidal cells to noradrenalin in the hippocampus [37].

In rats, environmental enrichment has been demonstrated to increase the size of the cerebral cortex, the efficacy and number of synapses and the problem-solving ability [38,39]. However, the neonatally REM sleep-deprived rats subjected to this enriched environment did not display any significant plasticity effect after weaning [33]. Hippocampal plasticity has been studied in rats by using the kindling model, in which kindling causes a prolonged decrease in latency and increase in sensitivity for epileptogenesis by electrical stimulation in the hippocampus. When compared with kindled controls, neonatally REM sleep-deprived kindled rats displayed an increase in latency and a reduced excitability ratio [40].

REM sleep and the visual system development.

Relationship between sleep and synaptic plasticity during brain maturation have been extensively studied in the developing visual system, since this system has provided a model for much of understanding of the mechanisms of neural development [41]. Development of central visual pathways occurs at ages when sleep amounts are very high or during landmark changes in sleep expression [9]; it requires both endogenous and exogenous (visual experience) sources of neuronal activity. The interplay of these two factors has been well documented in the lateral geniculate nucleus of the thalamus (LGN) and primary visual cortex (V1). It has been shown that early development of both LGN and V1 depends upon endogenous or spontaneous neural activity. For example, in cats, the segregation of retinal afferents in the LGN, which normally occurs between embryonic day 45 (E45) and birth (E65), well before eye opening, was impaired by the infusion, during this period, of tetrodotoxin, which prevents spontaneously generated action potential activity involved in this segregation [42]. A similar

stage of development is reported in V1, where the segregation of LGN afferents into ocular dominance columns begins well before the onset of visual experience [43]. Visual experience is required at later critical periods for the maintenance and refinement of selective and strong visual responses and precise columnar structure in the cortex. For instance, while rudimentary orientation selectivity can develop in the absence of patterned visual experience, this response property rapidly deteriorates if visual experience is prevented during a period that begins ~ 2 weeks after eye opening [44,45].

To test the role of REM sleep in brain development, several works have studied the effects of REM sleep deprivation or the elimination of the REM sleep PGO waves, on subsequent visual system development. A first study found that brainstem lesions in kittens that eliminated PGO waves resulted in smaller LGN volumes and reduced LGN soma sizes [46]. This result was then confirmed and extended in a second study, in which PGO waves suppression in developing cats produced much slower LGN responses to stimulation of the optic chiasm [47]. These morphological and functional changes in LGN cells are consistent with a delayed maturation of the LGN and suggest that REM sleep activity provides a source of endogenous neuronal activity necessary for normal LGN development. More recent works used various forms of selective REM sleep deprivation or suppression of PGO waves combined with monocular deprivation (MD). One commonly used method to selectively deprive kittens of REM sleep is the 'flower pot' or 'pedestal' technique that consists of placing the animal on a platform emerging from water. On this pedestal, the animal can generate non-REM sleep but not REM sleep because, at onset of REM sleep, the animal falls in the water due to muscular atonia. Using this technique, Oksenberg et al. [48] showed that 1 week of REM sleep deprivation in kittens enhanced the effects of MD on LGN cell morphology: LGN cells receiving input from the occluded eye were smaller when REM sleep deprivation was combined with MD compared to MD alone, therefore resulting in greater difference in the size of LGN cells activated by the open and deprived eyes. A similar increase in LGN cell size disparity has been reported when MD is combined with brainstem lesions that eliminate PGO waves in kittens and, in this case, LGN cells receiving input from the open eye appeared to increase in size [49]. These studies suggest that at least some effects of REM sleep deprivation on LGN cell size are mediated by the phasic processes of REM sleep. Another interesting result showed that REM sleep deprivation for 1 week decreased immunoreactivity for the calcium binding protein parvalbumin, which has been demonstrated to

influence certain forms of neuronal synaptic plasticity [50], in GABAergic inter-neurons of the developing LGN [51]. Therefore, all these studies indicate that REM sleep may influence plasticity in the LGN during critical periods of visual system development [52].

REM sleep has also been reported to modulate the expression of *long-term potentiation* (LTP) elicited during the critical period for visual system development [53]. In this type of LTP, high-frequency white-matter stimulation in neocortical slices from juvenile rats (post-natal days (P) 28–30) produces LTP in upper neocortical layers; this effect wanes with age (P35+), and is no more observed in adult neocortex. Shaffery et al. [54] used a less stressful version of the ‘pedestal’ technique (‘multiple small-platform’) and found that 1 week of REM sleep deprivation extended the critical period for this developmentally regulated form of LTP in visual cortex; this type of LTP was observed in slices of visual cortex from REM sleep deprived rats at ages P34–40, when it is not normally found. This result is in line with the concept of a maturational delay and suggests that REM sleep deprivation impairs or retards normal brain maturation.

Non-REM sleep

Non-REM sleep is characterized by events which potentially induce synaptic plasticity, such as synchronized bursting in thalamocortical circuits, transient increases of intra-cellular calcium and, in some mammals, the release of somatotropins [13,55,56]. A role for non-REM sleep in developmental cortical plasticity is suggested by maturational changes in non-REM sleep that coincide with periods of heightened cortical plasticity. In the cat, there is a steep decrease in REM sleep and a sharp increase in non-REM sleep amounts near the beginning of the critical period for visual system development [9]. The beginning of this period also coincides in rats with the development of non-REM sleep homeostasis. Before the 4th post-natal week, non-REM sleep EEG does not intensify following sleep deprivation, indicating that the regulatory relationship between wake and non-REM sleep matures in parallel with periods of heightened cortical plasticity [57].

A more recent study has highlighted a relationship between non-REM sleep and developmental cortical plasticity *in vivo* [58]. MD not only induces morphological changes in the LGN during the critical period for visual system maturation, but also provokes rapid changes in neocortical responses to visual stimulation at this time [52]. Frank and colleagues [41,58] combined MD with periods of ad lib sleep or sleep deprivation. Cats at the peak

of the critical period had one eye sutured shut and were kept awake in an enlightened environment for 6 hours. This MD period was used as a standard stimulus for the induction of plasticity. The authors wanted to determine whether the effects of MD would be enhanced by a period of sleep occurring immediately thereafter. Both optical imaging of intrinsic cortical signals and extra-cellular unit recording showed that sleep nearly doubled the effects of MD on visual cortical responses. In this study, it was not possible to determine the exact contribution of REM and non-REM sleep to this process. However, the enhancement of cortical plasticity was highly correlated with non-REM sleep time and intensity, suggesting an important role for non-REM sleep in the rapid cortical synaptic remodelling elicited by MD. Another study has shown that non-REM sleep electrical activity itself underwent changes as a consequence of waking experience during a late critical period (P30–60) in cats and mice [59]: dark-rearing induced during sleep a huge and reversible decrement of delta activity (1–4 Hz) that was restricted to the visual cortex. Interestingly, this modulation was impaired by gene-targeted reduction of NMDA receptor function, potentially reflecting that NMDA receptor activation participates in the adjustment of non-REM sleep rhythms by sensory experience during a late critical period for visual system development [59].

Taken together, these findings could suggest that non-REM sleep consolidates waking experience; a process that might begin during critical periods of brain development when the animal is most sensitive to waking experience and is retained throughout life [41].

Summary and further considerations

REM sleep deprivation produces several anatomical and electrophysiological changes in the developing visual system and modulates cortical plasticity. Non-REM sleep seems to be necessary for the consolidation of visual traces during critical periods of experience-dependent cortical plasticity *in vivo*. These results suggest that both sleep states may be important for neural development, although the contribution of each state is likely to be different. If the precise role of each state is still unclear, current findings indicate that the relative amounts of REM sleep and non-REM sleep during early life both influence brain maturation [41]. REM sleep is maximally expressed at ages when endogenous neuronal activity is crucial for the establishment of fundamental neuronal circuitry in the visual system. Non-REM sleep, on the other hand, is present at later stages of development, quickly matures after eye

opening [9,10] and become homeostatically regulated by wake in a way similar to adult non-REM sleep during critical periods of experience-dependent synaptic plasticity [57]. Therefore, it is possible that, while REM sleep helps establish early patterns of neural circuitry, non-REM sleep in part consolidates changes in neural circuitry elicited by waking experience [41].

Although the findings discussed above strongly support a role for sleep in neural development, some considerations should be kept in mind [41,52]. First, the potential secondary effects of the experimental manipulation used in a study should be considered. For instance, sleep deprivation induces stress and has multiple behavioural or neurochemical effects that may in turn influence the results of an experiment [60,61]. Another issue is that manipulations performed in one sleep state may also influence neural processing in other vigilance states, making it difficult to determine which vigilance state is responsible for the observed effects. For example, REM sleep deprivation can alter non-REM sleep architecture, increasing sleep fragmentation and suppressing deeper stages of non-REM sleep, even when total amounts of non-REM sleep are preserved [62–64].

At present, available experimental evidence strongly suggests a role for sleep in brain development, but further studies are still needed. In particular, we currently know little about the cellular and molecular mechanisms by which sleep exerts its effects during neural maturation. The confirmation of such data might have important public health implications [16]. For example, the use of antidepressants or anti-hypertensive drugs such as clonidine during pregnancy and lactation can suppress foetal and neonatal REM sleep. Moreover, infants born prematurely suffer from sleep disturbances during a long stay in the neonatal intensive care unit [16]. Some behavioural and physiological consequences in adulthood in these individuals may be caused by sleep deprivation in early life and may consequently be better prevented by the development of appropriate clinical interventions, with the aim of improving the neurobehavioural outcome of high-risk infants.

Brain plasticity in adulthood: The role of sleep in learning and memory

Sleep has also been implicated in the plastic cerebral changes that underlie learning and memory in the adult brain. Three sequential steps may be considered to test this hypothesis: exposure to a new stimulus, processing of memory traces and performance at re-test. In this design, sleep would

participate to the consolidation of memory traces [65]. Consolidation refers to the processing of memory traces during which the traces may be reactivated, analysed and gradually incorporated into long-term memory [66]. According to this hypothesis, the memory trace stay in a fragile state until the first post-exposure sleep period has occurred [67]. At present, the major debate is whether memory trace consolidation during sleep relies on specific patterns of neuronal activities and their effects at the sub-cellular level or on time-dependent factors unrelated to sleep itself (e.g. circadian rhythms, stress hormones, etc.) [65]. In the following lines, we will give a summary review of relevant works, assessing the role of sleep in learning and memory in adult animals and humans and presented following the different levels of description, from the behavioural to the molecular scale.

Behavioural level

While not suited to highlight the underlying neurobiological mechanisms, behavioural studies of sleep probe the impact of sleep on learning and memory. There are three overlapping broad categories of findings at this level [52]. First, sleep amounts, in particular REM sleep, are reported to increase following a learning task or exposure to an ‘enriched’ environment known to trigger synaptic remodelling. Secondly, learning performances in specific tasks are enhanced following certain periods of sleep. And, thirdly, sleep deprivation following a learning task impairs task acquisition. We shall present a brief and non-exhaustive overview of animal and human data covering these topics.

Animal data. The general architecture of sleep may be altered during the post-training night. In animals, mainly rodents, it has been shown that training on various learning tasks is followed by an increase in REM sleep duration [68,69], which reverts back to normal levels once the animal masters the task [70,71]. More recently, Datta [72] reported increases in REM sleep and non-REM–REM transitional states following conditioned avoidance learning in the rat and found that levels of performance improvement between practice sessions was positively correlated with the density of pontine waves (i.e. the PGO waves component recorded in the pons) occurring in both the intervening REM sleep and non-REM–REM transitional sleep. This last finding suggests that the potential positive effects of REM sleep on memory may depend more on changes in REM sleep phasic events than on absolute REM sleep amounts [52]. In animals, an important issue is whether the stress response possibly

accompanying the training sessions could explain post-training changes in sleep parameters. Indeed, it is known that stress in itself can lead to an increase in REM sleep and that an acute stress may favour memory formation [73,74]. However, an argument against a prominent influence of stress on the results obtained in sleep/memory studies is that post-training REM sleep rebound seems closely related to learning processes [65]. Indeed, there is no detectable increase in REM sleep when material cannot be learnt (pseudo-conditioning) [70,71] or when the animal does not reach a sufficient level of learning in the task [75,76], despite the fact that the experimental conditions induce stress levels similar to that experienced by animals that successfully mastered the requirements of the task.

To a lesser extent, rodent studies have also shown changes in non-REM sleep amounts and architecture following exposure to enriched environments [77,78] or learning tasks, in some cases coincident with increases in REM sleep [79–81].

Conversely, sleep deprivation appears to profoundly interfere with learning and memory consolidation in mice and rats, with the greatest effects reported in ‘demanding’ learning tasks that presumably require more complex cognitive processing [82]. However, the sleep deprivation methodology in animals is inherently affected by non-specific effects of sleep deprivation, such as increased brain excitability and stress response, that might also lead to memory impairment [83]. Although these indirect effects cannot be ruled out, several arguments favour a genuine role for the lack of sleep [65]. For instance, learning is impaired by sleep deprivation only if the task entails a new behavioural strategy [75,82]. Also, impairment of performance is observed only if the sleep deprivation occurs during specific periods of time, called paradoxical sleep windows, whose characteristics vary with the strain and type of the organism used in the study, the type of learning task and the number of training trials per session [77,78]. Similar deprivation outside these sleep periods has no effect on subsequent performance, despite similar stressful conditions [84].

Human data. In humans, long-term memories belong to multiple memory systems, roughly categorized in two main types: declarative and non-declarative memories [85,86]. In declarative memory, information encoding and retrieval are carried out explicitly, i.e. the subject is aware that the stored information exists and is being accessed. Conversely, non-declarative memories can be acquired and re-expressed implicitly, i.e. although the subject is not necessarily aware that new

information has been encoded or retrieved, its behavioural performance may be affected by the new memory [87]. This distinction is only schematic since several sub-categories of these memory types are described. Declarative memory is composed of episodic memory, i.e. autobiographical memory for events that occur in a specific spatial and temporal context, and of semantic memory which refers to general knowledge about the world [86]. On the other hand, non-declarative memories gather very different cognitive forms such as skills and habits, priming and simple conditioning [87]. However, it should be kept in mind that categorization of learning tasks among memory systems is not always clear-cut. For instance, no task can really be deemed specific to a single memory system, because explicit or implicit contributions to the performance cannot be completely segregated. Moreover, not all non-declarative memories are implicit. For instance, in the serial reaction time (SRT) task, a paradigm of implicit learning in which subjects are asked to react to the appearance of successive stimuli, the task could be assimilated to explicit learning if the subjects are aware that there is a sequence to learn [88].

Human studies have evidenced post-training REM and non-REM sleep modifications or performance impairments following sleep deprivation for a variety of learning tasks that cannot be easily segregated in homogeneous categories [87]. The following lines will give an overview of these data relating sleep stages to declarative and non-declarative memories. A peculiar situation concerns learning of emotionally-charged contents that will be discussed separately.

Declarative learning tasks have been shown to induce REM and non-REM sleep changes. Post-training REM sleep modifications were observed for example in Morse code learning [89], intensive learning period in college students [90] and textbook passage study [87]. On the other hand, effects on subsequent non-REM sleep have also been described: positive correlation between the non-REM–REM sleep cycles and memorization of word lists [91], stage 2 sleep and EEG spindles increases after maze learning [92] and positive correlation between EEG spindles increases following memorization of word lists and overnight improvement in the number of recalled words [93,94]. Moreover, performance on several declarative tasks has been shown differentially modulated by sleep, depending on the specific sleep stage that is predominant or selectively reduced [87]. For instance, recall of sentences and prose passages is impaired following selective REM sleep deprivation [95] and likewise poorer recall of short stories [96] was observed following REM sleep, but not SWS, deprivation. Conversely, several studies indicate that the recall of

paired-associate lists of words [97–99] is better after sleep during the first part of the night (early sleep; SWS predominant) than after sleep during the second half of the night (late sleep; REM sleep predominant). In the same way, the recall of spatial memory in a declarative mental spatial rotation task is better following early than late sleep [100]. Thus, there are some discrepancies in human studies about the respective role of non-REM and REM sleep for declarative learning tasks. Also, Ficca et al. [101] showed that morning recall of pairs of unrelated words was impaired after fragmented sleep leading to cycle disorganization, but not when awakenings during the night preserved the sleep cycle, suggesting the importance of whole night organization of sleep rather than a specific sleep stage in this type of declarative learning task.

Concerning non-declarative tasks, discrepancies also exist about the respective role of non-REM and REM sleep. Examples from two learning types will be presented here, visual perceptual learning and procedural motor learning. In the first type, selective REM sleep deprivation, but not SWS deprivation, abolishes the overnight performance improvement during a texture discrimination task [102], suggesting that processes of memory consolidation strongly depend on REM sleep in this task. No performance improvement was noticed after one night of sleep deprivation followed by two full nights of recovery [103], suggesting that the first night after the learning session is mandatory to the formation of the memory trace in this perceptual task. On the other hand, with the same task, the performance improvement was not altered by late sleep deprivation but rather by early sleep deprivation, dominated by SWS, and even more so by total sleep deprivation [104]. Repeated testing on this task four times in a single day leads to a performance deterioration, which seems to be training-specific and not due to a generalized fatigue [105]. Mednick et al. [105] demonstrated that this deterioration can be prevented when subjects take a mid-day nap between the second and third sessions. The authors argued that SWS had a central role in this process, because there was a significant increase in SWS during the 60-minute naps taken on the day of the texture discrimination task, compared to baseline naps taken on a different day, while the increase in REM sleep was not statistically significant. Still the same authors further showed a better improvement to the task when naps incorporate both SWS and REM sleep episodes [106]. Data from procedural motor learning tasks may also diverge, concerning the respective roles of REM and non-REM sleep in consolidation of motor skills [107]. For example, sleep after practice of a sequential motor task, the finger-to-thumb opposition task, enhanced speed of sequence performance and

reduced error rate; this effect was specific to the motor sequence learned and independent of whether sleep was placed during daytime or night time [108]. In the visuo-motor sequential learning task, the serial reaction time task (SRT), subjects are asked to react as quickly and accurately as possible to the appearance of successive stimuli by pressing the spatially corresponding key. The sequential structure of the stimuli is manipulated unknown to the participants. Cajochen et al. [109] yielded evidence that implicit learning of these structured sequences is modulated by circadian phase and sleep type, as performance improved after multiple naps and in particular after naps that followed the circadian peak of REM sleep. Huber et al. [110] found a performance improvement in another procedural implicit motor learning task, the rotation adaptation task, after a night of sleep; most importantly, this post-sleep performance enhancement was positively correlated with a local increase in EEG power that was specific to the delta frequency range (1–4 Hz), the major electrophysiological marker of SWS. In a recent study, Robertson et al. [88] showed that offline skill improvements, following explicit sequence learning of the SRT task (that is, when subjects were aware that there was a sequence to learn), were only observed when the interval until re-test included sleep and the overnight improvement was correlated with the amount of non-REM sleep. Intriguingly, they also found that when subjects had little awareness for the sequence (implicit learning), an offline learning was observed regardless of whether the interval until re-test did or did not contain a period of sleep. They concluded that offline learning was sleep (and mostly non-REM sleep) dependent for explicit skills but time-dependent for implicit skills, a conclusion awaiting further experimental support. Stage 2 sleep has also been implicated in motor learning. For instance, proficiency in learning to tap simple finger sequences at an increasingly rapid rate while avoiding errors has been reported to positively correlate with stage 2 sleep, especially that in the last quadrant of the night [111]. As alluded above in the field of declarative memory [101], one possible hypothesis to take into account the role of both REM and non-REM sleep is a sequential processing of memory, in which non-REM sleep would prompt memory formation and REM sleep would possibly but not necessarily consolidate these memory traces [81,87]. In line with this concept of the importance of a full night of sleep, Stickgold et al. [112] showed that improvement in the visual texture discrimination task in subjects who slept for 8 hours during the night after training best correlates with the combined proportion of SWS in the first quarter of the night and of REM sleep in the last quarter.

It also seems that the emotional content of the material to be learnt influences the way sleep impacts on task performance [113]. With the early-night/late-night paradigm, Wagner et al. [114] have shown that, while early sleep is overall better for simple declarative memory retention, REM sleep enhances recall of emotionally charged memories. Moreover, REM sleep increases the negative reaction to previously viewed pictures with negative content [115]. These results are in accordance with a long history of evidence suggesting that REM sleep and possibly REM sleep dreaming in particular contributes to the processing of affective memories [116–119]. In addition, shortenings of sleep latencies and increases in REM densities have been reported in major depression [120,121], the state of bereavement [120,122], war-related anxiety [123] and more generally in post-traumatic stress disorder (PTSD) [124]. These findings suggest that REM sleep processes emotional memories and that changes in REM sleep might lead to dysfunctional processing of traumatic memories during sleep, which might in turn contribute to PTSD [125].

In summary human behavioural studies at the general level provide evidence for the role of sleep in memory processing. Still, the results about the specific role of each sleep stage for the different types of memory processes are not univocal. Some findings support the dual process hypothesis that argues that REM sleep and non-REM sleep act differently on memory traces, depending on the memory system they belong to. In this concept, a classical view is that non-REM sleep would facilitate consolidation of declarative memory with no affective content, whereas REM sleep would facilitate consolidation of non-declarative memory and emotionally-charged declarative memories [78]. However, this distinction is not supported by all findings, some data suggesting a role of SWS in non-declarative memories. Finally, other studies emphasized the potential importance of the ordered succession of non-REM and REM sleep in memory consolidation processes, in line with the sequential hypothesis [81]. These hypotheses are not contradictory by themselves but deserve further clarification for a better understanding of the roles that sleep stages play in the consolidation of different memory types.

Brain systems level: Reactivation of neuronal ensembles during sleep

Neuronal activity patterns displayed during previous learning seem to be reinstated during sleep. These reactivations would allow for the adaptation of inter-cellular connection strengths between the elements of the network and the incorporation of

the new experience into long-term memory [65]. Consolidation of memory traces would involve not only the strengthening of some synapses but also the weakening of other, inappropriate, connections that overload cerebral networks ('reverse learning') [126,127].

Animal data

Hippocampus and sleep. At the level of individual hippocampal cells, the firing pattern during sleep depends on previous waking experience. An initial report in rats showed that hippocampal neurons activated during a waking period displayed increased activity during subsequent REM sleep and non-REM sleep [128]. Other studies in the rodent hippocampus have shown that pairs of hippocampal neurons whose activity is correlated during a learned behaviour are more likely to show correlated activity in non-REM sleep subsequent to the training period [129,130] and that the temporal sequence of neuronal firing during waking is preserved in subsequent non-REM sleep [131,132]. The replay of sequences is not specific to non-REM sleep. After repetitive exposure to a circular track, the patterns of discharges of multiple hippocampal units, reflecting up to several minutes of behavioural experience, are reproduced during REM sleep [133]. There is also evidence that the novel representations are strengthened while the older ones are weakened during REM sleep. For example, hippocampal firing for new experience during post-exposure REM sleep occurs in phase with the theta rhythm (4–7 Hz), a condition known to induce LTP, while cells coding for familiar environments tend to fire out of phase with the theta rhythm, a situation that may lead to depotentiation [127].

At the network level, simultaneous ensemble recordings in the posterior parietal neocortex and in the CA1 field of the hippocampus of rats showed that the ensemble activity-correlation structure within and between these areas during exploration of a simple maze resembled that of subsequent sleep, suggesting a re-expression of traces of recent experience in both neocortical and hippocampal circuits during sleep and a potential dialogue between these two systems along with this process [134]. In a recent work, recording of extra-cellular activity and local field potentials in multiple fore-brain areas of rats has allowed one to observe that the spatiotemporal patterns of neuronal ensembles activity initially produced by the exploration of novel objects recurred for up to 48 hours, not only in the hippocampus, but also in the cerebral cortex, putamen and thalamus; this reverberation was strongest during SWS, more variable across animals during REM sleep and decreased during waking [135].

Other studies in rats have shown that the activity of hippocampal cells is integrated in two types of macroscopic patterns [63,136,137]. First, gamma oscillations (40–100 Hz) and theta rhythm are recorded in the superficial layers of the entorhinal cortex, the gyrus dentatus and the CA3 and CA1 fields of the hippocampus during exploratory behaviour and REM sleep. On the other hand, sharp waves, crowned by high-frequency ripples (140–200 Hz) are initiated in CA3 and recorded in CA1 and the deep layers of the entorhinal cortex, during awake immobility and non-REM sleep. These observations have led to the elaboration of a two-stage model of hippocampal functioning through hippocampo-neocortical interactions during sleep [136,138,139]: during gamma and theta oscillations, neocortical inputs would transfer information about the external world to the hippocampal structures through the entorhinal cortex; and during ripples and sharp waves, hippocampal information is believed to be played back to the entorhinal cortex [136,137] and through it, to neocortical areas [140]. This model might, therefore, consolidate memory traces, as has been suggested by computational simulations [141].

If these results are interesting, they do not provide definitive evidence for the involvement of sleep in memory processes, as there is no evidence that these neuronal hippocampal activities actually modulate the behavioural adaptation to the new environment [65].

Cortical reactivations and thalamocortical interactions: Sleep spindles and slow oscillations. Neuronal reactivations do not only occur in the hippocampus. Cortical neuronal activities during sleep can also be modulated following training on a hippocampus-independent task [65]. For example, fast (30–40 Hz) neocortical oscillations in the cat can be enhanced by instrumental conditioning during wakefulness and a selective increase in these oscillations is observed during subsequent non-REM and REM sleep [142]. More generally, non-REM sleep oscillations (spindles, delta waves, slow rhythms) are associated with rhythmic spike bursts in thalamic and cortical neurons, which lead to persistent excitability changes [143]. These short-term plasticity processes might be used to consolidate memory traces acquired during wakefulness.

Sleep spindles are a prominent feature in the early stages of non-REM sleep. The neurophysiological mechanisms of spindle generation involves thalamic and corticothalamic networks, but their definitive functional meaning still remains to be elucidated [144]. It has been hypothesized that spindle activity would be related to massive Ca^{2+} influx into

dendrites of spindling cells [145]. This massive Ca^{2+} influx would produce high enough levels of $[\text{Ca}^{2+}]_i$ to activate protein kinases and, thereby, produce LTP. These changes would, thus, open the gate to subsequent long-term modifications in cortical networks.

During non-REM sleep, large populations of cortical neurons also fire synchronously in a slow oscillation (<1 Hz) that alternates phases of hyperpolarization and depolarization [146]. During the depolarization phase, it has also been hypothesized that bursting neurons would generate short periods of fast oscillations that would iteratively recall and store information embodied in the assemblies primed during spindling [145]. Timofeev et al. [147] proposed that the neurons recruited by the slow oscillations would preferentially be those with the highest number of synapses recently potentiated during previous wakefulness.

PGO waves. In animals, a distinguishing feature of REM sleep is the recording of (PGO) waves [148], i.e. prominent phasic bioelectrical potentials, closely related to rapid eye movements that occur in isolation or in bursts during the transition from non-REM to REM sleep or during REM sleep itself [149, 150]. Although observed from many parts of the animal brain [151], PGO waves are most easily recorded in the pons [152], the lateral geniculate bodies [153] and the occipital cortex [148], hence their name. PGO waves seem to represent a fundamental process of REM sleep in animals and notably would be important in central nervous system maturation, as seen previously.

PGO waves might also participate in learning and memory consolidation processes in adults. Indeed, *P*-wave (i.e. the pontine component of PGO-type waves recorded in rats) density not only substantially increases after aversive conditioning in rats [149], but the percentage of changes in *P*-wave density between REM sleep episodes was shown proportional to the improvement of task performance between sessions [72]. Moreover, activation of the phasic *P*-wave generator by carbachol micro-injections is coupled with enhanced performance improvement on a two-way active avoidance learning task [154] and would eliminate the learning impairment produced by post-training REM sleep deprivation [155]. When induced by brainstem stimulation [156], PGO waves synchronize high-frequency activities (20–50 Hz), the expression of which can be experience-dependent during sleep [142]. Hence, it is suggested that PGO waves during REM sleep represent a natural physiological process of memory [157], potentially through the synchronization of fast oscillations that would convey experience-dependent

information in thalamo-cortical and intra-cortical circuits [156]. Another intriguing fact is the temporal relationship, described as a phase-locking, during REM sleep, between PGO waves in cats and hippocampal theta waves, that have been hypothesized to drive the PGO waves generator [158–160]. Considering that high-frequency stimulation in area CA1 of the rat hippocampus during the positive phase of hippocampal theta rhythm has been shown to induce LTP, while stimulation on the negative phase can reverse previously established LTP [161], the interaction between theta and PGO waves has been proposed to be involved in the possible learning and memory functions of REM sleep [162].

Human data: Contribution of functional neuroimaging

PGO waves in humans? In humans, some data suggest that the rapid eye movements observed during REM sleep could be generated by mechanisms similar to PGO waves in animals. In epileptic patients, direct intra-cerebral recordings in the striate cortex have shown monophasic and diphasic potentials during REM sleep [163]. In normal subjects, surface EEG displayed transient occipital and/or parietal potentials time-locked to rapid eye movements [164] and source dipoles of magnetoencephalography (MEG) signal have been localized in the brainstem, thalamus, hippocampus and occipital cortex during REM sleep [165]. A recent MEG study has also identified an intermittent and prominent activation in mid-pontine nuclei (PNs) during REM sleep eye movements in normal human subjects [166]. Since definitive proof for the existence of PGO waves in humans would require *in situ* electrophysiological recordings, which is obviously precluded in healthy subjects, non-invasive functional neuroimaging techniques represent a suitable approach to unravel their presence in humans. Using cerebral blood flow (CBF) determination with positron emission tomography (PET) in normal sleepers, Peigneux et al. [167] showed that regional cerebral activity in the LGN and the occipital cortex was closely related to the production of spontaneous rapid eye movements more during REM sleep than during wakefulness. This result suggests that processes similar to PGO waves are responsible for rapid eye movements generation in humans. Unlike in animals, the direct demonstration of an association between PGO activity and memory consolidation is still unavailable. Nevertheless, this hypothesis is supported by several works showing an increase in the density of rapid eye movements during REM sleep following procedural learning [168] and intensive learning periods [90] or a correlation between retention levels after learning a Morse

code and the frequency of rapid eye movements during post-training REM sleep [89].

Experience-dependent changes in functional connectivity during post-training sleep. The reactivation during sleep of neuronal ensembles activated during learning appears as a possible mechanism for the off-line memory processing. Such a reactivation has been reported in several experimental situations: in the rat hippocampus [128–130,132–135], neocortex [134,135], putamen and thalamus [135], as described earlier, and in the song area of young zebra finches [169]; this suggests the generality of the reactivation in the processing of memory traces during sleep, across species [65]. However, functional significance of this reactivation in animals still remains obscure as it has not been shown in these experiments that experience-dependent modifications in neuronal populations were associated with subsequent behavioural change. This section will describe two types of human functional neuroimaging experiments. The first study shows that sleep deprivation during the first post-training night impairs the changes in regional cerebral segregation and integration that usually underlie the performance gains in subjects allowed to sleep on that night [170]. The second type of studies demonstrates the reactivation of both cortical and hippocampal structures during post-training sleep and provides evidence for a link between learning, as measured by behavioural methods and the activity of neuronal populations during sleep [171–174].

In a functional magnetic resonance imaging (fMRI) study, learning-dependent changes in regional brain activity after sleep or sleep deprivation were compared using a pursuit task (PT), in which subjects were trained to maintain a joystick position as close as possible to a moving target, whose trajectory was predictable on the horizontal axis but not on the vertical axis [170]. The time on target was used as the behavioural performance parameter. In the first group, subjects were totally sleep deprived during the first post-training night; while in the second group, they were allowed to sleep during the same night. Both groups were then re-tested after at least two more nights of normal sleep in order to recover a similar state of arousal across the two groups and between the training and re-test sessions. Indeed, the aim of the study was not to describe the immediate consequences of sleep deprivation on human performance or cognition, but to provide evidence that sleep deprivation alters the slow processes that lead to memory consolidation. The fMRI scanning session was recorded during this re-test, while subjects were exposed to the previously learned trajectory and also to a new one in which the

predictable axis was vertical. Behavioural results showed that the time on target was larger for the learned trajectory than for the new one in both groups during the re-test and that this performance gain was greater in the sleeping group than in the sleep-deprived group. The fMRI data displayed a significant effect of learning, irrespective of the group, in two regions: the left supplementary eye field (SEF) and the right dentate nucleus. The right superior temporal sulcus (STS) was found more active for the learned than for the new trajectory and more so in the sleeping group than in the sleep-deprived group. The functional connectivity also showed that the dentate nucleus was more closely linked to the STS, and the SEF to the frontal eye field (FEF), for the learned than for the new trajectory and more so in the sleeping group. Moreover, interactions between temporal cortex and cerebellum as well as between the FEF and the SEF are known to be both implicated in the standard pursuit eye movement pathways [175]. These results, therefore, suggest that the performance on the PT relies on the subject's ability to learn the motion patterns of trajectory in order to programme the optimal pursuit eye movements; sleep deprivation during the first post-training night would disturb the slow processes that lead to the acquisition of this procedural skill and alter the related changes in connectivity that are usually reinforced in subjects allowed to sleep on the first post-training night [170].

Reactivation studies were conducted using the PET technique. In a first PET experiment [172], variations in regional cerebral blood flow (rCBF) were determined in three groups of normal subjects under different conditions. The first group (A) was intended to provide a list of the brain areas that were activated during the execution of the probabilistic SRT task [176], as compared to rest. SRT is a paradigm of implicit sequence learning, in which the succession of stimuli followed a sequential pattern based on a highly complex probabilistic finite-state grammar, without the subjects knowing it. A second group (B) was similarly trained and then scanned during the post-training sleep to identify the brain areas more active in REM sleep than during resting wakefulness after practice of the SRT task. To ensure that post-training REM sleep rCBF distribution was different from the pattern of 'typical' REM sleep, a third group (C), not trained to the task, was scanned at night in the same conditions. The final analysis showed the regions (bilateral cuneus and adjacent striate cortex, mesencephalon and left pre-motor cortex) that were both more active during REM sleep (vs resting wakefulness) in the trained subjects compared to the non-trained subjects and activated during the execution of the SRT task, suggesting that memory traces were actually reprocessed during

REM sleep, because the subjects' performance on the task was improved in the post-sleep re-test session [172]. In addition, the rCBF in the left pre-motor cortex was significantly more correlated with the activity of the pre-supplementary motor area (pre-SMA) and posterior parietal cortex (PPC), two important structures in sequence learning, during post-training REM sleep than during 'typical' REM sleep [173], further supporting that sequential memory traces were replayed in the cortical network during REM sleep.

However, it was not possible to determine in the study of Maquet et al. [170] whether experience-dependent reactivations during REM sleep were related to a simple visuo-motor skill optimization or to the high-order acquisition of the probabilistic sequential structure of the learned material. In order to test this issue, a new group (D) was scanned during sleep after practice on the same SRT task, but using a completely random sequence. First, there was no significant reactivation during post-random SRT training, suggesting that the processing of recent memories during post-training sleep does not seem to be initiated unless the material to be learnt is structured. Most importantly, a probabilistic-specific activation during post-training REM sleep was found in the left and right cuneus, when comparing probabilistic- and random-SRT trained groups; rCBF in these reactivated structures was correlated to the amount of high-order learning achieved prior to sleep and functional connections were found reinforced between the reactivated cuneus and the caudate nucleus of the striatum, a structure known to be specifically involved in probabilistic sequence learning during wakefulness [177], suggesting its implication in the off-line reprocessing of implicitly acquired high-order sequential information during REM sleep.

A second PET study, recently published [174], looked for the brain areas reactivated during non-REM sleep following spatial learning in normal human subjects and the relation between this experience-dependent reactivation and the modifications in subsequent spatial behaviour. Again, rCBF was estimated in three experimental subject groups: first, during training to a declarative spatial memory task in which subjects learned to find their way inside a three-dimensional virtual town; secondly, during all stages of nocturnal sleep after an extended period of spatial training to the town; and, thirdly, during sleep without prior training. The right hippocampus and parahippocampal gyrus were found commonly activated during the wakefulness training session and during subsequent SWS (vs wakefulness or REM sleep) at a higher level in trained than in non-trained subjects, suggesting that hippocampal areas that are activated during a spatial

learning task are likewise activated during subsequent SWS, in agreement with animal studies showing the reactivation of neuronal patterns in hippocampal cell assemblies during non-REM sleep [129,130,132,134,135]. Moreover, this study critically and originally demonstrated a significant correlation between rCBF increases in the right hippocampus and parahippocampal gyrus during SWS (vs wakefulness) and the overnight gain in behavioural performance from the pre-sleep training to the post-sleep re-test session. These data suggest that hippocampal reactivation during SWS is learning-dependent and reflects the offline processing of recent spatial memory traces during non-REM sleep in humans, which eventually leads to plastic changes underlying a subsequent improvement in performance.

In summary, the above studies have found that cortical areas engaged in the implicit acquisition of motor procedural memories are reactivated during post-training REM sleep, but not during non-REM sleep [173], while a reactivation of the (para)hippocampal system occurs during post-training non-REM sleep, but not REM sleep, following intensive topographical/episodic learning [174]. These results are in line with behavioural data suggesting that non-REM sleep and REM sleep differentially modulate the consolidation of declarative and non-declarative memories, respectively, in the model called the dual process hypothesis [78,100]. However, these data do not allow one to discard the sequential hypothesis in which the ordered succession of non-REM sleep and REM sleep would be necessary for the consolidation of memory traces, whatever the memory system [81, 104,112]. These different models should not be viewed as mutually exclusive and deserve further investigation.

Cellular and molecular level

Consolidation of the memory trace is known to rely on particular patterns of neuromodulation as well as on gene expression and protein synthesis [178]. The intervention of sleep in these processes is conceivable but has not been systematically investigated [65]. The results mentioned here, therefore, set the stage for future research.

Neurotransmitter and neuroendocrine activity during sleep. During sleep, there are dramatic changes in the levels of modulatory neurotransmitters throughout the brain. Non-REM sleep is characterized by a decrease in the levels of norepinephrine, serotonin and acetylcholine (ACh), compared to waking [179–181]. Conversely, ACh increases

during REM sleep to levels comparable to that observed during waking, while norepinephrine and serotonin continue to drop to near zero [180]. Because of its differential modulation during sleep, ACh has been more thoroughly investigated in studies exploring learning and memory.

Experimental data show that ACh enhances cortical plasticity in adult mammals [182–184]. In contrast, scopolamine, an ACh antagonist, impairs subsequent performance on an avoidance task when administered to rats during certain periods of REM sleep [185]. Therefore, it has been suggested that ACh may modulate molecular mechanisms of memory consolidation [186]. Physiological evidence, including electrophysiological data and local measurements of ACh levels, supports a two-stage model for the functioning of the hippocampus-dependent memory system, modulated by cholinergic tone changes within the hippocampus across the sleep–wake cycle. High levels of ACh would promote the encoding of new information in the hippocampus during wakefulness, by partially suppressing excitatory feedback connections and then facilitating encoding without interference from previously stored information. Conversely, during non-REM sleep, and especially SWS, the lower levels of ACh would favour the release of this suppression and, therefore, allow spontaneously reactivated hippocampal neurons coding for an association to drive cells in the entorhinal cortex and neocortex without any assistance from sensory input, resulting in the facilitation of memory traces consolidation [65, 187]. Accordingly, a recent study has shown that the increasing in cerebral cholinergic activity during SWS-rich sleep by infusion of a cholinesterase inhibitor in human subjects prevented the consolidation of declarative memories for word pairs, but the treatment did not affect memory consolidation of a non-declarative mirror tracing task nor did it alter memory consolidation during waking. These data support the hypothesis that a low cholinergic tone during human SWS is critical for declarative memory consolidation [188].

Many other neuromodulators might play a role in sleep and memory consolidation. For instance, adenosine is a neurotransmitter that has been the focus of many studies in sleep/wake cycle regulation because of its potential role as a chemical mediator of the sleep-inducing effects of prolonged wakefulness and sleep deprivation [189–191]. It is known that caffeine, an adenosine receptor antagonist, promotes wakefulness and appears to increase arousal and attention [192]. Adenosine receptor agonists and antagonists also appear to alter learning and memory in rodents [191]: caffeine administered after training improves memory for a step-through inhibitory avoidance task in mice, via its action on A_{2a} receptors

[193]. Glutamatergic neurotransmission also seems to be involved in memory formation and particularly in the sleep associated consolidation of procedural skills: caroverine, when administered at low concentrations, is a fairly selective blocker of the AMPA glutamatergic receptor [194,195] and strongly reduced the overnight improvement in procedural visual texture discrimination skills, when infused in normal human subjects during the first 8 hour period of nocturnal sleep [196].

The neuroendocrine system is also differentially activated across the sleep-wake cycle. It is known that the hypothalamo-pituitary-adrenal (HPA) system and, thus, the release of adrenal cortisol are strongly suppressed during human SWS-rich sleep [197]. When infused during this sleep period, the elevated cortisol concentration blocked the increase in recall performance for word pairs, suggesting that the spontaneous decrease in cortisol release during early nocturnal sleep might be a pre-requisite for the consolidation of the hippocampus (a site of very high expression of corticosteroid receptors) dependent declarative memories [198]. More specifically, it has been demonstrated that the apparent detrimental effect of cortisol on declarative memory mainly results from an activation of the glucocorticoid receptors (GR) because administration of the GR agonist dexamethasone before the early retention sleep impaired post-sleep recall of declarative word pairs in the same way as cortisol, while the administration of canrenoate, a mineralocorticoid receptor (MR; the other receptor type that binds cortisol in hippocampal and associated cortical structures) blocker, fails to affect the recall performance [198,199].

DNA/RNA synthesis during sleep. Several studies have investigated the synthesis of DNA and RNA during different vigilance states or after sleep deprivation [200–205]. For example, studies in rabbits showed positive correlations between RNA synthesis in neocortical neurons and EEG synchronization during sleep [202,203]. Less consistent findings were reported in studies assessing DNA synthesis: a negative relationship was found between DNA synthesis and post-learning REM sleep amounts in non-learning rats in one study [204], but the investigators were unable to replicate these earlier results.

The interpretation of these studies is difficult because the presence of increased RNA following sleep does not *a priori* indicate a sleep-dependent increase in substances involved in synaptic plasticity, since the identity of these mRNA transcripts is unknown [52]. Nevertheless, these studies are noteworthy because they constitute some of the

first attempts to link sleep to molecular changes in the brain.

Gene transcription during sleep. Several studies, using techniques such as polymerase chain reaction (PCR), *in situ* hybridization or micro-array technology, have shown that many genes known to influence synaptic plasticity are up-regulated during wakefulness or sleep deprivation and down-regulated by sleep [206–208], thus pleading against the synaptic re-modelling during sleep. However, it has been described that a fraction of genes potentially important for synaptic plasticity are up-regulated by sleep and, thus, under certain conditions, sleep can promote the expression of these genes [209]. For instance, warm ambient temperatures, known to increase sleep in the rodent, also increased expression of the plasticity-related gene MMP-9 in hippocampus and cerebral cortex [210]. In a more recent study, using high-density micro-arrays, Cirelli et al. [211] have described a number of ~100 genes whose brain-specific expression increases during sleep and a similar number of genes whose expression increases during wakefulness. These genes belong to different functional categories suggesting that sleep and wakefulness may promote different cellular processes. Some of the wakefulness-related transcripts might help the brain to face high energy needs and would be involved in high synaptic excitatory neurotransmission, high transcriptional activity and synaptic potentiation in the acquisition of new information. On the other hand, there was a sleep-related increase of transcripts involved in membrane trafficking, synthesis/maintenance at different levels and myelin formation: exocytosis and neurotransmitter release, synaptic vesicle recycling, cycling between trans-Golgi network and plasma membrane, myelin structural proteins, cholesterol synthesis and transport, etc. The association between sleep and membrane trafficking, cholesterol and protein synthesis might not be incidental [211]. Indeed, recent evidence shows that glia-derived cholesterol may be the limiting factor for synapse formation and maintenance [212]. In this study, there was also a sleep-related increase in mRNA levels of calmodulin-dependent protein kinase IV that has been specifically involved in synaptic depression and in the consolidation of long-term memory [213,214]. These findings, therefore, suggest an association between sleep and different aspects of neural plasticity [211].

Interestingly, an experience-dependent expression of a gene during post-exposure sleep has been described [215]. The expression of *zif-268*, an immediate-early gene involved in neuronal plasticity, was shown up-regulated during post-exposure REM

sleep in the cortex and hippocampus of rats exposed to an enriched environment for 3 hours, whereas non-exposed rats showed a generalized decrease in zif-268 expression during non-REM sleep and REM sleep as compared to wakefulness. In a complementary study, Ribeiro et al. [216] also found a REM sleep-dependent activation of zif-268 following hippocampal LTP in previous waking. In this latter study, the expression of zif-268 in several extra-hippocampal areas also suggests a progressive activation of this gene along the hippocampo-neocortical network during REM sleep. One should remember that these results only provide evidence compatible with experience-dependent gene transcriptions and that one still does not know which cascade of cellular events they trigger and whether these processes would induce a subsequent behavioural modulation [65].

Protein synthesis during sleep. In addition to modifying gene transcription, sleep may also promote the translation of these genes into their active proteins. Studies in rodents and monkeys showed positive correlations between levels of cerebral protein synthesis and duration of non-REM sleep [217,218]. Cirelli et al. [211] found that sleep was associated with increased transcript levels of key components of the translational machinery, supporting the involvement of sleep in protein synthesis. The inactivity of the central noradrenergic (NA) system during sleep has been proposed to play a permissive role to enhance brain protein synthesis, because NA depletion increased the expression of the sleep-related gene encoding the translation elongation factor 2 [219]. Several data indicate that some proteins synthesized during sleep could influence synaptic plasticity. For example, both neurogranin and dendrin are believed to modulate synaptic plasticity [220–222] and are reduced in the cerebral cortex following 24 hours of sleep deprivation. However, a sleep-dependent increase in their synthesis has not been demonstrated conclusively. Sleep loss also affects concentrations of the neurotrophins NGF and BDNF. Notably, they seem to be affected by REM sleep, since 6 hours of REM sleep deprivation reduced BDNF in the cerebellum and brainstem and NGF in the hippocampus [223]. Once again, a direct sleep-dependent increase in the synthesis of these neurotrophins during sleep has not been reported. On the behavioural level, it's interesting to notice that an earlier study had reported a learning impairment in rats when anisomycin, a protein synthesis inhibitor, was administered during certain periods of REM sleep [185].

Conclusion

Sleep appears to play an important role in brain maturation during early development as well as in learning and memory processes throughout lifespan. Thus, while a huge number of studies support the relationship between sleep and brain plasticity at the different levels of description, several issues remain to be elucidated. First, as memory systems are complex, numerous and most probably depend on heterogeneous mechanisms, sleep may influence these systems differentially, as notably shown by behavioural and brain imaging data described in previous sections [100,104,110,174,173]. This also implies that not all memories would need sleep to consolidate. The characterization of task-dependent and regionally specific brain activities during post-training sleep should, thus, be continued, in order to assess whether and in which cases sleep is an absolute requirement or simply favours brain plasticity, in other words in which conditions sleep plays an executive or a permissive role for brain plasticity and memory consolidation. Secondly, it is critical to continue and refine the characterization of the precise contribution of each sleep stage in the processing of memory traces in the adult brain as well as in brain maturation in infants, although several models are proposed [41,78,81]. And, thirdly, it will also be necessary to re-evaluate the data that do not support the role of sleep in brain plasticity or memory processes, such as the supposed absence of deleterious effects on memory reported with the use of anti-depressant drugs, which importantly decrease the amount of REM sleep [61]. In this example, a careful neuropsychological study comparing patients under anti-depressant medications to matched controls has yet to be done.

A better characterization of sleep and its role during brain development or memory processes is critical for an optimal understanding and management, in paediatric and adult medicine communities, of the sleep disorders, which constitute a serious public health concern. Indeed, there is increasing evidence that sleep problems negatively impact cognitive, behavioural and emotional functioning in children [224]. These problems are often underestimated because young children do not manifest obvious symptoms of sleep loss such as yawning or excessive daytime sleepiness commonly observed in adolescents and adults, but rather show less specific symptoms like irritability, hyperactivity, short attention span and low tolerance for frustration [225]. There are some clinical and anecdotal evidence supporting an association between sleep loss and resultant cognitive and behavioural changes. For example, in children with severe learning disabilities, those with sleep problems show significantly more

types of challenging behaviour particularly of a higher severity than those without sleep problems [226], resulting in management difficulties for carers. A link seems to exist between typical symptoms of inadequate sleep in children and symptoms characteristic of attention deficit/hyperactivity disorder (ADHD) [225]; moreover children diagnosed with ADHD have been shown to have a higher incidence of sleep problems and shorter average sleep duration than children without ADHD [227]. These sleep problems and their related cognitive and behavioural effects in children also have potentially fatal outcomes [224]. Indeed, injury-related morbidity and mortality represents one of the most important health problems for children [228]. Sleep loss has been implicated in childhood injuries such as falls, pedestrian accidents and cycling accidents. For example, a study reported that children who experience sleep loss were more likely to have accidents in the subsequent 24 hours than children who get adequate sleep and the more hours children are continuously awake, the higher the risk for injury [229]. Despite the serious consequences of sleep disorders, there are very few experimental data concerning the effects of sleep loss on daytime functioning in children and their long-term cognitive, behavioural and physiological consequences in the adulthood. Yet, it has been shown that emotional and behavioural problems in children often significantly improve when underlying sleep problems are identified and rectified resulting in the attainment of adequate amounts of sleep [225]. Thus, defining the short-term and long-term neurophysiological and cognitive-behavioural consequences of sleep troubles in early life will help the diagnosis, treatment and prevention of sleep-related pathological conditions in paediatrics, but will also provide a set of data to integrate with the current knowledge about sleep, memory and plasticity. Conversely, it is obvious that the understanding of the complex relationship between sleep, development, learning, memory and brain plasticity is a pre-requisite for understanding the various effects of altered sleep in children.

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