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Increases in Number of REMS and REM Density in Humans following an Intensive Learning Period

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Summary: Animal studies have recently demonstrated that increases in rapid eye movement (REM) sleep and actual number of rapid eye movements (REMs) over normal levels followed successful learning of an avoidance task. These increases persisted for many days following the end of the training sessions. It was hypothesized that similar extended increases in REM sleep parameters would follow an intensive learning task in humans. Senior college students were sleep monitored following the end of their Christmas examinations. Results showed that there was a significant increase in the number of REMs observed following the exams as compared to baseline and control subject values. The number of extra REMs was most prominent during the fifth REM period of the night. A significantly increased REM density was observed at the fourth REM sleep period of the night. Results support the idea of REM sleep and/or the REMs themselves being involved in long-term memory processing several days after the end of training. **Key Words:** Rapid eye movement sleep—Rapid eye movement density—Learning—Memory—Humans.

There is now substantial evidence from animal studies of a relationship between rapid eye movement (REM) sleep and learning. Two major approaches have been used to study the REM sleep–learning hypothesis. One approach has been to train the animal and then to impose REM deprivation (REMD) in the hours following the learning session. The second approach has been to train the animal and then to monitor the sleep following the session for changes in REM sleep parameters. In general, the results have indicated that learning is followed by REM sleep increases (i.e. minutes of REM, % REM) over normal levels. REMD applied at these times of expected above-normal REM sleep increases has resulted in learning deficits (1–4). It has also been noted that the timing of these special REM periods, in terms of latency to onset and duration, depends on the strain and type or organism doing the learning, the type of task and the number of training trials per session. These critical REM sleep periods have been called REM windows (4).

It has also been observed that rats exposed to a two-way shuttle shock-avoidance task exhibited extra, above-normal, levels of REM sleep that persisted as long as 7 days after the last training session had ended (5,6). As well, the number of rapid eye movements (REMs) paralleled the increases in REM sleep (6). Further, in parallel studies using the same paradigm, REMD was found to significantly retard learning/memory when applied either during the 9–12-hour or 48–72-hour period after training (6,7). These results strongly suggest that memory processing continues for at least several days after the end of acquisition and that REM sleep is involved.

A variety of human recording studies have been done to examine sleep parameter changes following learning. The focus has almost invariably been upon sleep changes occurring the same night as the learning situation (2,8–14). The examination of sleep parameter changes several days after learning has, with the exception of one single-subject study (15), not yet been attempted in humans. One of the problems involved with human recording studies is that the task given by the experimenter must ethically be moderate in size and difficulty. It is unfortunately also likely to be emotionally neutral and personally irrelevant to the subject. This has given rise to mixed results from human sleep–learning studies (2). By contrast, the animal studies use naive subjects, and the learning situation imposed is likely to be the most important event in the entire life of the organism.

The present study tried to circumvent these problems by choosing a personally relevant, real-life situation of great importance to the subjects. It was decided to examine the sleep of honors psychology students,

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both when relatively little learning was going on in their lives and shortly after extensive learning had taken place. Several predictions were made. It was hypothesized that the amount of REM sleep and number of REMs would be higher following a major learning event (Christmas exams) in the life of the individual and that this type of sleep would persist for many days after the end of the learning situation, as was seen in the rat (5,6). It was also predicted that the REM sleep parameters would be higher in test subjects than for appropriate control subjects not in the exam situation. Further, it was predicted that REM sleep parameters for the test subjects would be higher following the learning situation than at baseline either the summer before or the summer after the learning situation.

METHOD

Subjects

The subjects (n = 11) were five males and six females. Control subjects (four females, one male) ranged in age from 21 to 27 ($\bar{x}$ = 22.8) yr, and the test subjects (two females, four males) had an age range of 21–24 ($\bar{x}$ = 21.7) yr. Subjects were given the Trent University Sleep Questionnaire followed by extensive personal interviews. Those finally chosen for the study were assessed to be relatively free of major personal problems (i.e. severe sickness in themselves or close family members, major monetary worries, etc.). All female subjects were asked to tell us approximately when their menstrual period normally began, in order that recording could take place at least 7 days preceding or following the beginning of the cycle. All subjects were found to be in good health and none was on medication of any kind.

Design and procedure

The basic design of the study can be seen in Table 1. Test subjects were given four consecutive nights of EEG/EOG/EMG sleep recording during the summer months after having been accepted into the honors program (Summer 1). These same subjects were recorded again several days after, rather than immediately after, their Christmas exams. Times were 1–3 days after the last exam for the start of the 4 consecutive days of recording. Thus, subjects had completed their exams and had time to recover from any self-imposed sleep deprivation, although none of the subjects was in the habit of staying up much beyond their normal bedtime. None of the subjects reported staying up even part of the night to "cram" for their exams. Likewise, they reported heeding our request not to stay up beyond their normal bedtimes in the 1–3 days after their exams had finished. Also, their use of alcohol in this time period was reportedly very modest (i.e. a glass of wine with dinner, etc.).

TABLE 1. *Basic experimental design*

	Summer 1	Christmas season, after exams	Summer 2
Test (n = 6)	No learning	Learning	No learning
Controls (n = 5)	No learning	No learning	No learning

These same subjects were then asked to come in at least 2 mo after the end of April (final) exams for a third recording session of four consecutive nights (Summer 2). Thus, both pre- and postlearning recording was carried out to provide a baseline for sleep that followed the Christmas exams. For controls, it was possible to choose subjects of virtually the same age who had just been through the honors program. These subjects had for financial reasons delayed their entry into graduate school and had found employment as teaching/research assistants in the department. Thus, they were very comparable to the test subjects. Their sleep recording took place, as with the test subjects, during Summer 1, just before Christmas and again during Summer 2. As with the test subjects, none was assessed to be involved in any major learning situation or to have significant personal problems of any kind.

The learning situation

The honors psychology program is specifically designed as a fourth year of specialized university undergraduate study for those students who plan to enter graduate school. The academic year runs from September of one year to April of the next year. Of approximately 200 students majoring in psychology, 10–15 are normally accepted into this program by the end of their third year. Thus, all subjects were clearly superior in their academic ability.

Honors students are all required to write at least two examinations in the second week of December, each being worth approximately 25% of the final grade in the course. These exams are in "core" psychology courses and are considered to be the most difficult offered at the undergraduate level (i.e. advanced biopsychology, perception). Student-based estimates of the number of actual hours of studying done in the last week to prepare for these exams ranged from 25 to 40. It was further estimated that about 50% of the studying done in the final week was done in the 2 days prior to the exam. All subjects reported spending "all day" (about 10 hr of study time) on the day prior to each exam. Students found the material to be challenging, and marks ranged from the low 70% range to the low

90% range, indicating that grasp of the material was good, but not perfect.

Recording and scoring of sleep records

All subjects were requested to refrain from any alcohol ingestion on recording nights, although normal levels of smoking were allowed. They reported to the laboratory one hour before "lights out" for electrode application. One EEG (C3/A2), two EOG (horizontal eye movements) and one EMG (chin muscle) electrode pair were used. Activity was recorded using a Beckman (Model R-411) polygraph at a paper speed of 10 mm/sec. Subjects were allowed to go to bed at their normal bedtime (11 p.m.–1 a.m.). They were told that they could sleep as long as they wished. The sleep records were all scored by two scorers, and inter-rater reliability was greater than 95%. All records were scored using the standard method of Rechtshaffen and Kales (16).

The REMs were counted manually and were only included if at least one of the two EOG traces showed a deflection of at least 25 μV amplitude. No distinction was made between bursts and single REMs; all were counted and added to the final total and were not analyzed separately. The REM density was calculated by dividing the total number of REMs in a REM period by the total number of minutes of REM sleep in the period.

Our system for obtaining density values cannot be compared directly with those of other workers, because others have used slightly different methods of estimating densities. For example, Aserinsky (17) used the number of 2 sec mini-epochs containing at least one eye movement divided by the total number of mini-epochs of REM sleep. We were concerned that this method might not be sensitive enough. It was reasoned that if the eye movements were to increase in number but also increase in frequency as a result of learning, there could theoretically be a greater number of actual eye movements in the same period of time, but the calculated densities would not appear different. Thus, each and every eye movement of acceptable size was counted in the present study to avoid this possible problem. It was very difficult because of the small number of subjects and limited personnel with scoring expertise to score the records blind, but the attempt was made. However, all records were done completely independently by the two scorers.

Data analyses

Statistical analyses were all done in the following way. Mean values for all subjects for nights 3 and 4 were found. Then, difference scores were calculated, using the following formula: Mean Score at Christmas − [(Mean Score for Summer 1 + Mean Score for Summer 2)/2]. The subtraction scores thus obtained were then compared in a one-way analysis of variance of test versus control groups. Thus, both between- and within-subject comparisons were made.

One of our concerns was the possibility of a first-night effect (18). Thus, a randomized-blocks analysis of variance of total sleep time (TST) was done using all four nights and all 11 subjects at each of the three recording times. No significant night effect was found, either at the prelearning, Christmas or postlearning recording periods. However, there was a significant subjects effect at both the prelearning [$F(10,30) = 2.46$, $p < 0.05$] and Christmas [$F(10,30) = 4.48$, $p < 0.01$] recording periods. The same result was observed during the Christmas period when the analyses were repeated using only nights 2, 3 and 4 [$F(10,20) = 3.66$, $p < 0.01$].

These results indicated a great deal of variability between subjects on sleep-recording nights 1 and 2. When only the last 2 days at each of the three test times were analyzed in this way, there was no subject effect. There were no systematically lower scores on nights 1 or 2, as might have been expected of a first-night effect. However, several of the subjects did seem to have scores that were considerably lower on these nights. On the other hand, there were several subjects that apparently exhibited more TST on the first or second nights. Thus, the mean TST was not significantly different because the higher and lower scores cancelled each other. However, because of the larger variability on nights 1 and 2, it was considered prudent to omit these data.

RESULTS

The most obvious result in this study was the increase in the total number of REMs observed in the test subjects following the exam period [$F(1,9) = 6.3$, $p < 0.05$]. These results can be seen in Fig. 1. Also significant was the increased density of REMs [$F(1,9) = 6.5$, $p < 0.05$] occurring following the exam period for the test subjects. No other REM-related measure (minutes of REM sleep, % REM sleep or latency from stage 2 onset to any of the five REM periods) was found to be significant. Further, there were no changes in any of the other sleep parameters measured. Values for all of these measures can be found in Table 2.

In an attempt to find out more precisely when, during the sleep night, the extra REMs were concentrated, the two groups were compared for each of the five REM periods (REMP) separately. Subjects in both groups occasionally skipped the first or last REMP. When at least one of the baseline summer recordings was pres-

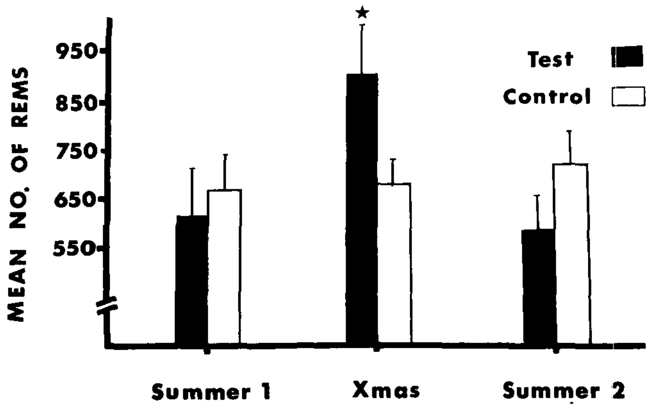

FIG. 1. Mean number of REMs for both test and control subjects (± SEM). Pre- and postlearning baseline values are shown for Summer 1 and Summer 2. The posttraining values of the test group and the nonlearning control values are shown at Christmas. *$p < 0.05$

ent for a REMP, that value was used in the comparison with the December test score to get a subtraction value. When the REMP was missing from the December score, the subject was omitted from the analysis of that particular REMP. Fortunately, this only happened once for stage 1 REMP in a control subject and once for stage 5 REMP in a test subject.

Analyses of variance revealed that the test subjects had significantly more REMs during the fifth (last) REMP of the night [$F(1,8) = 8.66$, $p < 0.05$]. REM density was found to be higher for the test group during the fourth REMP [$F(1,9) = 6.21$, $p < 0.05$]. Although the increase in REM density did not reach significance for REMP 3 [$F(1,9) = 4.18$] or REMP 5 [$F(1,8) = 4.86$], it did approach significance in each case ($0.1 < p < 0.05$). Graphs of the number of REMs and REM density for each of the five REMPs can be seen in Fig. 2 and 3.

A repeated-measures analysis of variance, using the total number of REMs measured, was performed on the pre- and postlearning baseline data for all 11 test and control subjects. No differences of any kind were observed indicating equivalence of the pre- and postlearning baseline values for the most sensitive measure, total number of REMs.

DISCUSSION

To summarize, the results clearly indicate an increase in the number of REMs for the test group 3–5 days following their exams. This is indicated by measure of both the number of REMs and REM density. The detailed analyses of each of the five REM periods show that the bulk of the extra REMs for the test group occurred during the fifth REM period. The REM density measure, on the other hand, showed the fourth period to be different for the test and control groups. No other sleep measure reached significance.

TABLE 2. *Sleep characteristics of test and control subjects*

	Test subjects		Control subjects	
Measure	Christmas	$\frac{\text{Pre + Post}}{2}$	Christmas	$\frac{\text{Pre + Post}}{2}$
TIB (min)	427	440	432	437
TST (min)	411	422	422	430
Latency to S1 (min)	8.00	6.95	4.40	5.92
S1 (min)	28.42	23.38	28.75	25.30
S2 (min)	219.61	213.05	234.10	205.62
S3 + S4 (min)	78.10	73.75	62.35	78.40
REM (min)	94.73	91.20	93.60	99.50
% REM	23.62	20.76	22.64	23.26
No. of REM periods	4.25	4.25	5.00	4.45
REM density	9.07*	6.17	6.94	7.48
Total no. of REMs	909.83*	599.50	674.82	692.07
Lat. S2–REM1[a] (min)	84.67	72.05	73.50	78.17
Lat. S2–REM2 (min)	158.91	165.25	163.20	157.95
Lat. S2–REM3 (min)	258.75	251.01	265.60	248.75
Lat. S2–REM4 (min)	344.83	351.13	365.20	348.73
Lat. S2–REM5 (min)	415.10	445.59	448.93	419.84

[a] Lat. S2–REM = latency from stage 2 onset to the REM period onset.

* $p < 0.05$. Statistical differences are both between and within groups.

It was predicted that there would be a very prolonged postlearning increase in REM sleep parameters for test subjects compared to controls and to the test subject's own baseline values. This prediction appears to be generally correct. Even though test subjects were measured anywhere from 3 to 5 days after their last exam, the increase in number of REMs and REM density appeared to be present for all of them. On the other hand, there was no lengthening of the actual time spent in REM sleep.

Only one human study (15) on a single subject is directly comparable to the present data, but results are quite similar. Sleep monitoring was done 1 mo before,

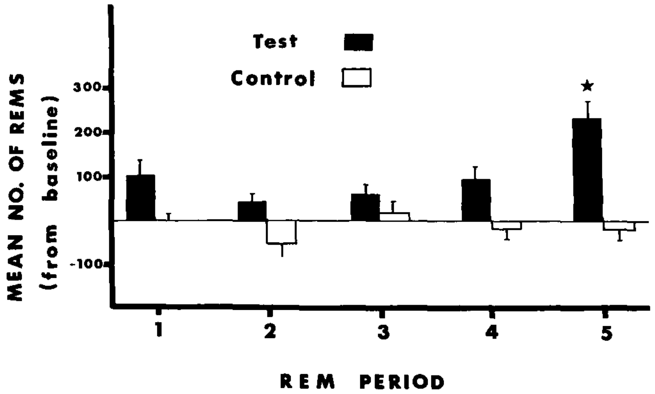

FIG. 2. A detailed examination of the two groups at Christmas (postlearning for the test group), showing mean numbers of REMs compared to the subjects' own pre- and postlearning baseline values for each of the five REM periods (± SEM). Test subjects had significantly higher numbers of REMs than did controls of REM period 5. *$p < 0.05$.

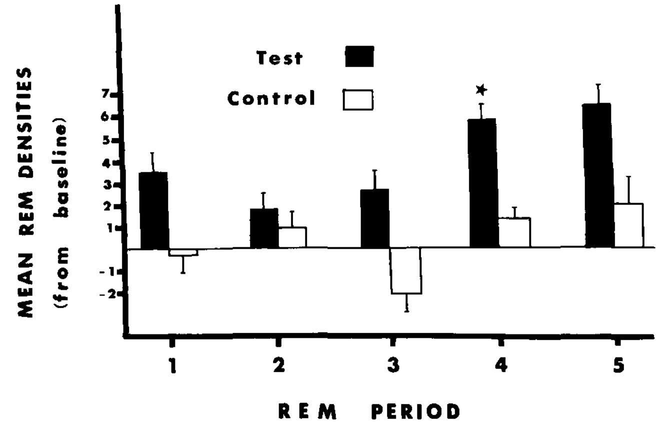

FIG. 3. A detailed examination of the two groups at Christmas (postlearning for the test group), showing mean REM densities compared to the subjects' own pre- and postlearning baseline values for each of the five REM periods (± SEM). Test subjects had significantly higher REM densities than did controls at REM period 4. $^*p < 0.05$.

1 wk before and 1 mo after the end of the doctoral examinations of a 29-yr-old male. Increases in REM sleep time, REM density and % REM sleep were observed both 1 wk before and 1 mo after the exams. There are no other human recording studies that have reported a REMs/REM density increase at times so distant from the last training/learning session.

The parallel between the present human findings and those in animal studies with comparable training–recording times (5,6) is of interest here. Although the two rat studies showed prolonged increases in % REM sleep for many days following the end of training and the present human study did not, one animal study (6) also showed an increase in number of REMs, paralleling the rise in REM sleep.

If it is assumed that the REM sleep parameter increases seen the same day as the training situation (as reported in most studies) are a reflection of the same general process as those seen several days after the end of training (present experiment), then more human studies can be compared with the present data. A number of human studies have reported changes in REM sleep parameters the night immediately following various intellectual/cognitive tasks. Unfortunately, there does not seem to be any obvious factor that would predict whether these effects will take the form of a change in the time spent in REM sleep or a change in the number of REMs within the REM periods or both. One group (8) has reported an increase in the ratio of high-frequency to low-frequency REMs following acquisition of a computer language. Another laboratory (9) has reported that visual learning induced intensification of oculomotor activity as well as REM sleep time increases. On the other hand, these same workers reported that auditory learning did not result in an increase in the number of REMs, although there was an increase in REM time. DeKoninck et al. (10) reported that conversational second language learning resulted in an increase in the relative amount of REM sleep. To complicate the picture, both REM sleep duration and number of REM sleep episodes were increased in a task with both visual and auditory components. However, no REM density increases were reported (11). Where emphasis was on the emotional/stressful aspect of the task and the learning component was minimal, Baekeland et al. (12) reported REM density increases within REM periods following the viewing of an anxiety-producing film. However, Koulack et al. (13) reported a REM density decrease in subjects adapting to a stressful "intelligence" test. In a perceptual–motor visual inversion study, Prevost et al. (14) observed modest increases in REM sleep time as well as reduced REM densities. Thus, present studies do not provide us with any simple pattern of REM sleep changes correlated with obvious variables, such as type of task or emotional component.

The fact that the measure of significance in the present experiment was number of REMs (rather than number of minutes of REM sleep or % REM sleep) presumably bears some relation to the nature of the learning situation. The type of task would probably also dictate when the extra REMs were most prevalent. This would be consistent with the different kinds of REM sleep changes seen in the animal literature, which vary with the strain and type of animal, nature of the task and number of training trials per session (4).

One technique for verifying the REM sleep–learning hypothesis is to apply REMD after task acquisition and then look for relearning–memory deficits. Of course it was not possible to expose students in the present paradigm to REMD. However, several comparable studies have been carried out, imposing REMD many hours after less demanding tasks. One human study has reported that total sleep deprivation 48–72 hr after training in a complex logic task resulted in deficits 1 wk later (19). A partial REMD study has been done in which moderate alcohol consumption followed logic task acquisition. The alcohol induced a significant drop in the amount of REM sleep in the first two REM periods of the night and induced memory deficits of 20–30%. These deficits occurred whether the alcohol was consumed on the same night as the learning task or 48 hr after task acquisition (20). In the only comparable animal study, REMD applied 48–72 hr after the end of a two-way shuttle avoidance task in rats resulted in significant deficits when animals were retested 7 days later (7). Thus, several REMD studies support the idea that the REMs increases observed in the present study represent some kind of learning–memory processing many hours after the end of the learning session.

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