## **SLEEP and SLEEP DISORDERS**

### Implicit Memory and REM Sleep: A Pilot Study

C cile Colin, R.A., Jos Morais, Ph.D., and Myriam Kerkhofs, Ph.D.

This study examined implicit and explicit memory for words presented during Rapid Eye Movement (REM) sleep. Ten subjects were presented two lists of twenty words: one list just before they fell asleep and the other during the second and the third periods of REM sleep. The next morning, they were given an implicit memory task (word stem completion) and an explicit memory task (recognition). While they were carrying out these tests, their electrodermal activity was recorded. The implicit task showed no learning effect for either list of words, whereas the explicit task showed a learning effect only for the list presented before sleeping. Moreover, the recording of the skin conductance variations gave no significant results. Thus, no evidence of implicit memory during REM sleep was observed. (Sleep and Hypnosis 1999;1:82-87)

Key words: REM sleep, implicit memory, learning, cognition

#### INTRODUCTION

T he relationships between sleep and memory have been commonly described while investigations of learning during sleep are quite rare. Four different types of paradigm have been used to uncover the role of REM sleep in the memorization processes.

#### 1. The Postlearning Sleep Effect on Retention:

Jenkins and Dallenbach (1) carried out the first study in this field. They found retention to be considerably better for subjects who slept immediately after learning compared to subjects who were awake for an equal interval. Since then, this finding has been replicated (2,3,4). More recent investigations were aimed at specifying the stage of sleep that would be responsible for this beneficial effect on retention. It was found that performances were enhanced if learning had been followed by a period of REM sleep

Address reprint requests to: Dr. Myriam Kerkhofs, Sleep Laboratory, C. H. U. A. V sale Route de Goz e 606 6110 Montigny-le Tilleul, Belgium. Phone: 32 71 29 5193, Fax: 32 71 29 5604 email: mkerkhof@ulb.ac.be

Acknowledgements: We are very grateful to the Professor A. Herchuelz and to the whole staff of the Sleep Laboratory of A. V sale Hospital. Without their precious help, this study could not have been achieved. We also thank Professor V. De Maertelaer (IRBHN, Free University of Brussels) for excellent statistical help. Accepted February 17, 1999. instead of another stage of sleep (5,6).

#### 2. The Postlearning REM Sleep Deprivation Studies:

Since REM sleep seems to have a beneficial effect on retention, it has been proposed that REM sleep deprivation could produce a detrimental effect. Studies on animals (7,8) and on humans (9) supported this prediction; moreover, they suggested that the degree of impairment depended on task complexity.

#### 3. The Effect of Learning on Subsequent REM Sleep:

As showed by Hennevin and Leconte (7) and by Bloch, Hennevin and Leconte (10), in rats and cats, acquisition sessions are followed by an increase of time spent in REM sleep. This REM sleep increase was correlated with learning. As a matter of fact, when animals are unable to learn, they do not present any REM sleep increase. Moreover, REM sleep duration returns to its normal value when the learning task has been totally mastered; by contrast, each new task is followed by a new REM sleep increase. Thus, the REM sleep increase seems to occur if and only if a new situation requires a restructuration of the memory representations. However, the results of similar studies on humans are more confused and even contradictory (11,12).

## 4. The Effects of Postlearning REM Sleep Stimulations on Retention:

In rats, reticular electrical stimulations, delivered during postlearning REM sleep, led to performance improvement (13). In humans, an auditory stimulation, applied during

From the Laboratory of Experimental Psychology, Universit Libre de Bruxelles, Belgium and Sleep Laboratory, H pital Erasme, Brussels and C.H.U. A.V sale, Montigny-le-Tilleul, Universit Libre de Bruxelles, Belgium.

postlearning REM sleep, also led to performance improvement, especially if those stimulations were REMs contingent (14,15), that is to say, applied during periods at which central activation was the most intense.

Thus, by means of these four paradigms, REM sleep has been shown to play a significant role in the memory consolidation processes. Consequently, one may wonder if this sleep stage is also favorable for the acquisition of new information in humans.

Only a few studies investigated the possibility of learning during sleep in general, and during REM sleep in particular. Many of these studies concerned the learning of foreign languages. Rubin's works (16), for example, yielded some positive results, but a fundamental criticism ought to be raised against his studies: the lack of electroencephalograph (EEG) analysis of the subjects' sleep. Hence, the Rubin's subjects positive results could have been caused by the fact that they were awake, instead of asleep, when they were presented the stimuli.

Simon and Emmons (17) presented question-response pairs at different levels from wakefulness to deep sleep (the state of the subjects was controlled by EEG). They found results on recall and recognition tests to be correlated with the depth of sleep. The percentage of correct responses decreased as the amount of alpha waves decreased and delta appeared, that is as the sleep level deepened. However, this kind of methodology is subject to another criticism: the retention material was assessed only by means of explicit tests of memory. In other words, subjects were aware of being tested about a material they had learnt earlier.

Yet, for a few decades, it has been pointed out that memory can be assessed in implicit ways. In an implicit memory task, subjects are not instructed to intentionally recollect the items presented earlier; they are instead given some apparently unrelated task. The underlying assumption is that performance in this task is facilitated by the information acquired during the previous study episode. In other words, implicit memory is disclosed when performance on a task is facilitated by a specific learning episode in the absence of deliberate recollection.

Wood, Bootzin, Kihlstrom and Schacter (18) performed the only study that used implicit tasks to examine retention of words presented during sleep. These authors presented homophones pairs and category-instance pairs to 19 experimental subjects (during REM sleep or stage 2) and to 12 control subjects (who were awake). Retention was implicitly assessed by asking the subjects to spell homophones or to name an instance from a category but no evidence of implicit memory for verbal material presented during sleep was observed.

The present study was aimed to find evidence of implicit memory during REM sleep using, however, a slightly different method. Verbal material was presented during REM sleep only and its retention was assessed by explicit and implicit memory tests. An implicit perceptual memory test (word stem completion) was chosen which enabled to use a priming paradigm and to maintain the same modality (auditory) between the learning and the test, since implicit memory seems to be sensitive to a modality change between these two episodes (19). In a word stem completion test, subjects are asked to complete stems with the first words that come to their mind; they are not required to use the words presented during the learning episode. The word stem completion test is considered as an implicit memory test because subjects have to perform a task which is unrelated to the prior study phase (20). The aim of the stem completion task was to compare performance for the words presented before sleeping to performance for the words presented during REM sleep; we expected the words presented before sleeping to be used more often than the words presented during sleep.

The explicit memory test was a recognition task, which consist in presenting target words among distractors. For each item, the subjects have to indicate if they recognize the word or not. The recognition test is considered as an explicit memory test because the testing procedure requires the subjects to reflect consciously on a previous learning episode (21). Performance on the recognition test served as a control for effective learning. Whether implicit retention occurred or not, we expected performance for the words presented before sleeping to be better than performance for the words presented during REM sleep.

Furthermore, while the subjects were carrying out the two tasks, their electrodermal activity (EDA) was recorded. We used this activity as another measure of implicit memory. EDA is thought to reflect underlying psychological processes. Indeed, electrodermal changes are an index of the activation of reticulocortical system and are observed after a sensory or an emotional stimulation (22). EDA has already been used successfully in studies with control subjects, amnesics or prosopagnosics (23,24). Moreover, the great utility of this tool lies in the fact that changes in EDA are not available to consciousness. An implicit memory effect could occur at this psychophysiological level even if there were no learning effect for both behavioral tests.

#### METHODS

#### **Subjects**

Ten healthy students (plus an extra one for a pre-test), aged between 20 and 23 without sleep complaints were tested. The PSQI (25) was administered to all subjects in order to detect sleep complaints. The subjects were paid for their participation to the study and were informed on the aims. Particularly, they were told that verbal stimuli will be presented to them before, during and after sleep.

#### Learning Material:

One hundred and twenty trisyllabic words have been selected and divided into sixty word pairs. The two words of each pair were matched in frequency and began, at the phonetic form, with the same two first syllables (we selected word that alliterated because of the requirements of our implicit memory task: a word stem completion test). We chose to use word pairs in order to dispose of two similar categories of words, so that half of the subjects would be submitted to words from one category and the other half would be submitted to words from the other category This precaution enabled us to control for possible biases in the stimuli construction. Implicit Memory and REM Sleep

Furthermore, the sixty word pairs were divided into three lists of twenty word pairs. Lists A were presented before sleeping (half of the subjects were given A1, corresponding to the first category of words, and the other half A2, corresponding to the second category); lists B (B1 and B2) during REM sleep; and lists C (C1 and C2) were not presented during a learning episode but served as distractors for the recognition test and as new stems for the word stem completion test.

#### Sleep

The study was performed at the Sleep Laboratory, C.H.U. A. V sale, Montigny-le-Tilleul, Belgium. The subjects spent two consecutive nights in the sleep laboratory. The first night was an habituation night during which no material was presented. The second night was the experimental night. Sleep was recorded according to standard criteria (26). Three EEG recordings (central, occipital and parietal leads), two electrooculogram (EOG) and one chin electromyogram (EMG) were performed. Scoring of sleep stages were performed following standard criteria (23).

#### Procedure

The subjects were submitted to two different lists of 20 words played over loudspeakers, one before sleeping and the other one during sleep. The words were presented at 3 seconds intervals.

#### **Before Sleep**

The first list was presented only once, more or less ten minutes before sleeping. The subjects were awake, in bed and listening to the tape, but no EEG recording was made.

#### **During Sleep**

The second list was given after ten minutes of the second and the third periods of REM sleep in order to be sure that the REM sleep was well installed. If the sleep record showed any signs of arousal, the tape was immediately turned off and restarted when the subject had resumed REM sleep.

#### After Sleep

The next morning, the subjects had to complete a questionnaire about the quality of their sleep. Then, they were connected to a psychogalvanometer which recorded their skin conductance variations while they were carrying out two tests: word stem completion followed by recognition. The choice of this test order was justified by the following assumption: if the recognition test had been given before the first, then the word stem completion test might have displayed a greater priming effect for those items that would have been recognized previously.

During the word stem completion test, the subjects were asked to complete 60 word stems in order to form trisyllabic words. Among the 60 word stems, 20 corresponded to the 20 words heard before sleeping (list A), 20 others corresponded to the 20 words heard during REM sleep (list B) and 20 were new (list C). For every

item, the subjects had 20 seconds to give three completion possibilities.

For example, if they had previously learn paragraph, they could be presented the stem para and answer whatever come to their mind including paragraph of course.

Our recognition test was made of the 20 words heard before sleeping mixed up with the 20 words heard during REM sleep and with 20 distractors. For each item, the subjects had three seconds to say heard before, heard during or not at all. It is rather unusual to give a recognition test with three answer possibilities. Usually, there are only two possibilities: the subjects have to say if they recognize the items or not (yes or no). However, in this case, it was worth dividing the category recognized into before and during. Indeed, some subjects might not remember having heard a word before sleeping but have a diffuse recollection, which could justify the during answer.

While the subjects were carrying out these two tasks, their skin conductance variations were recorded in order to know if an implicit memory effect could appear at this psychophysiological level.

#### Statistical Analysis

ANOVA analysis was performed with power (the probability of rejecting the null hypothesis in case of actual difference) analysis.

#### RESULTS

The subjects slept quite well (they claimed their sleep was deep and not much agitated). Nevertheless, they told us that they did not sleep as well as at home (more awakenings). As a matter of fact, five of them woke up because of the tape and were aware of having heard words but could not give us any example. Detailed information about the sleep variables are given in Table 1. Due to a technical flaw, data concerning subject 4 were lost after the recording night. Total sleep time was 448 min in average and percentages of sleep stages were in normal ranges.

It should be noted that for five subjects, we observed, during the tape presentation, a REMs disappearance (the EMG remaining at the required level for the REM sleep) together with the appearance of alpha waves.

#### Word Stem Completion Test and Recognition Test

Results for the 10 experimental subjects are given in Table 2 (for the word stem completion test) and in Table 3 (for the recognition test). For the word stem completion test, we totalized the number of target responses (when a subject completes a stem with a word heard before or during sleep) and the number of non target responses (when a subject completes a stem with a word from the other category). We did not take into account other words produced by our subjects (belonging thus neither to the category they heard, neither to the category (serie) they did not hear).

For the recognition test, we totalized the number of correct and incorrect responses for every type of words ( A ,  $\,B\,$  and  $\,C$  ).

#### Table 1. Sleep polygraphic variables in 9 subjects

Sleep Variables	S1	S2	S3	S5	S6	S7	S8	S9	S10
Total Sleep Time	462	430	416	426	429	460	477	466	467
Sleep Efficiency	87	81	80	85	92	94	95	96	84
Stage 1 (%)	8	5	7	2	3	3	4	4	11
Stage 2 (%)	59	47	41	55	54	44	58	49	40
Stage 3 (%)	13	9	11	14	14	13	5	4	9
Stage 4 (%)	6	12	14	6	8	14	9	15	11
Stage REM (%)	4	13	16	18	16	24	20	25	14

We applied an ANOVA analysis on the data from the two behavioral tests. We took the type of stimulus (list A vs list B) and the type of task (word stem completion vs recognition) as within-subjects factors, the type of category (1 vs 2) as between-subjects factor and the frequency of correct answers as dependent variable.

#### **Electrodermal Activity**

The electrodermal response is a phasic phenomenon, relatively brief and exhibited between 1 and 5 seconds after starting the stimulus. For the recognition test, the subjects had three seconds to give their answer for each item.

		List A			List B		A+B
Subj.	Target resp.	N-target resp.	Total	Target resp.	N-target resp.		Total
1	5	5	10	4	5	9	19
2	7	6	13	3	1	4	17
3	10	3	13	2	4	6	19
4	10	5	15	5	2	7	22
5	5	7	12	4	3	7	19
6	8	2	10	2	2	4	14
7	3	6	9	2	3	5	14
8	16	6	22	4	7	11	33
9	6	5	11	4	4	8	19
10	10	11	21	5	10	15	36
tot.	80	56	136	35	41	76	212

The list effect was significant (F(1,8)=21,32, p<.01) indicating that for both tasks, the percentage of correct responses was much higher for the A list (words presented before sleep) than for the B list (words presented during sleep).

Table 3. Number of correct responses in the recognition test for the 10 subjects

Subj.	List A	List B	List C	Tot.
1	7	6	11	24
2	12	4	14	30
3	16	7	7	30
4	8	11	12	31
5	8	4	11	23
6	6	0	16	22
7	13	8	8	29
8	15	3	15	33
9	13	0	17	30
10	7	4	15	26
tot.	105	47	126	278

Consequently, the skin conductance variations, relative to a particular item, could have happened during the time devoted to the following item. Because of this, we decided to consider the words two by two. This way of processing gave us 9 different categories of word pairs (A word followed by a B word or C word, B word followed by a A word , ...). We computed the skin conductance values for every category. We based our computations upon the hypothesis according to which, as we already mentioned it, there should be no explicit or implicit learning during sleep. Consequently, a A word was supposed to be known, while a B word or a C word was supposed to be unknown by the subjects. We thus supposed that there would be a skin conductance variation between a supposed known word and a supposed unknown one, and conversely. We found no significant differences between the different categories of words. When a word supposed known was followed by a supposed unknown one, it produced the same skin conductance variations as if the contrary occurred. We did not analyze the results for the word stem completion test. Indeed, the subjects had 20 seconds to complete each stem. As the electrodermal response occurs only between 1 and 5 seconds after starting the stimulus, we did not know how to analyze the remaining 15 seconds, more especially because the variations continued during this interval.

# Correlations Between Sleep Parameters and Tests Performance

Positive correlations (r = 0,85 and r = 0,80) were found between the remaining sleep time after the last occurrence of the tape (ST) and the performance for the two tests, but only for the subjects who woke up during the tape, suggesting that the longer ST was the better the results were. In addition, REM sleep percentage was positively correlated to the performance for the word stem completion test, but, only for the subjects who did not wake up (r = 0,74). However, none of these correlations did reach statistical significance (p=0,065; p=0,1; and p=0,149, respectively).

#### DISCUSSION

At the explicit level, the words presented before sleeping were considerably more often recognized than the words presented during REM sleep. We thus concluded that the words presented before sleeping seem to have been well memorized, whereas no trace of explicit memory for the words presented during REM sleep was found. Concerning the implicit level, the same pattern was observed: the items learnt before sleep were much more often used to complete the stems than the ones learnt during REM sleep.

We may thus infer that, for the word stem completion test, the subjects seem to have used preferably the words heard before sleeping to complete the stems, possibly under the influence of intentional retrieval. Another explanation can be that the words presented during REM sleep were not perceived, at least by the five subjects who did not wake up during auditory presentation.

Finally, there was no significant skin conductance variations either, for both types of words and for both tasks. These results confirm thus our hypothesis and are congruent with previous studies showing that, in presence of an EEG control, no learning, either explicit or implicit, during sleep seems to happen (17,18). Nevertheless, our study raised important methodological issues. Firstly, the sound intensity had to be adjusted so that the subjects

could hear the words, without any awakening. In order to do this, we asked our pre-test subject to listen to the tape. The sound was reduced till he did not hear the stimuli anymore. We adjusted the sound intensity slightly above this threshold, however, it is not proved that this threshold would be adapted to all the subjects. Even with an adapted threshold nothing could prove that the stimuli had been effectively perceived during REM sleep.

The second issue concerned the recall interval that was longer for the pre sleep stimuli (8 hours) than for the stimuli presented during sleep (4 hours). However, a longer interval would impair the performance, which was not the case in our study.

The third issue concerned the analysis of the skin conductance variations data. We choose a method that was probably not the only one possible and perhaps not the best one. Moreover, we had to face the lack of specificity of the psychogalvanometer. Indeed, it reacts to every stimulation, not only to the psychological ones: intellectual activity, muscular activity, noise, emotions also provoke electrodermal activity. The skin conductance variations we computed were thus probably not provoked by a physiological recognition of the stimuli only.

Finally, the disappearance of the REMs in five subjects while they were submitted to the tape could reflect the processing of the auditory information presented during this sleep stage. Furthermore, we observed an alpha wave simultaneously with the REMs disappearance. According to Cipolli (27), the alpha wave may play an important role in learning processes, as there seems to be a correlation between retention rate and the level of EEG activation after stimulus presentation. This EEG activation should be higher than a minimal level so that learning could occur. Thus, learning during REM sleep could be possible if the stimuli provoked an EEG acceleration. However, we did not find such a correlation between learning and alpha waves in our study. Nevertheless, the precise role of the alpha waves in this work is difficult to determine since we systematically attempted to avoid them.

In conclusion, we failed to obtain any implicit learning effect during REM sleep, with either behavioral or psychophysiological measures. However, due to the relatively small size of our sample, these results should be confirmed on a larger sample.

#### REFERENCES

- Jenkins J, Dallenbach K. Obliviscence during sleep and waking. Journal of American Psychology 1924;35:605-612.
- 2 Benson K, Feinberg I. Sleep and Memory: Retention 8 and 24 hours after initial learning. Psychophysiology 1975;12:192-195.
- Benson K, Feinberg I. The Beneficial Effect of sleep in an extended Jenkins and Dallenbach Paradigm. Psychophysiology 1977;14:375-384.
- 4. Ekstrand BR Effects of sleep on memory. Journal of Experimental Psychology 1966;75:64-72.
- 5. Grosvenor A, Lack LC. The Effect of sleep before or after learning on memory. Sleep 1984;7:155-167.
- 6. Scrima L. Isolated REM sleep facilitates recall of complex associative information. Psychophysiology 1982, 19:252-259.

- 7. Hennevin E, Leconte P. Etude des relations entre le sommeil paradoxal et les processus d'acquisition. Physiology and Behavior 1977;18:307-319.
- 8. Leconte P, Hennevin E, Bloch V. Analyse des effets d'un apprentissage et de son niveau d'acquisition sur le sommeil paradoxal cons cutif. Brain Research 1973,49:367-379.
- Empson JAC, Clarke PRF. Rapid Eye Movement and remembering. Nature 1970;227:287-288.
- Bloch V, Hennevin E, Leconte P. Relationship Between Paradoxical Sleep and Memory Processes. In: Brazier MAB, ed. Brain Mechanisms in Memory and Learning: From the Single Neuron to Man. New-York, Raven Press, 1979;1182-1191.
- 11. Guerrien A. REM Sleep and memory processes in humans. Acta Psychiatrica Belgica 1994;94:75-82.
- Smith C, Lapp L. Increases in number of REMs and REM density in humans following an intensive learning period. Sleep 1991;14:325-330.
- Hennevin E, Hars B, Bloch V. Improvement of learning by mesencephalic reticular stimulation during postlearning paradoxical sleep. Behavioral and Neural Biology 1989;51:291-306.
- 14. Dujardin K, Guerrien A, Mandai O, Sockeel P, Leconte P. Facilitation mn sique par stimulation auditive au cours du sommeil paradoxal chez l'homme. C.R Acad. Sci. Paris, t. 307, S rie III, 1988:653-656.
- 15. Guerrien A. Sommeil et cognition: effets de modifications induites du sommeil paradoxal chez l'homme. Th se pr sent e pour l'obtention du Doctorat en Psychologie. Universit Charles De Gaulle, Lille III, 1990.
- 16. Rubin F. Learning and Sleep: The Theory and Partice of Hypnopaedia. Baltimore: Williams and Wilkins Company, 1971.
- 17. Simon CW, Emmons WH. Responses to material presented during various levels of Sleep. Journal of Experimental Psychology 1956;51:89-97.

- Wood JM, Bootzin RR, Kilhstrom JF, Schacter DL. Implicit and explicit memory for verbal information presented during sleep. Psychological Science 1992;3:236-239.
- Moscovitch, M, Vriezen, E., Goshen-Gottstein, Y. Implicit Tests of memory in patients with focal lesions or degenerative brain disorders. In: Boller F, Grafman J, eds. Handbook of Neuropsychology, Vol. 8, Elsevier, 1993;133-173.
- 20. Rajaram S, Roediger III HL. Direct comparison of four implicit memory tests. Journal of Experimental Psychology: Learning, Memory and Cognition 1993;19:765-776.
- Parkin AJ, Reid TK, Russo R. On the differential nature of implicit and explicit memory. Memory and Cognition 1990;18:507-514.
- 22. Fowles DC, Gruzelier JH. Progress in electrodermal research. Roy JC, Boucsein W, eds.NATO ASI Series, 1993.
- 23. Bauer RM, Verfaellie M. electrodermal discrimination of familiar but unfamiliar faces in prosopagnosia. Brain and Cognition 1988;8:240-252.
- 24. Verfaellie M, Bauer RM, Bowers D. Autonomic and behavioral evidence of "Implicit" memory in amnesia. Brain and Cognition 1991;15:10-25.
- 25. Buysse DJ, Reynolds III CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. Psychiatry Research 1989;28:192-213.
- Rechtschaffen A, Kales A.A. NIH Publication Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects. Washington, D.C.: U.S. Government Printing Office, 1968.
- 27. Cipolli C. Sleep, Dreams and Memory: An overview. Journal of Sleep Research 1995;4:2-9.