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Mesograde Amnesia During the Sleep Onset Transition: Replication and Electrophysiological Correlates

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Summary: The present study was designed to explore mechanisms of amnesia for meaningful auditory material presented during the sleep onset transition. Thirty undergraduate subjects (17 female, 13 male) were presented with auditory stimuli in an oddball paradigm until sleep onset. Subjects were allowed to accumulate either 30 seconds or 10 minutes of sleep, then awakened and tested on free recall and recognition memory for the meaningful stimuli. After 10 minutes of sleep, but not after 30 seconds of sleep, subjects had profound amnesia on free recall for stimuli presented in the 4-minute window prior to sleep onset. Increased beta electroencephalograph (EEG) power during the sleep period correlated positively with recall of stimuli in the 4-minute presleep window. Event-related potential recordings provided suggestive evidence that subjects continued to process the auditory stimuli to some extent during the sleep onset transition. When allowed to sleep for 10 minutes, subjects evidenced a mixed anterograde and retrograde amnesia for auditory stimuli presented in the 4-minute window prior to sleep onset. The results are discussed in terms of stimulus encoding, consolidation, and retrieval. **Key Words:** Sleep—Memory—Amnesia—Event-related potentials—Sleep onset.

There is mounting evidence that information presented during the presleep day is further processed during subsequent major sleep periods (1–5). However, the ability of subjects to demonstrate, explicitly or implicitly, long-term retention of meaningful auditory stimuli presented during sleep has failed to be shown (e.g. 6–8). The study of memory functioning during the sleep onset transition can directly examine wake versus sleep state changes in memory function (9–12).

Our laboratory conducted a two-phased study specifically examining the issue of memory for auditory stimuli presented immediately prior to sleep onset (13) employing current memory-assessment procedures. In trials where subjects were allowed to sleep for 10 minutes following stimulus presentation, they had nearly complete amnesia for stimuli presented during the 3-minute window prior to sleep onset in contrast with trials where they were allowed to sleep for only 30 seconds. This finding supported a conclusion that retrograde amnesia had taken place, as the difference between the conditions was the amount of sleep that accumulated after the termination of the stimulus presentation. However, a comparison group of awake control subjects showed performance well-above that of the subjects in both sleep conditions, suggesting that the decrease in arousal level occurring during stimulus presentation also contributed to the memory deficits observed. Taken together, these results suggested that central nervous system (CNS) processes both prior to and following electroencephalograph (EEG) sleep onset were related to the degree of memory impairment; anterograde and retrograde amnesia, respectively, had been observed.

Recent advances in the field of psychophysiology have demonstrated the utility of event-related potential (ERP) recordings in the study of human memory performance. The relative amplitude of one long latency or late positive component (LPC), P300 or P3b, elicited to the initial presentation of meaningful verbal stimuli in a rote memory task, has been shown to be highly predictive of later retrieval (14,15). Further, it has been shown that the repetition of previously viewed stimuli during various memory tasks elicits higher amplitude late positive components than does the presentation of new stimuli (16–18). Taken together, these results show the potential of employing ERP recordings to yield electrophysi-

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ological correlates of memory task performance, both during encoding and retrieval of stimuli.

This article reports a conceptual replication and expansion of our earlier work. The protocol has been supplemented with spectral analysis of the sleep EEG, in order to further examine characteristics of poststimulus sleep associated with retrieval failure (retrograde amnesia component). The protocol was also supplemented with ERP recordings during initial stimulus presentation, as an index of on-line cognitive processing (anterograde amnesia component). It was hypothesized that the free-recall findings would be replicated, that on-line processing would appear relatively intact during the presleep stimulus presentation, and that some index of sleep depth would correlate with the strength of amnesia for the presleep stimuli.

METHODS

Subject selection

Thirty undergraduate subjects enrolled in Introductory Psychology courses at the University of Arizona were recruited by a poster advertisement. Inclusion criteria were: 1) English as first or primary language, 2) no self-report of major sleep pathology (i.e. chronic insomnia, sleep apnea, narcolepsy), 3) average reported nocturnal and nap sleep latencies of 20 minutes or less, 4) average reported total sleep time >5 hours and <9 hours, 5) self-reported right handedness, and 6) self-reported interest in participating in a laboratory-based sleep experiment. Exclusion criteria were: 1) any current use of nicotine by any route of administration, 2) average reported weeknight bedtime before 9:00 p.m. or after 1:00 a.m. 3) self-report of past head injury with or without concussion, 4) self-report of hearing or memory deficit, or 5) current use of any over-the-counter or prescription medication possessing stimulant, depressant, or amnestic properties.

Subjects were scheduled for two appointments: one for standardized memory testing (described below) and another on a separate day for the laboratory sleep study (also described below). Subjects were given a research consent form to sign at the first appointment and were asked to conform to the following rules for the 3 days prior to the second appointment (sleep study): 1) no alcohol or illicit drug use, 2) no napping, 3) bedtimes to be between 10:00 p.m. and 1:00 a.m. and 4) waketimes to be between 6:00 a.m. and 9:00 a.m. Subjects were also asked to limit their caffeine intake to the equivalent of 100 mg or less on the morning of the sleep study, prior to 10:00 a.m.

Apparatus

Auditory stimulus material

The target (or rare) auditory stimuli for the study were single-syllable, concrete nouns (e.g. "desk" and "wall") culled from Carol, Davies, and Richman's (19) book of word frequencies. Words were chosen to have moderate to high frequency of occurrence in the normative sample of ninth-graders, the closest normative sample available. Words were divided into nine matched lists that allowed for three presentation–sleep–testing trials per subject; of any three lists, one served as target words to be encoded during the presleep presentation phase and words from the other two lists served as matched distracters to be presented after sleep during the recognition task. The "frequent" auditory stimuli for the presentation phase were 1,000 Hz sine wave tones of 50 msecond duration (rise time and fall time were each 10 msecond).

Audio recording and playback equipment

The word stimuli were recorded onto an IBM-type personal computer, using an AdLib Gold 1000 audio card. Individual word recordings were parsed to be 1,500 mseconds or less and saved as individual files that could be presented in randomized order. All auditory stimuli were presented to the subjects from the personal computer via two small bookshelf speakers located at the headboard of the bed at a distance of approximately 2 feet.

Electrophysiological monitoring equipment

All electrophysiological signals, the auditory stimuli, response button presses, and an event marker (for auditory stimuli) were simultaneously written onto chartpaper by a Grass Instruments Company model 8-16E electroencephalograph (hereafter referred to as the "polygraph") with AC differential amplifiers with low- and high-frequency filters set to 0.3 Hz and 35 Hz, respectively. Electroencephalographic signals were simultaneously digitized at 128 Hz using a Dell 310 personal computer with a data translation DT-2801 A–D board governed by Stellate System's Rhythm software (version 8.0).

Gold-plated, silver disk-type scalp EEG electrodes were placed at sites necessary to conduct both polysomnographic (PSG) and ERP recordings using scalp locations (Fz, C3, Cz, C4, Pz, and O1) measured according to the 10–20 system (20). Gold-plated, silver disk-type electrodes were placed for ground, linked-earlobe reference (A1–A2 to refer-

TABLE 1. *Trial instructions*

• "Now we are ready to begin. I would like you to get into your favorite body position for falling asleep".
Pause for subject to get comfortable
• "I am going to play you some words over the speaker now and ask you to help me set the appropriate volume. The words should be loud enough so you can hear them clearly but quiet enough for you to be able to fall asleep".
Proceed with volume calibration
• "In a few minutes, I will turn out the light and begin playing you words and tones. You should try to listen to them, but you don't need to concentrate on remembering them. Your primary task is just to relax and not to resist the urge to fall asleep".
• "At some point, at which you may or may not be asleep, I will call you over the intercom and ask you some questions. You will answer some of the questions verbally and some with the buttons taped to your thumbs. You won't need the buttons until then".
• "Do you have any questions?"
• "In order for us to get a good recording, please lie quietly, with your eyes closed, and try to fall asleep".
• "Good night".
Lights out
Wait 15 seconds
Proceed with presentation

ence Fz, Cz, and Pz), individual mastoid reference [A1 and A2 to reference horizontal electrooculogram (EOG) and C3, C4, and O1], left and right horizontal EOG, right vertical to horizontal EOG, and bipolar submental electromyogram (EMG). All electrodes were verified to have impedances below 5 K Ohms.

Response press equipment

For obtaining behavioral responses during the recognition memory testing, the subjects had miniature, momentary switches taped to their thumbs in such a position as to be easily pressed by their index fingers. A computer connected to the switches recorded the onset of each stimulus word, the delay until behavioral response, and the accuracy of each response.

Baseline memory assessment

In order to assess the generalizability of the results from this study to the general population and to provide a measure of the subjects' waking explicit-memory performance, all subjects were given the Wechsler memory scale revised [or WMS R (21)] during the week prior to the sleep study as a test of waking memory performance. All WMS-R administrations took place between the hours of 1:00 p.m. and 6:00 p.m., the same time period as the laboratory testing sessions, to control for possible circadian and time of day fluctuations in memory performance and subjective and objective arousal. Baseline memory testing occurred at least 2 days, but not earlier than 1 week, prior to the sleep trials.

Laboratory procedures

Orientation and preparation

On a given study day, the subject arrived at the laboratory at 12:00 p.m. or 3:00 p.m. for orientation and electrode application. At approximately 1:00 p.m. (or 4:00 p.m.), the subject was assisted to the bedroom for the testing session. The subject remained lying in bed in the dimly lit bedroom (<50 lux) for the three presentation–sleep–testing trials, lasting a total of approximately 2 hours.

Pretrial procedures

Following the subject calibrations, the subject gave verbal feedback about the volume of sample auditory presentations. The volume level was adjusted until the subject rated the level as loud enough to hear the words clearly, but quiet enough so that he/she could fall asleep during the stimuli. Then, the subject was read a set of instructions for the trials (Table 1). If there were no questions, the experimenter started all recordings and activated the computer program for the presentation phase of the first trial.

Stimulus presentation

During the presentation phase, the target stimuli were presented at a rate of one target word per 10-second block, yielding a total of six target words per minute. Frequents (tones) were presented four times per 10-second block, yielding a total of 24 frequents per minute. The ordering of the frequents and the target within each 10-second block was randomized by the computer such that infrequent stim-

TABLE 2. *Memory testing instructions*

Free recall
• "Speaking slowly and clearly, please tell me any words you remember hearing during this trial".
Recognition
• "You are now going to hear more words. Some of these you've heard before—others are new. When you hear a word you've heard before, please press the button in your right hand. When you hear a new word, please press the button in your left hand".
• "When responding to the words, please press the buttons as quickly as possible but without sacrificing accuracy".
End
• "OK. You will not hear these words again, so you do not need to remember them".

uli never appeared more than two times in succession.

Each presentation phase was continued until the experimenter observed 15 seconds of continuous sleep EEG on the polygraph. At that point, the subject was allowed to accumulate up to either 30 seconds or 10 minutes of sleep, determined by random selection. Following the sleep period, the experimenter called the subject's name over the intercom loudly and repeatedly until a verbal response was obtained from the subject.

Spectral analysis of the EEG

The EEG data from the 30 second and 10 minute trials were spectrally analyzed in 4-second, nonoverlapping epochs. Sections of the EEG containing muscle or other artifacts, based on a visual inspection on the computer monitor, were rejected from the analyses. Relative spectral power was calculated on the following bandwidths: delta (0.75–2.75 Hz), theta (2.75–7.50 Hz), alpha (7.75–11.75 Hz), sigma (12.00–14.00 Hz), and beta (14.25–30.00 Hz). Analyses were conducted on the sleep EEG from sites C3, C4, O1, Fz, Cz, and Pz. For the 10-minute condition, the spectral analyses were limited to the data from the first 3 minutes of sleep. This time period was selected ad hoc, based on the duration of amnesia observed in previous research (13). Data were eliminated from the analyses if the subject did not have a valid trial of that sleep condition or if sweat artefact skewed the delta band power.

Memory testing

Immediately after obtaining a verbal response from the subject, he/she was asked over the intercom to recall any words heard during the trial (Table 2). After the subject stated that no further words were recallable, the recognition task instructions were given (Table 2).

During the recognition task, target words from the last 5 minutes prior to sleep onset were presented with twice as many matched distracters (1:2 ratio), at a rate of one word per 4 seconds. Subjects were instructed to press the button strapped to their right hand when they heard a word that was presented before (repeated) and to press the button on the left hand after they heard a novel, unpresented word (unrepeated). Subjects were asked to respond as quickly as possible without sacrificing accuracy. Following this task, the subjects were informed that another trial would begin after a brief delay (approximately 1 minute), during which the experimenter reset the computer audio program and EEG acquisition program for another trial. Testing was repeated for a maximum of three trials (presentation–sleep–memory testing). Subjects were then debriefed and dismissed.

RESULTS

Memory performance

Waking memory performance

The means and standard deviations (SD) for the verbal memory and delayed recall indices were 99.43 (11.99) and 106.03 (12.42), respectively. Based on these results, our subjects were representative of the WMS-Rs normative sample (21).

Free-recall task

Free-recall responses were scored as correct when the subject replied with the exact target word or any form of the word (e.g. subject responded "walls" for the target "wall"). Incorrect responses consisted of either the subject failing to free recall a given target word or recalling a word not presented during that trial. Free-recall responses were sorted in terms of proximity to sleep onset, into 1-minute bins corresponding to each of the 5 minutes prior to sleep onset (minute 5 to minute 1). Data were omitted for all subjects (n = 2) who did not have data for both sleep conditions.

In a within-subjects 2 × 5 analysis of variance

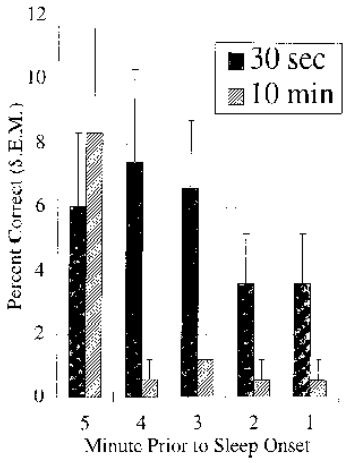

FIG. 1. Free-recall task organized by minute prior to sleep onset. After being allowed to sleep for up to 10 minutes, subjects were nearly completely amnesic for meaningful auditory stimuli presented during the 4-minute window prior to sleep onset. Columns represent the mean percent (SEM) of correct recall of words presented during each of five 1-minute bins prior to sleep onset.

(ANOVA) (sleep condition, minute) there was a significant effect of sleep condition, with subjects recalling significantly more words following 30 seconds of sleep versus 10 minutes of sleep [$F(1,27) = 6.718$, $p = 0.0152$; 30-second mean = 5.25%, 10-minute mean = 2.03%]. Figure 1 shows subjects' recall of words from each of the 5 minutes prior to sleep onset. Thus, these results replicate our earlier findings.

Recognition task: explicit responses

For the recognition task, each target word was presented in a three-word group with two randomly selected distracter words. To control for response bias, an adjusted recognition score for each target word was calculated by subtracting the average recognition score of the matched distracter words from the target-word score. For data analysis, the target-word responses were sorted into 1-minute bins according to the target-words' presleep presentation order (as described above for the free-recall task).

A within-subjects 2 × 5 ANOVA (sleep condition, minute) was conducted on the adjusted recognition data, as explained above. There were no significant effects. However, based on a visual analysis of the data, a further analysis was conducted, limited to the 3-minute window prior to sleep onset. A similar within-subjects 2 × 3 ANOVA (sleep condition, minute) was conducted. There was only a significant main effect of minute, with recognition performance being worse for words presented closer to sleep onset (see means in Table 3). To further explore the recognition data, a within-subjects 2 × 3 ANOVA (sleep condition, minute) was conducted on the data unadjusted for false-positive rate. Again, there was only a significant main effect of minute, as in the adjusted data (see means in Table 3 and Fig. 2 for unadjusted data).

Sleep data

Polysomnography

All sleep data from the 30-second and 10-minute trials were staged in 30-second epochs, based on a central EEG lead, according to standard criteria (*22*). Sleep scoring was performed by a single trained sleep researcher (J.K.W.).

Each of the 30 subjects had at least one 10-minute

TABLE 3. *Memory testing data*

Recognition: main effect of minute

		Percent correct for each minute prior to sleep onset		
		Minute 3	Minute 2	Minute 1
Adjusted data[a]	$F(1,26) = 7.425$; $p = 0.0015$	20.06%	6.79%	5.09%
Unadjusted data	$F(1,26) = 7.564$; $p = 0.0013$	49.69%	39.81%	36.73%

Correlations: relative EEG power (beta)

	EEG sites[b]					
Free recall	Fz	Cz	Pz	C4	C3	O1
Average for all words	$r = 0.451$, $p = 0.0140$	$r = 0.447$, $p = 0.0150$	$r = 0.487$, $p = 0.0074$	$r = 0.525$, $p = 0.0035$	$r = 0.431$, $p = 0.1096$	$r = 0.482$, $p = 0.0081$
4 Minutes prior to sleep	$r = 0.368$, $p = 0.0498$	$r = 0.497$, $p = 0.0061$	$r = 0.559$, $p = 0.0016$	$r = 0.479$, $p = 0.0085$	$r = 0.493$, $p = 0.0066$	$r = 0.523$, $p = 0.0036$

[a] EEG, electroencephalogram. Data adjusted to account for positive endorsement bias.
[b] Scalp locations.

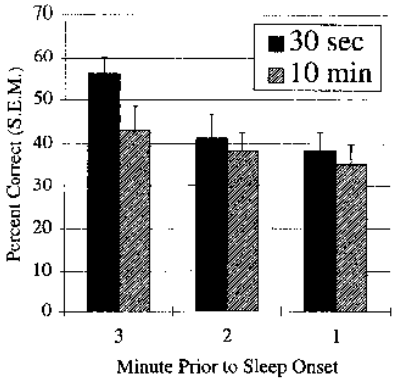

FIG. 2. Recognition task organized by minute prior to sleep onset. In both sleep conditions, subject recognition performance was significantly worse for words presented in closer proximity to sleep onset. Columns represent the mean percent (SEM) of correct recognition of words presented in the three 1-minute bins prior to sleep onset.

sleep trial that met the criterion of at least one-half of the 30-second epochs being scored as sleep. Within the 10-minute sleep condition, averaged across subjects, 17.07% of the epochs were scored as wakefulness. Within the first 3 minutes of sleep, this percentage of epoch scored as wakefulness rose to 25.56%. Within the 30-second sleep condition across subjects, 100% of the single 30-second sleep epochs were scored as sleep.

The average sleep latency for the 30-second trials was 8.8 minutes versus 8.4 minutes for the 10-minute sleep trials, which were not significantly different (paired t test; df = 27, paired t = 1.39, p = 0.176).

Combined results

Polysomnography as a predictor of free-recall performance

As the results of a previous version of this protocol had found a 3 minute window of amnesia in the 10-minute sleep condition, it was hypothesized that effective memory consolidation might require approximately 3 minutes of a sufficiently high level of arousal following the stimuli. We explored the relationship between impaired recall of words presented immediately prior to sleep onset (the 4-minute amnesia window) and the subsequent arousal state during sleep; the percentage of 30-second epochs during the first 3 minutes of the 10-minute sleep period scored as wakefulness were correlated with the recall percentages of words presented in the 4-minute window prior to sleep. A higher percentage of epochs scored as wakefulness was associated with better recall of the words from the window (r = 0.428 p = 0.0182).

EEG spectral power as a predictor of free recall performance

Based on the correlation reported above between wakefulness during the first 3 minutes of the 10-minute sleep period and free-recall performance for words presented in the 4 minutes prior to sleep onset, similar correlations were performed with the spectral EEG data. Only relative power in the beta band, in all EEG sites, correlated significantly with recall from the 4-minute window (Table 3). In all cases, higher relative beta activity was associated with better recall of words from the 4 minutes prior to sleep onset. Also, within subjects, higher relative beta power correlated significantly with free-recall percentage for all words presented in that trial.

Waking memory performance as a predictor of free recall-performance

Only a small minority of subjects were able to recall any words presented during the 4 minutes prior to the 10 minutes of sleep. To assess whether these subjects had recall of material from this period due to above-average waking memory performance, a stepwise regression was conducted. The relevant WMS-R subscale and index scale scores were entered as predictor variables against the percentage of words recalled from the 4 minutes prior to sleep onset. The only variable to enter the regression was delayed verbal-paired associates [$F(1,27)$ = 13.99, R squared = 0.341]. However, the direction of this relationship was counter to expectation; the higher the delayed verbal-paired associates score, the lower the recall of material from the 4 minutes prior to sleep onset. Thus, the data failed to support a conclusion that memory effects observed in the 10-minute sleep condition were related to level of waking memory performance.

Event-related potential data

For event-related potential analyses from the presleep period, 2,400 msecond-epochs including 500 mseconds prior to and 1,900 mseconds following the stimulus onset were examined. Epochs with artefacts were excluded if the average amplitude of any two continuous samples differed from the average amplitude of the entire epoch by 70 μV or more. Epochs were then digitally lowpass filtered [72 point, finite impulse response (FIR) with a half-amplitude

frequency of 7 Hz] and linearly detrended. As the target stimuli were of variable length, the late components varied greatly in latency. Hence, simple averaging of the epoch yielded grand average waveforms that were not well defined and appeared to have latency jitter. In order to correct for this confound, each EEG segment was shifted with a Woody adaptive filter (23) to maximally align epochs based initially on a positive sine wave template potential occurring 500–1,000 mseconds after the stimulus onset and subsequently on the average waveform. A maximum of 15 iterations were conducted, terminating after the change in the correlation coefficient was less than 0.01. To determine the amplitude of the LPC (also known as P300 and P3b), the highest positive potential within the search window on which the template was formed was selected. Late positive component amplitudes of the six-word stimuli per minute were averaged for each of the 5 minutes prior to sleep onset. Similarly, LPC amplitudes for each of the 24-tone stimuli per minute were averaged for each of the 5 minutes prior to sleep onset. All analyses were conducted on ERP data from site Pz, since LPC amplitudes are maximal at Pz.

For the analyses in this and the following sections, data from subjects who did not have a valid trial from both sleep conditions were eliminated from the analyses. Data from subjects who did not have ERP data for all 5 minutes prior to sleep onset were also excluded from the analyses.

In a within-subjects 2 × 2 × 5 ANOVA (sleep condition by stimulus type by minute prior to sleep onset), there was not a significant difference in LPC amplitude for the main effect of sleep condition. This, taken with the nonsignificant interaction of sleep condition with stimulus type suggests that the subjects were processing the auditory stimuli similarly in the trials where they were later allowed either up to 30 seconds or 10 minutes of sleep.

There was a significant main effect of stimulus type [$F(1,18) = 24.804$, $p = 0.0001$], with word stimuli being associated with larger amplitude LPCs than the tones. The main effect of minute was nearly significant [$F(4,72) = 2.419$, $p = 0.0563$], with the stimuli producing larger amplitude LPCs as sleep onset neared. These results suggested that differential cognitive processing of meaningful (words) from nonmeaningful (tones) stimuli continued during the sleep onset transition. These effects are shown in Fig. 3 below.

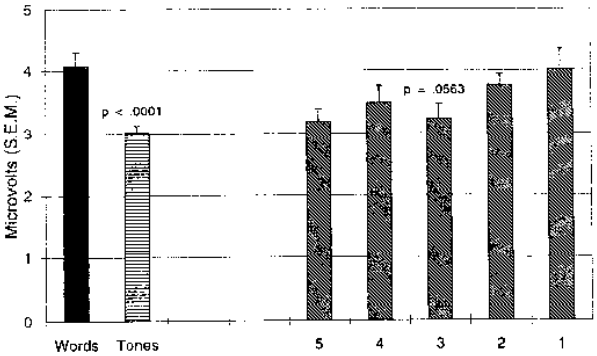

FIG. 3. Late positive component (LPC) amplitudes (SEM) to words and tones (left portion) and to all stimuli as a function of proximity to sleep onset (right portion). Late positive component amplitudes (SEM) elicited to different stimuli during initial stimulus presentation. Meaningful auditory stimuli (words) elicited significantly larger amplitude LPCs than did unmeaningful stimuli (tones). This suggests that subjects continue to differentially process auditory stimuli during the sleep onset transition. The increasing amplitude seen in the LPC component (collapsed across word and tone stimuli) provides further evidence that subjects continued to process auditory stimuli during the transition to sleep.

DISCUSSION

Summary of major findings

The results of the present study replicate earlier findings in this area. It was found in the earlier study that in trials where subjects were allowed to sleep for a 10-minute period following the presentation of auditory stimuli, performance was significantly worse on free-recall and recognition tasks for these stimuli than when tested after being allowed to sleep for up to 30 seconds (13). In the present study, we found that subjects were almost completely amnesic on free recall of words presented during the 4-minute window prior to sleep onset. These results are comparable with our previous results showing a 3-minute window of amnesia. In the present study, the subjects' "yes/no" responses in the recognition task did not replicate our earlier findings. The finding of a difference between the two sleep conditions in the free-recall task in both studies could well be explained by a retrieval problem due to insufficient processing during initial presentation. However, increased retrieval cues provided during the recognition task may have been sufficient to partially compensate for this deficient encoding and could account for the similar results between the two sleep conditions on this task.

Differences in the designs between studies may also have been responsible for the difference in the length of the window of amnesia. This study entailed presenting the subjects with far more stimuli more frequently (one word per 10-second window vs. one pair of words per minute in the previous study). In addition, subjects in the present study were not required to repeat the words aloud. It is possible that this was associated with more shallow encoding of the stimuli. However, in the present study, there was

also evidence in the event-related potential results suggesting that the subjects continued to process the meaningful auditory stimuli, to some extent, up until sleep onset.

There were also significant correlations between the sleep parameters and memory performance within the 10-minute sleep condition. Subjects who were able to recall stimuli during the window of amnesia had more indications of wakefulness during the first 3 minutes of their sleep. Higher relative EEG power in the beta band, which is usually indicative of shallower sleep, was associated with better free-recall performance. Similarly, subjects whose initial sleep period was fragmented, as judged by visual scoring of the polysomnograms, had significantly better free-recall performance.

Stimulus encoding during the sleep onset transition

Arousal level: sleep latency

The sleep latencies in both sleep conditions were not significantly different. This supports a conclusion that the subjects had comparable levels of sleepiness in the 30-second and 10-minute trials. Thus, arousal level during stimulus presentation cannot account for the difference in free-recall performance between the sleep conditions.

ERPs during stimulus presentation

There was not a significant decrease in the amplitude of the LPCs elicited to the meaningful auditory stimuli across the minutes prior to sleep onset. The LPCs elicited by word stimuli were significantly greater in amplitude than those elicited by the tones. These findings provide convergent evidence that the subjects were continuing to differentially process auditory stimuli during the sleep onset transition.

The finding of an overall increase of LPC amplitude during the 5-minute period preceding sleep onset could be interpreted in at least two ways. In keeping with the traditional view of interpretation of increases in LPC amplitude (24), it could be the case that as working memory function decreases near sleep onset, the subjective probability of a relevant stimulus' presentation also decreases. Second, as arousal decreases as sleep onset nears, the momentary perturbation associated with the stimulus increases that stimulus' significance. Either of these possibilities could account for the increase in LPC amplitude. However, increased LPC amplitude could simply be an artefact of the increasing synchronizability that is seen during the sleep onset transition of cortical neurons that underlie the scalp EEG. Hence, increasing spectral power is seen during this period (25) and could be reflected in the amplitude of the LPC if there were phase-locking between it and EEG frequency bands that overlap with the LPC.

Consolidation

Free-recall data

In agreement with previous findings, subjects in this study were nearly completely amnesic for words presented in the 4-minute window prior to sleep onset, when stimulus presentation was followed by a 10-minute sleep period. As described above, there is no evidence that initial encoding deficits alone, or at least deficits in rote memorization, can account for the deficit of free-recall performance in the 10-minute condition versus the 30-second condition.

Delay between presentation and testing: previous findings

Although not specifically addressed in the present investigation, results from a similar protocol (13) supported the conclusion that the difference in free-recall performance between the sleep conditions could not be attributed to the different delays between presentation and testing. In our earlier work, free-recall performance was not significantly different in trials when subjects remained awake for either 30 seconds or 10 minutes following stimulus presentation. Thus, it is unlikely that delay or simple "forgetting" due to elapsed time could account for the impairment observed in the free-recall performance in the 10-minute trials from the present study.

EEG spectral power versus memory performance

Within the 10-minute sleep condition, there were strong positive correlations between relative EEG power in the beta band during the first 3 minutes of sleep and free recall performance. This relationship held for all words presented prior to sleep and for only those words presented during the critical 4-minute window prior to sleep onset. Stated another way, subjects who evidenced a lesser degree of free-recall impairment had more EEG indications of wakefulness during the first part of their sleep period. It could be hypothesized that this higher level of wakefulness allowed for better consolidation of information from short-term to long-term memory.

Sleep fragmentation versus memory performance

Within the 10-minute sleep condition, there were also significant positive correlations between the number of 30-second epochs during the first 3 minutes of sleep scored as awake and subsequent free-recall performance for the words presented in the 4-minute window prior to sleep onset. As with the finding for beta EEG power reported above, this result suggests that lighter or more fragmented sleep allows consolidation to occur. Conversely, deeper or less fragmented sleep that immediately follows the presentation of auditory stimuli is associated with poorer memory for those stimuli. It is important to note that this result and the one discussed immediately prior represent the use of a single-trait (EEG), multiple-method design (EEG spectral power and sleep staging). Further research could benefit from broadening the protocol to measure other indices of arousal and attention, thereby using a multiple-trait, multiple-method design. For example, convergent evidence from measurements of other signs of arousal level (such as galvanic skin response or heart rate change) could strengthen conclusions.

Confounding variables

In order to provide for maximal generalizability of our results, attempts were made to control confounding subject variables. All subjects were screened for negative reported use of medications with known or suspected effects on sleep, alertness, or memory. Subjects were also instructed to abstain from alcohol and illicit drugs for the 3 days prior to the sleep study. Also, subjects were screened to have a negative self-report of head injuries to screen out organic causes of memory or sleep abnormalities. Further, our sample appeared representative of their age group's normative sample on a standard neuropsychological memory-assessment device.

As with many physiological and endocrine functions, short-term memory has been shown to follow a circadian pattern. Short-term memory performance has been shown to be at average daytime levels during the time window the subjects slept in the present study and to decline in successive hours (26). This makes the memory deficits observed in this study more impressive, as they occurred during the time of day when short-term memory has been shown to be relatively intact. One could hypothesize that further research utilizing the present protocol at nocturnal sleep onset might find even greater memory deficits due to time of day or circadian effects on short-term memory.

Conclusions

In intact humans, memory can fail for a variety of reasons. Complete failure to encode information can occur as a result of traumatic brain injury or with the use of certain surgical anesthetics (27,28). Encoding can also be shallow during situations where the subject is either hyper- or hypoaroused. The memory consolidation process itself can also be blocked, keeping information from being transferred from short-term memory to permanent or long-term memory. Additionally, various factors can interfere with the process by which information is retrieved from long-term memory. The memory deficit observed in the present study could be attributed to poor use of encoding strategies, impaired transfer of information from short-term to long-term memory, retrieval deficits, or a combination of the three factors.

With regard to the issue of memory consolidation, the factor best addressed by this study, several explanations are possible. It could be that memory consolidation was impaired in a graded fashion as a function of arousal level. In this way, the functioning of consolidation could be viewed along a continuum between fully awake/intact consolidation and deep sleep/no consolidation. Alternatively, memory consolidation may have been blocked after arousal level fell below a certain critical threshold level. This threshold may have occurred during the sleep onset transition, at sleep onset, or at a certain depth of sleep. Other research utilizing FFT and ERP measures found mixed evidence of gradual increases in delta, theta, and sigma power values during the sleep onset transition, with an additional abrupt increase in power in those bands at the onset of sleep itself. Increased amplitude of the late ERP components was also seen at sleep onset in that protocol (25). These data support the conclusion that arousal level during initial presentation may be decreasing concurrent with increasingly synchronized scalp EEG activity, first in a gradual fashion and later more rapidly at actual EEG sleep onset.

The linear correlations between beta EEG power while asleep and subsequent recall of presleep information support the arousal continuum hypothesis. Previous findings that memory performance is worse following just 30 seconds of sleep versus 30 seconds of wakefulness suggest that the downward slope of the function begins prior to sleep onset, as many researchers would not consider 30 seconds of sleep to be a "true" sleep onset. Support for the threshold hypothesis comes from the correlation observed between the percentage of awake epochs in the first few minutes of sleep and free recall for the stimuli

presented in the 4-minute window prior to sleep onset. It could be the case that a certain duration of unfragmented sleep is required to completely block memory consolidation from occurring. Further research could vary the length of the sleep conditions to look for a dose–response relationship to memory impairment.

These two hypotheses—the continuum of arousal and consolidation hypotheses—could also be described as anterograde and retrograde amnesia, respectively. In keeping with the presleep arousal continuum hypothesis, anterograde amnesia would be said to have occurred; while subjects were still awake but below a critical threshold, it is likely that encoding and consolidation processes were impaired. The alternative hypothesis of retrograde amnesia states that a certain depth and/or duration of sleep is necessary to block consolidation of material presented prior to sleep onset. As the results of this study and its predecessor (13) provide support for both hypotheses, a new term is proposed to describe the complex nature of this amnesia—"mesograde amnesia".

The concept introduced here, mesograde amnesia, encompasses all aspects of normal memory functioning—encoding, consolidation, and retrieval. Several factors can lead to deficient encoding, including impaired attention and concentration, both of which might be mediated by arousal level. Arousal level likely decreases in a gradual function during the sleep onset transition, as the midbrain and forebrain receive less activation from the brainstem reticular activating system. Similarly, consolidation (beyond short-term or working memory) of ongoing stimuli might require a sufficient duration and level of arousal. Finally, if stimuli receive poor encoding and consolidation is weakened or blocked due to an insufficient level of arousal, then subsequent spontaneous retrieval would be nearly impossible.

In addition to studying the normal changes in memory function during the sleep onset transition, the present protocol could be utilized to examine memory dysfunction associated with the "automatic behavior" seen in two disorders associated with excessive daytime sleepiness: narcolepsy and severe obstructive sleep apnea. Furthermore, the finding of increased beta activity in association with better recall of information presented during the transition to sleep could be studied in association with the finding of increased beta activity in insomniacs during transitional sleep (29), perhaps providing insight into the overestimation of sleep latency seen in many insomniacs.

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