Learning and sleep: the sequential hypothesis

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Summary During the last 30 years, paradoxical sleep (PS) has been generally considered as the only type of sleep involved in memory processing, mainly for the consistent increase of PS episodes in laboratory animals learning a relatively complex task, and for the retention deficits induced by post-training PS deprivation. The vicissitudes of this idea, examined in detail by several laboratories, have been critically presented in a number of review articles. However, according to a more comprehensive unitary proposal (the sequential hypothesis), memory processing during sleep does require the initial participation of slow-wave sleep (SS) in addition to the subsequent involvement of PS. The evidence supporting this hypothesis, largely derived from experiments concerning rats trained for a two-way active avoidance task, is reviewed here in some detail. Recent studies of human sleep are in full agreement with this view. In the rat, the main effect of learning on post-training SS consists in the selective increment in the average duration of SS episodes initiating different types of sleep sequences. Notably, following training for a two-way active avoidance task, the occurrence of this effect in sleep sequences including transition sleep (TS), such as SS→TS→W and SS→TS→PS, appears related to the processing of memories of the novel avoidance response. Conversely, the occurrence of the same effect in sleep sequences lacking TS may reflect the processing of memories of innate responses (escapes and freezings). Memories of innate and novel responses are assumed to engage in a dynamic competitive interaction to attain control of waking behaviour. Interestingly, in baseline sleep, variables of SS→TS→W and SS→TS→PS sequences, such as the average duration of SS, TS, and PS episodes, have proved to be good indices of the capacity to learn, as shown by their strong correlations with the number of avoidances scored by rats the following day. Comparable correlations have not been displayed by variables of baseline SS→W and SS→PS sequences.

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INTRODUCTION

The involvement of sleep in memory processing was suggested almost a century ago [1], but opinions are still not in agreement with regard to the type(s) of sleep involved, and to the operations performed by the sleeping brain [2–14]. This article illustrates the view that sleep is involved in memory processing in its entirety, that is not only during paradoxical sleep (PS), as initially proposed [2–5, 8–14], but also and primarily during slow-wave sleep (SS). Indeed, sleep processing of newly acquired memories is assumed to consist of two main steps initially requiring SS and eventually PS (the sequential hypothesis). This proposal was stated in an early
paper: “... some of the initial steps in information processing occur during SS and are required for the further processing that takes place during PS” [15], and was later expanded and formalized. A two-step memory processing mechanism has also been proposed on a theoretical basis [16].

Our review is largely focused on animal data, but recent human studies supporting the sequential hypothesis [17–20] will be briefly mentioned. In laboratory rodents, most recent progress has been derived from multiple recordings of hippocampal neuronal activity concurrent with electroencephalographic (EEG) waves during waking experiences and the subsequent sleep [21–28].

THE INVOLVEMENT OF PARADOXICAL SLEEP

The involvement of PS in memory processing was proposed 30 years ago on the basis of an increment in the number of post-training PS episodes in learning rats [29, 30]. This proposal has since been investigated in extenso and in depth [3, 5, 13], and critically discussed in several review articles expressing substantial support [3, 5, 8–13] or substantial reservations [2, 4, 14], respectively.

Two main lines of evidence support a PS involvement in memory processing: (i) the reproducible increase in PS time occurring after learning relatively complex tasks [3, 5, 8–13], or after exposure to an enriched environment [reviewed in 11, 13]; and (ii) the retention impairment elicited by post-training PS deprivation (PSD) [3, 5, 8–13]. The latter treatment is generally imposed by confining a rat or a mouse on a small platform surrounded by water (the pool method). While on the platform, the subject may enter SS, but the postural atonia induced by PS makes it fall in the water and awaken. The confounding effects induced by such a stressful condition have been examined and singled out using suitable control groups or platforms of adequate design [5, 8–13].

It is worth noting that even supportive review articles [3, 5, 8–13] acknowledge that learning simple behavioural tasks, such as passive avoidance, does not elicit increments in post-training PS. This apparent discrepancy has been accounted for by considering Seligman’s distinction between readily learned tasks, that rely on the innate configuration of brain circuitry in a given animal species, and complex tasks that are more difficult to learn due to the inadequacies of innate brain circuits [8]. Only the latter tasks are assumed to require increments in post-training PS to attain a suitable modification of brain circuits.

In early reports, PS time was observed to increase in rats trained for a two-way active avoidance task soon after the daily training session preceding a marked improvement in performance [31]. In later sessions, the effect waned and disappeared [3]. A post-training PSD encompassing the period of PS increase (2–3 h) led to a long-term retention impairment suggesting that PS was required for memory consolidation [12, 13]. The latter concept, based on a variety of experiments with amnesic or memory enhancing treatments [32], implies that memory traces remain sensitive to modulating influences for about an hour after the acquisition step while progressively giving way to consolidated, insensitive memories (long-term memory). The process of consolidation requires the activation of the gene expression system [33, 34].

The concept of PS consolidation was retained in later experiments with a variety of training tasks in which PS augmentations were also observed at much later times after training, in so-called intermittent “PS windows” lasting a few hours and present up to days after training. PSD treatments overlapping PS windows induced impairments of retention comparable to immediate post-training PSD, while no deleterious effects were elicited by PSD imposed between PS windows [12, 13]. Clearly, at such late times after acquisition, the concept of consolidation cannot retain its original sense, and should be considered as merely denoting a persisting positive influence of PS on memory retention. Indeed, even if activation of the gene expression system may lead to protracted effects [33, 35], the sensitivity of long-term memories to modulating influences may only encompass relatively brief intervals of time [32]. Hence, to assume that consolidation does occur during late PS windows, or that it may even persist for years in humans [36], memory traces consolidated soon after acquisition should be envisaged to undergo multiple labilization events prior to or at the onset of PS windows. This intriguing thought is supported by the observation that memory traces regain their sensitivity to modulating influences during PS episodes, but not during SS [3]. In this connection, it is of great interest that, in the amygdala, retrieval of consolidated fear memories has recently been shown to elicit the
need for reconsolidation mediated by a new wave of protein synthesis [37].

As the condition of memory labilization may also lead to memory loss, this peculiar feature should be viewed in the more general context of memory turnover, that is of memory clearing in addition to memory formation. Indeed, on the basis of theoretical considerations and analogies between brain operations and computer operations, an active process of memory elimination ("reverse learning") has been proposed as the main role of PS, leading brain circuits to clear their overloaded, non-adaptive traces [2]. To our knowledge, this hypothesis has not yet received experimental support, and in its present formulation is in contrast with extensive evidence favouring a positive role of PS in memory formation and maintenance [3, 5, 8–13] (but, see below the section on the Role of SS and PS in memory processing).

Interestingly, in a training protocol (brightness discrimination in rats), post-training PSD was reported to induce an improvement in performance rather than a retention deficit. This paradoxical effect could not be attributed to contingent factors, but appeared dependent on the bona fide deprivation of PS [38]. Hence, in this training task, PS should be viewed as adversely affecting the retention of adaptive memories, presumably by a mechanism comparable to that of reverse learning [2], albeit concerning adaptive memories rather than non-adaptive memories.

The extreme view that PS is not involved in memory processing [14] is mainly based on the following considerations: (i) in animals, the number of experiments supporting the role of PS in memory processing is approximately the same as the number of experiments with negative results; furthermore, the retention deficits induced by PSD are based on a procedure plagued by confounding effects; in addition, (ii) in humans, pharmacological treatments drastically reducing or fully abolishing PS for long periods of time are not associated with memory impairments. However, it may be pointed out that: (i) negative data are open to many interpretations and cannot be considered of equal weight to positive results; if simple tasks do not elicit increments in post-training PS, more complex tasks do, and this apparent discrepancy has been adequately interpreted [3, 5, 8–13]; in addition, (ii) memory impairments induced by prolonged decrements or complete loss of PS might be expected to occur only if PS is assumed to be the only vigilance state concerned with memory consolidation, thereby excluding any vicarious substitution by waking or by SS in such a role. These conditions may not apply (see the following section).

THE INVOLVEMENT OF SLOW-WAVE SLEEP: THE SEQUENTIAL HYPOTHESIS

At the time the sequential hypothesis was conceived [15] and later formalized [6], PS was the only type of sleep believed to be involved in memory processing [5, 8–11], in agreement with the prevailing implicit view of sleep as a mere aggregate of two different physiological entities, endowed with specific, albeit not mutually shared, roles [6, 15, 39]. On the other hand, the entire phenomenology of sleep, notably its temporal architecture and dependence on waking experience, supported the notion of a strict cyclic interweaving of the two sleep modalities. We were also impressed by a number of additional observations, such as: (i) the concomitant increment in SS and PS in rats exposed to an enriched environment or learning an appetitive task [reviewed in 11, 13]; (ii) the role of SS in the retention deficit induced by PSD [40]; and (iii) the marked increase in brain energy flow selectively occurring during SS in rats exposed to a novel environment but not in habituated rats [41]. These considerations indicated that brain activity during SS was modulated by the previous waking experience (just as accepted for PS), and suggested that memories acquired during waking were processed during sleep in two main sequential steps respectively occurring during SS and PS (the sequential hypothesis) [6, 7, 15].

Initial supporting evidence

The hypothesis was initially examined in rats trained for a two-way active avoidance task in a massed session, by comparing variables of post-training SS with baseline values, as well as with home caged controls. The same variables were also compared between rats attaining the learning criterion during the training session (fast learning or FL rats) and rats failing to learn (non-learning or NL rats). FL rats were shown to retain long-term memory of their behavioural responses. The main change in SS
Table 1  Spearman non-parametric correlation coefficients among number of avoidances scored by FL rats during the training session and variables of SS and PS episodes recorded in each hour of the post-training sleep session

<table>
<thead>
<tr>
<th>Sleep variable</th>
<th>SS→(W) 1 h</th>
<th>SS→(W) 2 h</th>
<th>SS→(W) 3 h</th>
<th>SS→(PS) 1 h</th>
<th>SS→(PS) 2 h</th>
<th>SS→(PS) 3 h</th>
<th>PS 1 h</th>
<th>PS 2 h</th>
<th>PS 3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-0.515</td>
<td>-0.318</td>
<td>-0.555*</td>
<td>0.470</td>
<td>0.402</td>
<td>0.727**</td>
<td>0.546*</td>
<td>0.620*</td>
<td>0.449</td>
</tr>
<tr>
<td>N</td>
<td>-0.661*</td>
<td>-0.322</td>
<td>-0.390</td>
<td>0.383</td>
<td>0.596*</td>
<td>-0.501</td>
<td>0.383</td>
<td>0.596*</td>
<td>-0.501</td>
</tr>
<tr>
<td>D</td>
<td>0.573*</td>
<td>0.351</td>
<td>0.137</td>
<td>0.163</td>
<td>-0.073</td>
<td>0.809**</td>
<td>0.489</td>
<td>0.160</td>
<td>0.591*</td>
</tr>
</tbody>
</table>

SS→(W) and SS→(PS), episodes of slow-wave sleep followed by waking or by paradoxical sleep. A, N, and D, total amount, number, and average duration of the sleep episodes. 1 h, 2 h, and 3 h, first, second and third hour of the post-training sleep session. The above data concerned an experiment performed before the identification of transition sleep; as a result, SS→W and SS→PS sequences were still including the corresponding SS→TS→W and SS→TS→PS sequences. *P<0.05; **P<0.01. Modified from [43].

episodes consisted in their marked lengthening, while their number tended to decrease and their total amount to remain invariant [42, 43]. These modifications concerned the SS episodes of either one of two sleep sequences (SS→W and SS→PS), although their onset times and/or kinetics markedly differed between FL and NL rats with regard to each sequence. Comparable data had previously been observed in NL rats [44, 45]. In brief, the shortening of SS episodes of the SS→PS sequence prevailed in FL rats, while the lengthening of SS episodes of the SS→W sequence prevailed in NL rats. Increments in PS number and total amount were only observed in FL rats. In addition, in FL rats, the number of avoidances was inversely correlated with the total amount and the number of SS episodes of the SS→W sequence, but directly correlated with the total amount and the number of SS and PS episodes of the SS→PS sequence (Table 1). Furthermore, the correlation between avoidances and average duration of SS episodes of the SS→W sequence became significant in the first post-training hour, while the correlations between avoidances and the average duration of SS and PS episodes of the SS→PS sequence became significant only in the third post-training hour (Table 1).

The pattern of post-training SS modifications observed in FL rats could not be attributed to sleep deprivation or stress, as it markedly differed from that of NL rats, experiencing a similar amount of sleep deprivation during training, and an even greater amount of stress due to the higher number of footshocks they received [42]. This conclusion was supported by the results of an experiment with a group of old rats trained for the same active avoidance task, that displayed only freezing responses. Despite their degree of sleep deprivation and stress being comparable or even greater than that of FL rats, variables of post-training SS were not significantly modified [46].

The above data were interpreted to suggest that “adaptive behavioural responses favour the appearance of fewer SS→W episodes of longer duration, and of more numerous SS→PS and PS episodes of longer duration. On the contrary, non-adaptive responses may favour the appearance of more numerous SS→W episodes of somewhat longer duration.” As a result, it was proposed that “non-adaptive memory traces may be destabilized during SS→W episodes, and eventually cleared from the brain. On the other hand, adaptive memory traces may be destabilized during SS→PS episodes, to be stored again in more suitable form (better integrated with pre-existing memory traces) during the ensuing PS episode” [43]. On the whole, the data supported the involvement of SS in memory processing, and suggested that processing operations were not the same in all SS episodes, but differed in some relevant feature according to the type of sleep sequence in which SS episodes were included.

It is of relevance that a significant lengthening of post-training SS episodes was also detected in rats exposed to a 10-min training session for a spatial habituation task that did not involve administration of footshocks, but merely the exploration of a closed, square corridor [47]. The effect only concerned rats retaining long-term memory of their experience, and consisted in an early, short-lasting
Figure 1  Behavioural and sleep data of adult rats exposed to a 10-min non-associative spatial habituation task in a closed, square corridor. Left column, number (N) of corner crossings (CC) and rearings (R) per minute in the initial trial (■) and in the retention trial 3 h later (□). The decrement in number of movements of the latter trial (shaded area) is a measure of long-term habituation. Right column, total amount (A) of SS episodes followed by W or by PS determined the day before the initial trial (open bars) and soon after the initial trial (shaded bars). All data are presented as mean ± SEM. Modified from [47].

but dramatic appearance of rather long SS episodes of the SS→PS sequence, together with a simultaneous less pronounced increment in the total amount of SS episodes of the SS→W sequence (Fig. 1). The data demonstrated that SS was also involved in the processing of memories of a non-associative task inducing a minimal amount of stress, and that its involvement consisted in the lengthening of SS episodes, as had previously been shown for the more complex and stressful two-way active avoidance task.

More recent clarifying data

1. Identification of a group of slow-learning rats

A key observation clarifying the interpretation of post-training sleep data in rats trained for the two-way active avoidance task concerned the identification of a subgroup of NL rats that attained the learning criterion the day after the training session, during a retention test (slow-learning or SL rats) [48]. As a result, the remaining NL rats that failed
to learn even during the retention test, are here identified as persistent non-learning rats or NL* rats. Slow-learning rats were actually displaying a delayed improvement of performance without additional training (reminiscence) [49], comparable to that described in BALB/c [50] and C57BR mice [51]. Reminiscence in mice was known to be associated with an increment in post-training PS, and to be abolished by post-training PSD.

Comparison of post-training sleep between SL and NL* rats demonstrated that only the former group displayed modifications of SS episodes comparable to those previously observed in FL rats, albeit markedly delayed and persisting for a longer time [48]. The main effect consisted again of the shortening of SS episodes of SS→W and SS→PS sequences, but its onset occurred in the third post-training hour, rather than in the first hour, as observed in FL rats. In addition, PS time selectively increased in SL rats, but only in the sixth post-training hour. Hence, the data demonstrated the primary involvement of SS in the reminiscence phenomenon, thereby confirming its basic role in memory processing.

2. Identification of transition sleep
An additional, perhaps more relevant observation emerged from EEG analyses of higher temporal resolution that led to the identification of transition sleep (TS). These brief episodes follow SS epochs and are characterized by the sudden mixing of theta and alpha waves with the previous delta waves. As TS episodes were followed by either W or PS, they identified two additional sleep sequences (SS→TS→W and SS→TS→PS) [52]. As a result, the former SS→W and SS→PS sequences were distinguished into TS-containing sequences and true SS→W and SS→PS sequences.

Variables of these four sleep sequences were initially determined in the baseline sleep of FL, SL, and NL* rats, and were correlated with the number of avoidances scored during the training session scheduled the following day [53]. The higher priority assigned to analyses of baseline sleep had been suggested by previous data indicating that, in baseline sleep, SS episodes of the SS→W sequence were markedly longer in FL rats than in NL rats, and that PS episodes were also longer in FL rats. In addition, the average duration of the SS episodes included in SS→W and SS→PS sequences had been found to correlate with the number of avoidances scored the following day [54]. Remarkably, these results demonstrated that SS variables were good indices of the rat propensity to learn the active avoidance task. However, at the time these data were gathered, SL rats had not yet been separated from NL* rats and were still included in the group of NL rats; in addition, TS episodes had not yet been identified and the former SS→W and SS→PS sequences were still including SS→TS→W and SS→TS→PS sequences, respectively.

Our more refined sleep analyses [53] indicated that, in the baseline session, the total amount of SS episodes of the SS→TS→W and SS→TS→PS sequences, and of TS and PS episodes of the SS→TS→PS sequence was higher in FL rats than in SL and/or NL* rats (Table 2). In addition, the total amount of SS and TS episodes of the SS→TS→W sequence strongly correlated with the number of avoidances scored by rats the following day, notably during the first training period (Table 3). On the other hand, the total amount of SS and PS episodes of the SS→PS sequence tended to prevail in NL rats (Table 2), while the total amount of SS of the SS→W and SS→PS sequences did not correlate with avoidances (Table 3). Interestingly, the average duration of SS episodes of the SS→PS sequence inversely correlated with the avoidances of the first training period (ρ = −0.542, P<0.05).

These data suggested that SS and PS were good indices of the capacity to learn the avoidance task as long as they belonged to TS-containing sequences, while they reflected a tendency to retain innate responses when they were associated with TS-lacking sequences. As a result, the former subtypes of SS and PS episodes were assumed to be involved in the processing of memories of the novel behavioural response (avoidances) while the latter subtypes were assumed to be involved in the processing of memories of innate responses (escapes and freezings).

The latter hypothesis was supported by the results of comparable analyses of post-training sleep [55] indicating that, in FL rats, several variables of the SS→TS→PS sequence, including the total amount of SS, TS, and PS episodes, were markedly higher than in SL rats, and that some of these variables, including the total amount and the average duration of SS and TS episodes, were markedly higher than in baseline sleep. Conversely, the average duration of SS episodes of the SS→W
Table 2  Total amounts of SS, TS, and PS episodes recorded in the baseline session (7 h) of FL, SL, and NL rats

<table>
<thead>
<tr>
<th>Sleep variable</th>
<th>FL</th>
<th>SL</th>
<th>NL</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS→(W)</td>
<td>5047 ± 555</td>
<td>5721 ± 682</td>
<td>5233 ± 685</td>
</tr>
<tr>
<td>SS→(PS)</td>
<td>446 ± 129</td>
<td>456 ± 132</td>
<td>1021 ± 373</td>
</tr>
<tr>
<td>SS→(TS→W)</td>
<td>3281 ± 375</td>
<td>1630 ± 208</td>
<td>1788 ± 455</td>
</tr>
<tr>
<td>(SS)→TS→(W)</td>
<td>872 ± 81</td>
<td>783 ± 198</td>
<td>578 ± 130</td>
</tr>
<tr>
<td>(SS)→TS→(PS)</td>
<td>612 ± 121</td>
<td>370 ± 106</td>
<td>333 ± 45</td>
</tr>
<tr>
<td>(SS)→PS</td>
<td>571 ± 273</td>
<td>751 ± 202</td>
<td>981 ± 153</td>
</tr>
<tr>
<td>(SS→TS)→PS</td>
<td>2667 ± 179</td>
<td>1958 ± 501</td>
<td>1967 ± 224</td>
</tr>
</tbody>
</table>

Results (in seconds) presented as average values ± SEM. FL, SL, and NL, fast learning, slow learning, and non-learning rats. SS, slow wave sleep; TS, transition sleep; PS, paradoxical sleep; W, waking. In parenthesis, sleep episodes preceding or following the sleep component considered. Intergroup comparisons were based on Student’s t-test for unpaired data. *P<0.05; **P<0.01 with regard to the group shown in parenthesis. Modified from [53].

Table 3  Spearman non-parametric correlation coefficients among total amounts of baseline SS, TS, and PS episodes and number of avoidances scored during the training session

<table>
<thead>
<tr>
<th>Sleep variable</th>
<th>Avoidances</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS→(W)</td>
<td>0.216</td>
<td>0.001</td>
<td>−0.079</td>
<td>−0.157</td>
<td>−0.232</td>
</tr>
<tr>
<td>SS→(PS)</td>
<td>−0.118</td>
<td>−0.112</td>
<td>−0.078</td>
<td>−0.713</td>
<td>0.345</td>
</tr>
<tr>
<td>SS→(TS→W)</td>
<td>0.600**</td>
<td>0.740**</td>
<td>0.721**</td>
<td>0.713**</td>
<td>0.345</td>
</tr>
<tr>
<td>SS→(TS→PS)</td>
<td>0.264</td>
<td>0.435</td>
<td>0.382</td>
<td>0.412</td>
<td>0.329</td>
</tr>
<tr>
<td>(SS)→PS</td>
<td>0.480*</td>
<td>0.580**</td>
<td>0.421</td>
<td>0.412</td>
<td>0.329</td>
</tr>
<tr>
<td>(SS→PS)</td>
<td>0.094</td>
<td>0.464</td>
<td>0.316</td>
<td>0.481</td>
<td>0.279</td>
</tr>
<tr>
<td>(SS→PS)</td>
<td>0.028</td>
<td>−0.266</td>
<td>−0.390</td>
<td>−0.481</td>
<td>0.279</td>
</tr>
<tr>
<td>(SS→TS)→PS</td>
<td>0.106</td>
<td>0.404</td>
<td>0.286</td>
<td>0.279</td>
<td></td>
</tr>
</tbody>
</table>

The baseline session started at about 9.00 am and lasted 7 h. The training session was scheduled the following day at the same time of the day, and included four training periods lasting 30 min, separated by rest intervals of the same duration. T1, T2, T3, and T4, first, second, third, and fourth training period. SS, slow wave sleep; TS, transition sleep; PS, paradoxical sleep; W, waking. In parenthesis, sleep episodes preceding or following the sleep component considered. *P<0.05; **P<0.01. Modified from [53].

sequence selectively increased in the post-training sleep of SL and NL rats in comparison with baseline values. Correlative analyses indicated furthermore that the number of avoidances correlated with the duration of SS episodes of the post-training SS→TS→PS sequence and with the duration of TS episodes of the SS→TS→W sequence, but inversely correlated with the number, amount and/or duration of SS episodes of the SS→W and SS→PS sequences.

The above data supported the view that TS-containing sleep sequences were involved in the processing of memories of the novel avoidance response, while TS-lacking sequences were involved in the processing of memories of innate escape and freezing responses. In either case, the initial processing step appeared to require the involvement of sleep sequences lacking PS, such as SS→W and SS→TS→W, respectively, while sleep sequences terminating with PS episodes appeared to reflect the emergence of the second processing step and of its eventual completion. The latter conclusion was borne out by additional post-training data too detailed to be summarized here [55], as well as by two independent sets of results which appear worth mentioning.
3. “On-line” memory processing relies on delta waves

The first set of data concerned the identification of a significant increase in the delta region of the EEG power spectrum that selectively emerged in FL rats (but not in SL or NL rats) during the first training period, that is at the time of their first encounter with the active avoidance task [7]. Interestingly, the delta increase was strictly associated with a dramatic improvement in performance that occurred during the second half of the initial 30-min training period [manuscript in preparation]. Apparently, the attainment of the learning criterion that takes place “on line” only in FL rats requires a selective power increase in the EEG delta waves. The latter effect provided a reasonable explanation for the lack of lengthening of SS episodes of the post-training SS→TS→W sequence expected in FL rats. Indeed, the selective increment in delta output displayed by FL rats during training could be interpreted to subserve the same role of the increment in average duration of the latter subtype of SS episodes.

4. Identification of trains of sleep sequences

The second set of data indicated that, in rats, sleep sequences are not randomly distributed in baseline sleep [56] or in post-training sleep [manuscript in preparation], but are actually structured in long non-random clusters of variable composition (trains). Trains containing the SS→TS→W sequence in addition to the other three sleep sequences, prevailed in the baseline sleep of FL rats, and variables of their sleep sequences and components correlated with the number of avoidances scored the following day. On the other hand, trains lacking the SS→TS→W sequence, but containing the other three sleep sequences, prevailed in the baseline sleep of SL and/or NL rats, and variables of their sleep sequences and components did not correlate or inversely correlated with the number of avoidances scored the following day [57].

Strong, independent support to our suggestion that TS-containing sleep sequences are involved in the processing of memories of novel avoidance responses [7, 53, 55, 57], has recently been provided by the observation that ponto-geniculate-occipital waves occurring during the SS→TS→PS sequence dramatically increase in number in the post-training sleep of rats mastering the two-way active avoidance task; furthermore, they display strong correlations with the number of avoidances [58]. This seminal study should open the way to a better understanding of the mechanisms mediating memory processing during sleep.

Neurophysiological studies

Starting with the pioneering data by Pavlides and Winson [21], hippocampal place cells active during a waking experience have been shown to resume activity during post-trial sleep. The SS replay of the spatio-temporal sequences of activation of these neurons during waking has been confirmed and detailed in a number of experiments dealing with multiple recordings of hippocampal and entorhinal neurons. Notably, the correlations between active place cells sharing overlapping perceptual fields during waking were reported to be strengthened during post-trial SS in comparison with pre-trial SS [22–27].

These data should be viewed in the more general framework of the flow of information entering the hippocampus from the neocortex via the superficial layers of the entorhinal cortex, and eventually returning to the neocortex via the deep layers of the entorhinal cortex. The hippocampal inflow is associated with the theta waves of active waking and PS, while the outflow is associated with the hippocampal sharp waves of quiet waking and SS. Hence, this bidirectional traffic intermittently oscillates in one or the other direction depending on the vigilance state. Memories are assumed to be selectively strengthened during their hippocampal journey, presumably by septal inputs conveying motivational and emotional elements of the same experience [22–24]. On the whole, the above data provide strong independent support to the involvement of SS in memory processing. In turn, they indirectly confirm the validity of the sequential hypothesis.

Recent human studies supporting the sequential hypothesis

A critical assessment of the early human studies examining the involvement of sleep in memory processing has been published [12], and has now been brought up to date [59]. The distinction between episodic/declarative memories and
procedural memories was initially thought to provide a plausible framework for the interpretation of studies supporting PS in the processing of procedural memories and of the fewer studies supporting SS in the processing of episodic/declarative memories. This interpretation has recently been challenged by data indicating that, in human subjects, the processing of procedural memories requires the combined initial involvement of SS and the subsequent substantial contribution by PS [18–20]. An additional human study emphasizing the key role of sleep cycles in memory processing is in good agreement with this interpretation [17].

ROLE OF SS AND PS IN MEMORY PROCESSING

A basic premise of current views on long-term memory formation holds that pre-existing brain circuits become remodeled by potentiating a set of synapses and/or sprouting new synapses, while a different set of synapses may undergo depression and/or elimination [33]. This view implies that pre-existing brain circuits serving innate or previously acquired perceptual or motor programs dynamically compete with memories of novel experiences for the control of the corresponding programs. If this conceptual framework is accepted, memory processing would require the operation of two opposing mechanisms, respectively involved in the potentiation/formation of one set of synapses and in the depression/elimination of a second set. A preliminary mechanism of selection is likewise required to tag either one of the two sets of synapses, presumably on the basis of motivational and rewarding cues that may remain effective throughout the memory processing time.

We have initially proposed that the role of SS consists in the weakening/elimination of one set of memories, thereby leading to an increment in the weight of the other set of memories and to a corresponding sharpening of the signal-to-noise ratio. In agreement with the prevailing literature [3, 11–13], we have also assumed that the role of PS consists in the integration of the prevailing sharpened set of memories within the multidimensional network of associative brain circuits [7]. We have further suggested that, in the rat, the initial processing of memories of the novel avoidance response takes place during SS→TS→W sequences, while the subsequent processing is brought to completion during SS→TS→PS sequences. Conversely, the processing of memories of innate behavioural responses was assumed to initiate during SS→W sequences, and be eventually completed during SS→PS sequences [52–57].

The suggestion that the consolidation of recent memories may take place during SS [60], now indirectly supported by experimental evidence [61], may seem in contrast with our view of SS as the sleep state promoting the weakening/elimination of memories [7, 52–57]. However, memory consolidation during SS may only occur in the hippocampus [61], as neurophysiological studies of thalamic/neocortical circuits during SS are only suggestive of the occurrence of short-term plasticity [62, but see 63 for a different view]. In addition, the expression of immediate early genes in brain is strongly elicited by waking, but drastically reduced by SS [64, 65]. The activation of these genes is generally believed to herald the formation of long-term memories [33]. Hence, available data do not support the concept of memory consolidation in the neocortex during SS. Nonetheless, the potentiation of waking experiences during hippocampal sharp waves [61] might be instrumental for the fine tuning of the corresponding memories, as well as for the protection of their neocortical counterparts from the putative erasing power of delta waves indiscriminately acting on non-protected, less relevant features of the same experience. Some support for the latter possibility may be found in the observation that expression of immediate early genes in the neocortex is substantially but not fully reduced by SS [64, 65]. The few cells still showing a persisting activation during SS might be the neocortical counterparts of the relevant features of a waking experience, following their hippocampal potentiation.

In conclusion, in view of the above considerations, and of the evidence related to the marked decrement in brain metabolic rate during SS [66], and to the ontogenetic pattern of SS development [6, 7, 15, 39], the postulated process of weakening/elimination of memories is still likely to take place during SS. What then of the role of PS? To answer this question, it is appropriate to keep in mind that the beneficial enhancement of the weight of one set of memories versus the competing set, presumed to occur during SS [7, 22–27], does not necessarily imply that the prevailing memories have already
achieved their optimal integration with pre-existing brain circuits.

To attain this goal, and allow memory retrieval to be efficiently triggered by a more widespread range of stimuli, novel memories would need to be challenged and processed with pre-existing memories sharing mutual features. This process is again likely to require the simultaneous strengthening and weakening of different sets of connections, and eventually to lead to a partial dislocation of the initial memory pattern and/or to an increase in its complexity. The key role of PS in memory integration has been recently emphasized, notably in human sleep, with regard to declarative/episodic memories and procedural memories [67]. In agreement with a previous explicit proposal of a differential role of SS and PS in the intermittent transfer of information between hippocampus and neocortex [60], it has been suggested that only the former set of memories participate in the hippocampal–neocortical dialogue [67].

Episodes of PS are likely to subserve a complex integrating role, as they resemble waking in most but not all features [68], and the goal of integration is to allow waking stimuli to gain easy access to integrated memories. The marked PS reactivation of several regions of the human brain active during a previous learning session [69], is in full agreement with such a role. Further support is provided by the renewed induction of brain immediate early genes during the episodes of PS that follow exposure of rats to an enriched environment [70], as well as by the divergent mentation that selectively occurs in human subjects during PS [71].

As mentioned above, the extensive remodeling of brain circuits presumably promoted by PS would require the concurrent weakening/elimination of some connections proposed by the “reverse learning” hypothesis [2] and suggested by recent experimental data [28], in addition to the potentiation/formation of other connections [3, 5, 8–13]. The coexistence of these two opposing mechanisms might be rationalized by assuming that the reactivation of memory traces occurring during PS may down-regulate the corresponding synaptic connections, thereby back leading them to their pre-consolidation sensitive state. As a result, a new wave of modulating influences would be allowed to selectively operate on the activated traces [3]. The substantial silence of brain noradrenaline and serotonergic systems during PS might contribute to the labilization process, in view of the key role these systems play in waking consolidation. Interestingly, the occurrence of an analogous process of memory labilization followed by reconsolidation has recently been demonstrated for fear memories upon their waking retrieval [37].

The more general question of how the sleeping brain may simultaneously harbour opposing mechanisms of modulation of synaptic strength will certainly require further sophisticated investigations to be fully answered. For the time being, it seems reasonable to assume as a premise that each set of synapses or even each synaptic connection may be unique from the point of view of its relations to the rest of the brain and of its past history. As a result, modulation may differentially be exerted on different synapses on the basis of known (and still unknown) rules, including motivational and rewarding factors, familiarity or novelty of single items of past experiences, etc. The differential modulation of the synaptic connections of hippocampal place cells reactivated during PS [28] is a striking example of how this may be achieved on the basis of their selective involvement in the representation of novel or familiar items of past experiences.

An analogous process of remodulation of brain circuitry, based on concurrent processes of downregulation and upregulation of different sets of synaptic connections, might likewise take place in foetal and neonatal stages of brain development during active sleep and PS [72]. These operations might be required to adapt newly formed basic brain circuits to the ongoing modifications induced by growth and differentiation.

**CONCLUSIONS**

The experimental evidence accumulated in studies with laboratory animals during the last 30 years leaves little doubt that recently acquired memories are actively processed during sleep [3–13, 58, 70]. Furthermore, the initial view of an exclusive role of PS in memory processing is giving way to the more comprehensive proposal of a unitary, albeit differentiated participation of sleep to these events (the sequential hypothesis [6, 7, 15]). The recent flourishing of human sleep studies supporting the combined contribution of SS and PS in memory processing [17–20] substantially adds to the experimental data supporting the validity of the
sequential hypothesis in laboratory animals [7, 42–48, 52–57].

Hence, the main issue now at stake appears to concern the nature of the processing operations performed by SS and PS, rather than the demonstration of their involvement in these mechanisms. If our deductive speculations prove correct, either type of sleep may turn out to promote the potentiation/protection of one set of synaptic connections together with the weakening/elimination of an additional set. However, the mechanisms underlying these opposite functions are likely to substantially differ in SS and PS. Indeed, they appear to be based on the prevailing SS tendency to depress recently acquired synaptic connections with the exception of those related to salient features of waking experiences, while the mechanisms prevailing during PS might rely on a back switching of memories to their pre-consolidated state, thereby allowing their differential modulation by contingent influences. The powerful neurophysiological methods of multiple neuronal recordings during waking experiences and post-trial sleep [22–28], and the sensitive technologies of mRNA and protein analyses in brain [64, 65, 70] hold encouraging promises of solution for this extraordinary biological problem.

**Research Agenda**

1. Sleep sequences containing or lacking TS have been related to memory processing in adult rats. The role of innate and environmental factors in shaping the adult complement of these sequences remains to be investigated.
2. Variables of SS, TS, and PS have only been determined in rats trained for a two-way active avoidance task. To examine the general validity of these observations, comparable analyses should be extended to rats trained for different tasks, ranging from complex appetitive tasks to simpler non-associative tasks.
3. The expression of brain intermediate early genes is known to be markedly reduced after a period of slow wave sleep following waking in a familiar environment. It is still not clear whether the brain expression of these genes would be comparably reduced after a period of slow-wave sleep following waking in a novel environment.

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APPENDIX I.
GLOSSARY OF TERMS

Consolidation: the process by which a recently acquired memory trace becomes insensitive to previously effective modulatory influences.

Delta region: region of the EEG power spectrum containing delta waves (0.5–4.0 Hz).

EEG power spectrum: pattern of EEG voltage output as a function of EEG wave frequency (μV²/Hz).

Escape response: innate response to a footshock consisting in quickly moving to the other compartment of a two-compartment shuttle-box, thus interrupting the footshock.

Freezing response: innate response to a footshock consisting in the block of escape movements.

Passive avoidance: acquired behavioural response consisting in the suppression of an instinctive behaviour, resulting in the avoidance of a punishment (footshock).

Place cells: hippocampal neurons selectively activated when the animal is in a specific location in space.

Postural atonia: loss of contractile tone in the muscles responsible for the postural asset.

Synaptic potentiation or depression: enhancement or decrement of synaptic efficacy measured by the size of a postsynaptic potential elicited by a single presynaptic stimulus.

Two-way active avoidance: acquired behavioural response consisting in avoiding a punishment (footshock) by quickly moving to the other compartment of a two-compartment shuttle-box upon the delivery of a signaling stimulus.