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SOME CONSIDERATIONS ON SLEEP AND NEURAL PLASTICITY

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INTRODUCTION

In Kleitman's encyclopedic treatise on "Sleep and Wakefulness" (54), the subject of sleep and memory is referred to only twice, and just in passing. In the 1939 edition, based on the seminal study by Jenkins and Dallenbach (41), Kleitman mentioned that "going to bed immediately after memorizing suitable material favors retention, as against remaining awake for some time thereafter". In the subsequent edition of the treatise (1963), based on an intervening study by Simon and Emmons (96), Kleitman went on to notice that learning during sleep was "impractical and probably impossible". That Kleitman had just these two terse statements to offer is striking in view of the large number of hypotheses suggesting that a key function of sleep, especially of the stage of sleep he and Aserinski had discovered, is in the service of memory or synaptic plasticity. On the other hand, Kleitman's demurring may have been wise. Almost fifty years later, despite the flourishing of all kinds of ingenious hypotheses, there is still great uncertainty about the relationship between sleep, memory, and synaptic plasticity.

In approaching this complex subject, it may be useful to make a few basic distinctions. On the psychological side, one should distinguish at least between memory acquisition, consolidation, and maintenance, and consider how sleep may contribute in turn to each of these phases. It is also important to distinguish between declarative and non declarative memories and their relationships to different stages of sleep. On the neural side, plasticity is a broad term that could refer to any sort of short-lasting or long-lasting neural change in response to development or experience, from the transient strengthening of existing synapses to the permanent formation of new ones. Despite a burgeoning literature on all aspects of neural plasticity, the field is far from a definitive synthesis of the molecular mechanisms of memory acquisition, consolidation, and maintenance. Nevertheless, it may already be worth asking whether different molecular and cellular aspects of neural plasticity may be associated preferentially with different behavioral states. Finally, it is important to keep in mind the question of whether sleep offers specific advantages, at the system or at the cellular level, over quiet wakefulness. Only where such advantages can be identified can one assume that the reduced responsiveness to the environment that sleep enforces may be worth its price.

ACQUISITION

The ability to acquire long-term memories is a natural accompaniment of conscious waking. The question of whether learning is possible during sleep has been the subject of controversy for many decades. However, well-controlled studies have failed to demonstrate the transfer of any learning for declarative material from sleep to consecutive waking (88). When Simon and Emmons (96) ensured, by employing careful EEG monitoring, that verbal material was presented exclusively when subjects were asleep, they found essentially no recollection of the material the next day. Other studies demonstrated that recall became possible only if subjects had been awakened and kept awake for some time (from 25 sec to 5 min, 55, 82). A more recent study convincingly demonstrated reduced memory encoding in sleep with respect to waking (116). This study also provided evidence that short periods of sleep may interfere with the transfer of information from short- into long-term memory. Conversely, there are several indications that an increase in arousal level may facilitate the acquisition of information (14). For example, after a weak electrical stimulation of the reticular formation immediately following a learning trial, there is an enhancement of performance when memory is tested one day later (24). Taken together, these and other studies indicate that acquisition of declarative memories in a retrievable form only occurs during wakefulness, and is prevented by sleep. On the other hand, it may be possible to acquire some forms of nondeclarative memory during sleep. For example, subjects could learn a simple conditioned response (a K complex was produced by a conditioned tone after association with an unconditioned electric shock) and the learned response could be transferred to waking (6).

The difficulty of acquiring information about the environment during sleep can be ascribed in part to the curtailment of sensory inputs enforced by eye-lid closure and various behavioral means. Moreover, central neural responses to stimuli of fixed amplitude are diminished in sleep with respect to waking, and the sleeper becomes partly disconnected from sensory inputs. For example, visual neurons in the lateral geniculate nucleus, which faithfully transmit stimuli to the cortex during waking, stop doing so during slow-wave sleep (SWS), and their burst-pause pattern of firing considerably dampens responses to stimuli (62, 72). Evoked potential studies also indicate a reduction in the transmission of sensory information during SWS. During REM sleep, on the other hand, the amplitude of the primary evoked cortical responses is generally similar to waking, while the late potentials are abolished, as if the ongoing activity that generates cognition during dreaming prevented the early thalamocortical activation from being incorporated into the intrinsic cognitive world (63).

A degree of sensory disconnection, however, is only part of the reason why the acquisition of new information is impaired during sleep. For example, somnambulists can carry out complex motor tasks and respond to sensory input during deep sleep, but they do not recall their actions or responses once they awaken (40). Another factor is suggested by studies testing the induction of long-term potentiation

(LTP) by electrical stimulation during different behavioral states. In particular, LTP obtained by high frequency stimulation of the perforant pathway, the main source of cortical input to the hippocampal formation, is dramatically suppressed during SWS as compared to waking (60). In another study, high-frequency stimulus trains reliably elicited LTP during a still but alert waking state, while during SWS the same stimulation often produced no lasting change or LTD (10). These various effects may depend on when the trains are given in relation to the high-amplitude sharp waves that characterize the hippocampal EEG during SWS and that are due to synchronous bursts of pyramidal and granule cell populations (12, 13). During REM sleep, on the other hand, a high-frequency stimulus train was capable of eliciting LTP in 70-95% of cases (10; see also 37, 64). The low probability of LTP induction during SWS compared to waking and REM sleep may result from an active process because normal LTP can be elicited in deeply anesthetized animals (114). It may also be due to the reduced release of acetylcholine (47), as well as to the absence of the theta rhythm. Pulses applied at theta rhythm periodicity induce LTP in the dentate gyrus and CA1 field, especially if applied at the peak of the theta rhythm (77). Moreover, the elimination of theta rhythm in the rat, via lesioning or the infusion of cholinergic antagonists into the medial septum, produces retrograde or anterograde spatial memory deficits, respectively (see 115).

1. *Molecular mechanisms.*

The literature on the cellular and molecular bases of neural plasticity is extraordinarily vast and complicated (1, 26). A distinction is generally made between short-term changes, not requiring gene transcription and protein synthesis, and long-term changes that require transcription and translation and involve the cell nucleus. At activated synapses, local processes such as protein phosphorylation can lead to temporary changes in synaptic efficacy in a way that is independent of gene transcription. For example, local protein phosphorylation may be responsible for increased transmitter release as well as for increased postsynaptic responses (99). Local changes such as phosphorylation of receptors and kinases may also act as "synaptic tags" and label those synapses that are candidates for long-term changes (28). Although the nature of these synaptic tags is not well understood, they could serve as a recruitment factor for constituents sent from the cell body that lead to a remodeling of those synapses. If the synaptic activation is repeated, or is particularly strong, or is associated with various reinforcing factors, short-term synaptic changes are transformed into long-term changes that can persist for considerable periods of time. Such long-term plasticity, which is often associated with structural changes in neural circuits, requires the activation of gene expression in the nucleus as well as protein synthesis (102).

Although many aspects of the nuclear involvement remain mysterious, there is by now substantial evidence for the key role played by the activation of certain transcription factors, especially P-CREB. In species as different as *Aplysia, Drosophila*, mice, and rats, P-CREB and the activation of CREB-dependent transcrip-

tion play a crucial role in long-term activity-dependent mechanisms of synaptic plasticity and in particular in the formation of different forms of long-term memory in both hippocampus and cerebral cortex (reviewed in 95). The phosphorylation of CREB is often associated with the induction of several immediate early genes (IEGs), including *Fos, NGFI-A, ARC, and BDNF* within tens of minutes to a few hours. The induction of these genes is followed by the synthesis of various proteins that are needed for the acquisition of long-term memories, presumably through the structural remodeling of synaptic circuits. With a few exceptions, it is still largely unknown which proteins are required and when, although it appears that different "waves" of protein synthesis may be responsible for different aspects of memory acquisition and consolidation.

It is obviously important to know whether the expression of some of these molecular correlates of memory acquisition changes depending upon behavioral state. For example, it has been suggested that sleep, notably SWS, may be a time during which, possibly through a massive influx of calcium during burst firing, the expression of "plasticity-related genes" may be preferentially induced (13). However, studies examining the phosphorylation of P-CREB in the brain have found just the opposite: Levels of P-CREB in most cortical areas are much higher in animals sacrificed after periods of waking than in animals that had been asleep (21). Levels of protein serine and threonine phosphorylation in general are also higher in waking than in sleep (18). Moreover, several IEGs that have been variously associated with the acquisition of plastic changes, such as c-Fos, NGFI-A, BDNF, and ARC, are all expressed at higher levels during waking than during sleep (21). This has been demonstrated in several species and after variable periods of sleep and waking (20). However, it is not yet clear how the pattern of induction of IEGs varies depending on the learning paradigm, for example whether it differs for declarative vs nondeclarative material (76).

Just as LTP is more readily induced during REM sleep than during SWS, it is possible that REM sleep may be more conducive than SWS to the induction of some of these molecular markers. REM episodes of normal duration are not associated with the induction of c-fos (20). However, if the duration of REM episodes in the rat is considerably increased, either by neurochemical manipulations or by long-term sleep deprivation, Fos expression is induced in certain limbic areas (20). On the other hand, most other cortical areas remain devoid of Fos staining despite the EEG activation. It is possible that the dramatic rebound of REM sleep following long-term deprivation, which is associated with intense theta activity, a high proportion of phasic events, and presumably with the abundant release of acetylcholine, may be sufficient to trigger gene expression in these regions.

2. *The role of neuromodulatory systems.*

What is responsible for the fact that the phosphorylation of CREB and the induction of IEGs occurs during waking but not, or to a much lesser degree, during sleep? Recent evidence indicates that an important factor is the activity of the

noradrenergic system, which is high in waking and low in sleep (4). Awake rats in which the locus coeruleus had been lesioned unilaterally showed levels of CREB phosphorylation and IEG expression on the side of the lesion that were indistinguishable from those observed in sleeping animals, while on the intact side the levels were typical of waking (17). By contrast, lesions of the serotoninergic system have no effects on the expression of these genes nor on the phosphorylation of CREB (107a). Thus, it appears that the release of specific neuromodulators during waking may facilitate, if not enable, the chain of events that leads to long-term neural plasticity. It should be kept in mind, of course, that if synaptic activation is strong enough (tetanic stimulation, injection of kainate etc), the enabling action of these neuromodulators may not be necessary. Under more physiological conditions, however, these systems may play a key role. For example, some experiments indicate that subthreshold tetanic stimulation can induce LTP only if it is associated with subthreshold application of the muscarinic agonist carbachol (5). It is also interesting to note that some of these neuromodulators may play a key role in neural plasticity during development (e.g. 32, 50, 105) as well as in invertebrates (e.g. 8, 34, 68).

In summary, at least with respect to the acquisition of long-term memories, the behavioral and electrophysiological evidence goes hand in hand with the recent molecular findings. Altogether, the conclusion is that the acquisition of information is severely dampened if not blocked during sleep, certainly during SWS and possibly also during REM sleep. In particular, it appears that the reduced activity of certain neuromodulatory systems is an important determinant of whether neural activity is accompanied or not by the acquisition of lasting new memories through the phospshorylation of CREB and the activation of gene transcription and translation. From an evolutionary point of view, such an arrangement seems amply justified. If the nervous system needs to acquire information about its environment in order to adapt itself to it, it makes sense that such acquisition be confined to periods of waking and should not occur during sleep, when neural activity is at least partly disconnected from the external world.

CONSOLIDATION

Consolidation refers to the idea that memories do not form instantaneously at the time of learning, but instead develop gradually after initial learning. It was understood early on that long-term memories were not stored in an immutable form, but would be susceptible to changes due to various events, such as learning of new material (interference), association with old material (integration), rehearsal, and forgetting (73). The neurobiological concept of consolidation is largely based on studies of amnestic agents, such as inhibitors of protein synthesis, electroconvulsive shock, head trauma, and medial temporal lobe lesions. These agents affect recently acquired memories more than old memories. Retrograde amnesia pro-

duced by such agents extends back typically by a few hours. In some cases – especially temporal lobe lesions in humans – retrograde amnesia can be of much longer duration. On the other hand, certain drugs, stimulation of the reticular formation, and hormones are capable of enhancing retention compared to control conditions. Consolidation is thus considered to be the process by which memories become progressively resistant to amnestic factors (100).

The idea that sleep may help in memory consolidation is an old one. The study by Jenkins and Dallenbach (41) mentioned by Kleitman provided early evidence that learning sessions followed by sleep would improve memory retention. Specifically, two subjects learned nonsense syllables and were examined for retention 1, 2, 4, and 8 hours later. It was observed that, if the time between learning and testing was spent asleep rather than awake, the retention of the learned material was much better. Animal studies soon indicated that REM sleep deprivation could interfere with the conversion of an acquired response into a long-term memory as well as with the consolidation of the latter (see, for example, 27). More recent studies in humans have confirmed an effect of sleep on memory (7, 39) and added an important distinction by associating different kinds of sleep to different kinds of memories (80). Declarative memories are conscious memories for facts, ideas, and events, i.e. for information that can be brought to conscious recollection as a verbal proposition or as a visual image. Nondeclarative memories are unconscious and are expressed as a change in behavior, rather than as a conscious recollection. They include motor and perceptual skills, habits, various forms of conditioning, and simple nonassociative memories such as habituation and sensitization. Plihal and Born (80) showed that sleep during the early part of the night, which is rich in SWS, favors retention of declarative memories, such as memory for paired associates. Conversely, sleep during the late part of the night, which is rich in REM sleep, favors the retention of non-declarative skills, such as mirror-tracing.

Other studies have confirmed that REM deprivation selectively impairs the retention of non-declarative, procedural tasks, such as the tower of Hanoi (98). In addition, REM deprivation interferes with perceptual learning, another form of non-declarative memory, in that subjects deprived of REM sleep, but not of SWS, did not show the expected overnight improvement (49). It should be noted, however, that in this last paradigm the improvement attributed to REM sleep could be brought about by waking as well (48, 49). Moreover, according to another study, the overnight improvement was proportional to the amount of SWS during the first quarter of the night as well as to amount of REM in the last quarter, suggesting a combined role for both kinds of sleep (103, 104). Finally, another recent study indicates that the REM sleep-rich late sleep may also be important for the consolidation of declarative memories having an emotional content (112).

The notion that sleep may favor memory consolidation is supported also by the substantial animal literature indicating that periods of intense learning, especially of complex tasks, or exposure to enriched environments, are followed by an increase in the number or duration of REM periods (97, 98; cf. 31 for evidence of an increase in SWS; in humans, however, the evidence for an increase in REM

sleep is much less clear, cf. 38; see also 111). The increase is observed in various learning paradigms and animal species, occurs typically a few hours after the training session, and lasts for a relatively short time window. This increase has been interpreted as indicating a homeostatic need for REM after periods of learning, especially in view of the fact that sleep deprivation during this time window appears to interfere with memory consolidation (97). Moreover, stimulation of the mesencephalic reticular formation enhances performance if administered during the window of REM sleep increase (36).

1. *Cellular mechanisms of early consolidation.*

The line between the acquisition and the early consolidation of long-term synaptic changes is inevitably fuzzy and difficult to draw. Nevertheless, it appears that the phosphorylation of CREB and the induction of the first wave of IEGs / transcription factors can be considered as the earliest markers or tags indicating which neurons will be involved in the structural changes that accompany long-term synaptic plasticity. What are the subsequent molecular changes? At present, it is thought that the next step corresponds to the synthesis of the proteins encoded by the genes that are activated by the phosphorylation of CREB, although the identity of such proteins is largely unknown (102). Indeed, it is well known that, when given one to two hours after training, inhibitors of protein synthesis severely disrupt long-term memory (100). Thus, the making of new proteins can be considered to mark the very first consolidation of long-term memories. On the other hand, waves of gene transcription and protein synthesis go on for several hours after acquisition, and interfering specifically with some of these proteins can disrupt consolidation for a corresponding period of time (e.g. 3, 87). The way in which these new proteins are needed is not yet well understood, although it is likely that they are used to build new synapses or to strengthen existing ones.

In which way could sleep promote memory consolidation? The initial phase lasting 1-2 hours that surrounds memory acquisition and is susceptible to disruption by nonspecific inhibitors of protein synthesis would not seem to have any obvious relationship to sleep. On the other hand, the synthesis of proteins that are important for synaptic plasticity goes on for many more hours, in which case sleep may play a role. Although protein synthesis requires just a few minutes (see e.g. 2) and is energetically undemanding (86), some evidence suggests that it may occur at higher rates during SWS than during waking or REM sleep. In a study using L-[1-14C]leucine autoradiography in the rat, it was shown that rate of cerebral protein synthesis was positively correlated with the occurrence of SWS (83). In monkeys, leucine incorporation was positively correlated with percent time in deep sleep in essentially all brain regions (75). Older studies had also indicated an increase in protein synthesis during the inactivity period, but had generally not controlled for circadian effects (see e.g. 22, 84). Indeed, the relative weight of circadian time and behavioral state with respect to protein synthesis remains to be determined. In addition, it will be important to know whether local protein synthesis in the dendrites (53, 107) may be favored during periods of sleep.

Equally important, it will be essential to determine whether sleep is associated with the increased synthesis of specific proteins. In the meantime, it can be pointed out that prolonged sleep deprivation leads to an increase in the level of BiP (92), a chaperone molecule that is often induced when protein synthesis is impaired (59).

Other mechanisms, in addition to protein synthesis, may be important for the early consolidation of synaptic changes. One such mechanism is the insertion and clustering of new receptor subunits at potentiated synaptic sites. For example, silent synapses only have NMDA receptors, and are detected electrophysiologically only when the Mg^{++} block on NMDA receptors is removed by depolarization. After potentiation, AMPA receptor subunits are inserted and clustered at the synaptic site, and the synapse becomes functional even in the absence of postsynaptic depolarization. Consolidation may also depend on the fact that the "tagging" of certain synapses may occur at one time and be followed, if the same or other synapses on the same neuron are activated again within a few hours, by persistent facilitation accompanied by local protein synthesis and growth (15).

Some aspects of the delivery of newly synthesized proteins to the appropriate synapses, or of dendritic protein synthesis, or of receptor insertion, internalization, or clustering may well be activity-dependent. For example, in CA1 slices, NMDA-mediated postsynaptic activation is necessary to obtain the clustering of AMPA receptors (93). In hippocampal cultures, NMDA blockade decreased the number of AMPA clusters (61). It may be, therefore, that synapses tagged during waking may only become consolidated if there is sufficient reactivation that warrants the formation of a stable cluster of AMPA receptors. At this stage, however, these cellular processes are only beginning to be understood, and their study in different behavioral states will almost certainly warrant the use of *in vitro* models of sleep and waking.

2. *Selective reactivation of neural circuits and late consolidation.*

The early phase of memory consolidation occurs within a few hours of acquisition and is dependent on a complex series of cellular and molecular changes involving gene transcription and protein synthesis. However, memory consolidation does not stop after a few hours. It is well known that an effective way to consolidate learned material is repetition: practice makes perfect. Moreover, memories are susceptible to disruption for a considerable time by amnestic agents such as seizures, head trauma, or brain lesions.

A substantial literature points to a key role for the medial temporal lobe (MTL) in the ongoing consolidation of declarative memories. Such lesions often produce both anterograde amnesia and retrograde amnesia for periods that can extend to days, weeks, and even months. In addition to being conscious, declarative memories for facts and events have several unique characteristics that distinguish them from simpler, nondeclarative memories. They are often represented by arbitrary associations, which may involve groups of neurons that are not directly or strongly linked, and they are extremely flexible. Accordingly, their neural substrate appears to involve highly distributed regions of neocortex, as opposed to local circuits

only. They can be acquired rapidly, over a single trial, or gradually, as when learning a long list of items or when a rat learns a spatial location. Studies of neural networks and associative memories suggest that the cortical connectivity underlying declarative memories should be modified in a slow and interleaved fashion, thus favoring the integration of new memories with old ones without causing catastrophic interference (69). Finally, their consolidation is dependent on the integrity of MTL structures.

Although the precise mechanisms through which the MTL is involved in declarative memory are still unclear, a plausible model is the following (74, 101, 102, 106). Thanks to the highly convergent connectivity from associative multimodal areas, the medial temporal lobe and the hippocampus in particular are ideally suited to form a highly condensed "sketch" of which cortical neuronal groups were activated by a specific fact or event. The induction of synaptic changes in the hippocampus and other MTL structures may serve to temporarily store high-order categories (33) and to rapidly index each particular combination of activated cortical sites. On the other hand, thanks to the backconnections from the hippocampus to the entorhinal cortex and from there through the hierarchy of neocortical areas, MTL structures seem also capable of reactivating the corresponding groups of cortical neurons (12). Thus, "play back" of the appropriate spatiotemporal activity patterns in MTL structures would reactivate the corresponding distributed neocortical circuits, which would be slowly consolidated and integrated with other memories. Over time, such memories would be transferred from the MTL to neocortical sites and would become resistant to MTL lesions, while the MTL would be available for indexing and temporarily storing new declarative memories. Indeed, experiments by Miyashita (71) have given direct proof that the coactivation of neurons in the anterior inferior temporal (IT) cortex which encode paired associates requires the integrity of the MTL and of its backprojections to IT. This model is also consistent with the finding that, while LTP in the hippocampus is typically induced by brief massed stimulation, in rat association neocortex LTP is best produced by spaced stimulation repeated over several days (108).

3. *Sleep and selective reactivation.*

Based on these premises, it would seem that an ideal time for the selective reactivation or "play back" of MTL activity pattern to the cortex may be when an animal is asleep (11, 12, 13, 67, 70). To begin with, sleep would prevent interference, in the sense that important recent declarative memories could be consolidated without being overwritten by the unavoidable running commentary provided by conscious experience. This hypothesis would predict, for example, that anesthesia should provide benefits very similar to those of sleep for the consolidation of declarative memories. It would also predict that sleep deprivation should be more harmful when subsequent waking experiences are more likely to interfere than when they do not. Second, sleep would allow for the repeated reactivation in an off-line mode of the neural circuits originally activated during the memorable experience. This would eliminate the need to re-experience the situation or to

behave accordingly, which may be maladaptive. Undoubtedly, repetition helps memory consolidation, and sleep would permit systematic repetition at a low price. Third, sleep would offer the opportunity to reactivate the relevant neural circuits in a spaced and interleaved fashion. As mentioned above, this would favor the integration of new with old memories and would avoid catastrophic interference. Fourth, the high-frequency, coherent bursts that characterize sleep may be particularly important at both the system and cellular level. Depending on how many neurons are recruited through burst firing and end up firing together, the network of associations may enlarge. Finally, it is conceivable that synchronous bursts may provide ideal conditions for triggering mechanisms of synaptic consolidation.

Unfortunately, at the cellular level, there is presently no indication about what molecular mechanisms of consolidation would be favored or enabled by sleep. The simple idea that "reactivation" may trigger the same chain of molecular events that is triggered during the original activation in waking, such as the phosphorylation of CREB and the induction of IEGs (13), does not seem consistent with the available data, which indicate a dramatic reduction of the levels of these markers during sleep (19-21). Other molecular mechanisms, such as those mentioned with respect to early consolidation – dendritic protein synthesis, receptor insertion and clustering, synaptic capture – may well occur preferentially during SWS, although at present no direct evidence is available. Recent data indicate that the activation of NMDA receptors, which contributes to many forms of LTP, may not be necessary for memory consolidation during sleep, since their blocking by ketamine has no adverse effects. On the other hand, it appears that the facilitating effect of early sleep on the consolidation of declarative memories is abolished if blood cortisol concentrations are raised to levels similar to those observed during waking (9).

At the electrophysiological level, by contrast, some recent data are at least consistent with a possible role for SWS in the consolidation of spatial memories. Using single unit recordings, Pavlides and Winson (78) showed that hippocampal cells whose activity had increased during waking also increased their activity during a subsequent episode of SWS. Wilson and McNaughton (113) monitored the activity of 50 to 100 single cells in area CA1 of the hippocampus during spatial behavioral tasks and in SWS before and after these behaviors. Cells with overlapping place fields that were coactive during the spatial task showed a significant tendency to fire together during the subsequent SWS, compared to the preceding SWS episodes. The effect declined gradually during each post-behavior SWS episode. Cells that were inactive during behavior, or that were active but had non-overlapping spatial firing, did not show this increase. Correlations between coactive cells were significantly larger during ripples (synchronized bursts associated with EEG sharp-wave activity) as compared to the intervals between them. It is important to note, however, that these electrophysiological data do not constitute direct evidence for a role of sleep in memory consolidation. If certain circuits have been strengthened as a result of waking activities, it would be expected that spontaneous neural activity during sleep should bear a trace of such strengthening. Whether sleep-related activity leads to consolidation is a com-

pletely independent question. Indeed, population activity in the hippocampus during the sharp waves and ripples observed in quiet waking after training exhibits the same "trace reactivation" as observed in subsequent sleep (58).

Experiments by Chrobak and Buszaki (16) add an important twist to the story. Activity in the superficial layers of entorhinal cortex, which project to the hippocampus (via the dentate gyrus, CA3 and CA1 circuit), is well correlated with the theta waves observed in the hippocampus during active waking or REM sleep. On the other hand, activity in the deep layers of entorhinal cortex, which receive projections from the hippocampus, is well correlated with the sharp waves observed in the hippocampus during active SWS and during immobility. This has led to the suggestion that the flow of neural activity would be towards the hippocampus during active waking, when declarative memories are being laid down temporarily. During periods of inactivity, or even better during periods of SWS, in which there is no competition from environmental stimuli, the flow of neural activity would be inverted, and the bursts of hippocampal firing during the sharp waves (ripples) would bombard the cortex and favor the formation of associative links between distant areas. Over time, this bombardment would lead to the consolidation of declarative memories within the cortex in relative independence from the hippocampus (12, 13; see also 94). There is some evidence that changes in the balance of neuromodulators, especially ACh, could also favor the reversal of information flow between active periods (high ACh, encoding) and inactive periods (low ACh, recall; 35). Nevertheless, further tests are obviously needed to corroborate the hypothesis of a reversal of the flow of neural activity to and from the hippocampus between waking and sleep. Moreover, direct tests of the role of NREM sleep in the long-term consolidation of declarative memories are still missing in animal models (cf. however 31). A recent study has elegantly shown that reversibly inactivating the hippocampal formation for over a week interferes with the consolidation of spatial memories in the rat (85). A crucial experiment would be to reproduce the same consolidation deficit though a similar period of sleep deprivation.

Finally, it remains to be ascertained what role would be fulfilled by REM sleep. Notably, the reactivation of hippocampal firing patterns corresponding to recent experiences has been clearly demonstrated only for SWS and quiet waking. The evidence for REM sleep is still controversial, with negative (58) as well as positive reports (M. Wilson, personal communication), including the suggestion that REM sleep may be associated with a reactivation only after exposure to novel but not familiar environments (81), and some evidence for reactivation during REM sleep has been provided by PET studies in humans (65a). Based on the results of Karni (49) and Born (80, 112) on perceptual learning, skill learning, and emotional memories, one could hypothesize that REM sleep may serve to consolidate non-declarative memories through a reactivation of the appropriate circuits. However, since in this case the underlying neural circuits would be considerably more local, there would be no need for a coordinated reactivation of distributed neuronal groups through the rapid storage of neural "indexes" in MTL structures. Rather,

a generalized reactivation would suffice, perhaps not unlike the one provided by waking. REM sleep seems to be well suited to such a role. Moreover, the phasic phenomena of REM sleep, specifically PGO waves, could provide a sufficient degree of synchronous firing, comparable to the one provided by hippocampal ripples and the associated thalamocortical spindles (94). Consistent with this, the areas where PGO waves are most prominent include brain regions involved in perceptual and motor learning. The prominent theta rhythm observed in many species might also provide such a reactivation, especially in limbic structures, which are selectively activated during REM sleep (20, 65, 90). These anatomical observations would be consistent with the observation that REM sleep is predominant early in ontogeny, at a stage in which nondeclarative memories form the largest part of the memorial repertoire. Finally, the idea that brain activation provided by REM sleep may promote the consolidation of nondeclarative memories is consistent with the results of Roffwarg and colleagues (66, 91), who have provided solid evidence for a role of REM sleep in the activity-dependent development of the visual system.

MAINTENANCE

Through the process of consolidation, recent memories become resistant to amnestic agents of various kinds, and can then persist for very long periods of time, as witnessed by the enormous number of facts or events, and of motor, linguistic, and perceptual skills that we can recall even after a lifetime. This does not mean that consolidated memories are immune to change, as evidenced by elaboration, reconstruction, and some degree of forgetting. However, the mere fact that memories can persist for several years in the absence of obvious rehearsal poses the interesting biological problem of how they can do so in the face of the ongoing molecular turnover and remodeling of the fine connectivity of the nervous system.

It is perhaps not surprising that the recognition that the brain is active during sleep has led to the suggestion that this may constitute an ideal time for keeping consolidated neural circuits in exercise, especially if they do not have a chance to be exercised during waking. For example, Davis (23) suggested that sleep may serve the reinforcement of old memories, by periodically stimulating "decaying memory proteins". Kavanau has argued that the periodic maintenance of circuits not utilized during waking (called "dynamic stabilization") may be a key function of both NREM and REM sleep, with REM sleep specifically subserving the maintenance of motor pathways (51, 52). A related suggestion is that sleep may serve to stimulate synapses that are not used during waking (56, 57). These may or may not correspond to "old memories". Finally, Jouvet suggested that REM sleep may serve to ensure that useful instinctive patterns of behavior remain functional even if they may not be exercised during waking (42-44).

It is evident that a strict test of such ideas in terms of memory performance is difficult. The effects of sleep on memory maintenance would presumably be

incremental, cumulative, and slow to manifest themselves. In a few instances, however, it appears that a sufficiently stringent test has been performed. Long-term REM deprivation is frequently obtained during chronic treatment with MAO inhibitors. However, no study has yet reported a marked impairment of old memories, habits, or innate behaviors. Based on this lack of evidence, Jouvet has concluded that REM sleep may play a role in "psychological individuation" rather than in the programming of innate behaviors (45, 46). Turning to SWS, it would seem that, if the requirement of sleep for memory maintenance were correct, the need for SWS should increase with age, while the opposite is the case. Moreover, one would expect that the suppression of SWS for several months or years should lead to impairment of old memories. However, in cases of fatal familial insomnia, in which there is a selective degeneration of thalamic nuclei with relative sparing of neocortex, autobiographical memory and memory for old facts or events (such as TV serials) appear to be preserved. This is in contrast to the severe alterations in memory encoding and consolidation (29, 30).

It is of course possible that the old memories tested in these neuropsychological investigations may have represented a small subset of particularly stable memories, which did not require sleep, and that the bulk of other memories are subject to deterioration in the absence of sleep. Once again, an important neurophysiological test would be to substitute SWS with isoelectric anesthesia: would old memories run down quickly under these conditions, indicating that some kind of neural activity is needed in order to maintain a functional circuitry? And would it be the case that only memories not rehearsed during waking would be susceptible to decay? Although the hypothesis that sleep may serve to maintain old memories is not supported by much evidence, it is nevertheless worthwhile to consider certain molecular mechanisms that would be consistent with such a role.

Molecular mechanisms of synaptic maintenance: global scaling.

The notion that sleep may serve to globally maintain old, consolidated memories is conceptually very different from the one according to which sleep would serve to selectively consolidate recent memories. According to the latter hypothesis, it is essential that only recently activated circuits be subject to selective consolidation. Memory maintenance should instead affect all synapses rather indiscriminately, or at least the majority of synapses, corresponding to old or already consolidated memories.

This distinction finds an intriguing parallel in some recent studies conducted using cell cultures. These studies indicate that two kinds of plastic changes should be distinguished at the level of the synapse: synapse-specific, experience-dependent changes that selectively affect circuits involved in learning, and homeostatic changes that globally affect all synapses. The first process, which refers to the differential change in the strength or number of certain synapses and is responsible for acquiring new information in the form of specific synaptic patterns (learning), is the one traditionally investigated by studies of synaptic plasticity. The second process refers to the uniform, multiplicative strengthening or weakening of all

synapses onto a given neuron. Evidence for the latter process has been provided largely by studies of Turrigiano and coworkers (89, 109, 110). These studies have demonstrated a new form of synaptic plasticity that increases or decreases the strength of all of a neuron's synaptic inputs as a function of activity by scaling the quantal amplitude of AMPA-mediated synaptic inputs up or down. In particular, chronic blockade of cortical culture activity increased the amplitude of miniature excitatory postsynaptic currents (mEPSCs) without changing their kinetics. These changes were at least partly due to postsynaptic alterations in the response to glutamate, affected each synapse in proportion to its initial strength, and were mediated in part by BDNF. Further studies have shown that changes in sensitivity of voltage-dependent conductances are responsible for homeostatically regulating the intrinsic excitability of neurons to promote stability in firing (25).

If such a process of synaptic scaling or normalization were functional in vivo, it could have several important consequences. In the absence of mechanisms of synaptic normalization, neural networks undergoing plastic changes are liable to runaway synaptic potentiation or depression: synapses that grow stronger (or weaker) because of a positive (negative) correlation between pre- and post-synaptic activity tend to make that correlation even stronger (weaker), with the result that some neurons would be firing in a saturated manner and others would stop firing altogether. The homeostatic regulation of the number and strength of synapses would counteract these pathological changes during development or experience. More to the point, synaptic scaling would be an ideal mechanism to promote synaptic maintenance. If we assume that a certain amount of synaptic turnover and decay is inevitable, then the activity-dependent rescaling of synapses to maintain a constant synaptic input would counteract such decay. Most importantly, it would do so without changing the relative weight of different synapses, with the result of preserving old memories.

Could sleep be instrumental in promoting the global, homeostatically regulated scaling of synaptic inputs? Theoretical considerations suggest that it might. For example, sleep would be an ideal time in which most synaptic circuits in the brain could be exercised in an off-line mode, without interfering with ongoing behavior. Mechanistically, the highly synchronous, burst mode of discharge observed during sleep may offer a good opportunity for intracellular mechanism to sense the total amount of synaptic inputs to each neuron and to trigger the appropriate homeostatic regulations. Moreover, the inactivity during sleep of certain neuromodulatory systems with diffuse projections would ensure that such synchronous activity burst could occur without triggering mechanisms of experience-dependent amplification. Clearly, experiments to assess whether the homeostatic changes described by Turrigiano and colleagues *in vitro* may occur *in vivo*, and whether they may occur specifically or preferentially during sleep, would be as invaluable as they are difficult to perform. For the time being, however, empirical evidence in support of this hypothesis is almost entirely missing. Indeed, the little empirical evidence that is available is negative: BDNF, which plays a critical role in synaptic scaling *in vitro*, is expressed at higher levels in the waking rather than in the sleeping brain (21, 79).

CONCLUSIONS

The last few years have seen the development of new approaches that are beginning to reveal the molecular correlates of sleep and waking. As was emphasized in this article, it is likely that an understanding of such molecular correlates will be essential for unraveling the relationship between sleep, learning, and memory. Molecular studies are already indicating how and why the acquisition of long-term memories is impaired during sleep. It is also becoming possible to pose, in molecular terms, the question of whether sleep may promote the selective consolidation of recently acquired memories, or instead the maintenance of old memories through an ongoing, indiscriminate activation of neural circuits. Other possibilities not discussed here, for example that certain sleep stages may favor the erasure of spurious memories, the "detagging" of synapses tagged during waking, or the formation of new synaptic contacts to refresh the repertoire of circuits available for the selection and acquisition of new memories, could also be investigated at the cellular and molecular level. Finally, another relevant question for future investigation is whether prolonged sleep deprivation may lead to disruption of plasticity because of specific cellular alterations. What appears increasingly necessary is the development of a reduced model of sleep-like vs wake-like neural activity. Such a reduced model, either *in vitro* or in the slice, would permit the direct evaluation of synaptic consolidation and synaptic scaling. It would also permit the visualization of the underlying mechanisms, such as receptor insertion and clustering, under firing regimes corresponding to the different behavioral states.

SUMMARY

A role for sleep in memory processes and neural plasticity has been suggested many times and in many different forms. However, we are far from a consensus on what this role might be and why it would be fulfilled preferentially by sleep. In this review, we distinguish between memory acquisition, consolidation, and maintenance, and we consider how sleep may specifically contribute to each of these phases. We also distinguish between declarative and nondeclarative memories and their relationships to different stages of sleep. Finally, we discuss whether different molecular and cellular aspects of neural plasticity may be associated preferentially with different behavioral states. A consideration of such molecular aspects could lead to more conclusive experiments concerning the relationship between sleep and plasticity.

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