REM Sleep Deprivation Suppresses Acquisition of Classical Eyeblink Conditioning

Hiromi Ohno; Ryo Urushihara; Hiroyoshi Sei; and Yusuke Morita

Department of Physiology, School of Medicine, The University of Tokushima, Tokushima 770-8503, Japan

**Study objectives:** The aim of this study was to investigate the issue of whether REM sleep is involved in implicit learning through the cerebellum-related neural circuit via the use of classical eyelink conditioning (CEC).

**Design:** Subjects were divided into three groups: control (sleep without interruption), REM sleep deprivation (RD), and slow wave sleep (stage 3+4) deprivation (SD). The CEC was performed after 8 hours of ordinary nocturnal sleep or sleep disrupted at a selected sleep stage.

**Setting:** A university-based sleep laboratory.

**Patients or Participants:** Twenty-seven healthy volunteers (all men, aged 23.2±0.6 years).

**Interventions:** The CEC was measured after selective sleep deprivation or ordinary nocturnal sleep.

**Measurements and Results:** The eyelink reflex was conditioned using a classical delay conditioning paradigm. The conditioned response (CR) was determined by electromyography measurements of the orbicularis oculi muscles. The rate of appearance of the CR was compared among the three groups. Compared with the control subjects, RD subjects were significantly deficient in their capacity to acquire conditioned eyelinks, while no difference was found among the SD subjects.

**Conclusions:** This study suggests that RD suppresses the cerebellar function in CEC and that REM sleep is closely linked with the learning function in the cerebellum.

**Keywords:** sleep deprivation, classical eyelink conditioning, cerebellum, REM sleep, slow wave sleep, human

**INTRODUCTION**

RAPID EYE MOVEMENT (REM) SLEEP IS ASSOCIATED WITH MEMORY. In the studies dealing with acquisition process of memory, it has been reported that REM sleep deprivation (RD) produces a slower acquisition in some learning paradigms in animals. On the other hand, in the studies dealing with consolidation process of memory, REM sleep increases after training in learning tasks, such as a complex bar-press operand. and RD following learning is specifically deleterious to memory in both animals and humans. Although there are two distinct types of long term memory; implicit and explicit, this is particularly true for implicit learning, which is strongly dependent on REM sleep. Early nocturnal sleep, which is dominated by slow wave sleep (SWS), facilitates the consolidation of declarative (explicit) memory, whereas late nocturnal sleep, dominated by REM sleep, facilitates the consolidation of nondeclarative (implicit) memory in humans.

Implicit learning can stem from performing a repetitive task, such as learning to type, play the piano, or ride a bicycle. Amnesiac patients with hippocampal damage, which is associated with deficits in explicit learning, have been found to repeatedly perform the above-mentioned tasks as successfully as healthy controls. On the other hand, patients with cerebellar dysfunction, whose explicit learning is normal, are impaired in associative motor learning, which is classified as implicit learning. Evidence from studies in humans indicates that the cerebellar circuitry, which serves as the substrate for CEC, is similar to that in nonhuman primates. To date, a considerable body of evidence has accumulated to show that patients with cerebellar cortical or deep nuclear lesions are severely impaired in CEC. Brain imaging techniques have demonstrated that the human cerebellum is activated during the acquisition of the classical conditioned responses. Therefore, it is generally thought that the cerebellum is closely involved in acquisition of CEC in humans but that its retention is not.

Based on these earlier studies, we hypothesize that REM sleep is related to CEC through the cerebellum-related neural circuit and, to test this hypothesis, observed the effect of RD on its acquisition.

**METHODS**

Twenty-seven healthy volunteers (all men, aged 23.2±0.6 years) with no history of neurologic or chronic illness were studied after giving their informed consent. These subjects were divided into three groups: control (sleep without interruption), RD, and SWS (stage 3+4) deprivation (SD). The CECs were obtained after 8 hours of ordinary nocturnal sleep or sleep disrupted at a selective sleep stage.

It has been reported that some healthy subjects can acquire the conditioning of an eyelink reflex with difficulty. Fourteen subjects who did not acquire the conditioned response on the experimental day were tested again after 1 month. Nine of the 14 subjects did not acquire the conditioned response again (non-learners) and were omitted from further analysis in the present study (control: n=3, RD: n=2, SD: n=4). As a result, data based on 18 subjects were used (control: n=6, RD: n=6, SD: n=6).

**Sleep recordings**

The subjects were asked to arrive at the sleep laboratory 1 hour prior to their usual bedtime, and they slept in a soundproof and electrically shielded room for 1 night. On the experimental day, they were obliged to abstain from naps and alcoholic beverages during the day. Sleep was monitored using a computerized polysomnographic system.
Classical Eyeblink Conditioning

The eyeblink reflex was conditioned using a classical delay conditioning paradigm. The subjects were allowed to lie in bed comfortably with their eyes open and were instructed to relax during the session. The unconditioned stimulus (UCS) consisted of an electrical square-wave stimulus, 0.5 milliseconds in duration, delivered to the supraorbital nerve via conventional cutaneous surface electrodes with the cathode placed over the supraorbital foramen. The stimulus intensity was increased gradually until a stable eyeblink reflex was obtained with tolerable discomfort. The conditioned stimulus (CS) consisted of an acoustic signal 1kHz in frequency and 400 milliseconds in duration, applied through earphones simultaneously to both ears at an intensity of 60 dB (sound-pressure level). The stimulus protocol started with 5 UCS (UCS-alone trials) and 5 CS (CS-alone trials) in order to document their independence and the neutrality of the CS. These were followed by 64 CS-UCS paired trials (conditioning phase) and 10 CS-alone trials (extinction phase). The paired stimulation (CS-UCS) consisted of CS with a duration of 400 milliseconds and the UCS that was delivered just at the end of the CS (Figure 1). The time interval between the trials varied between 15 and 20 seconds in order to avoid any anticipation.

The eyeblink EMG responses were recorded using Ag/AgCl surface electrodes attached to the orbicularis oculi muscles. The ground electrode was placed around the left wrist. The EMG signals were amplified and filtered with a time constant of 0.01 second, without a highcut filter, through a bioamplifier (AB-622M, Nihon Koden, Japan). The signals were digitized and displayed on the monitor of the data acquisition system (MacLab System, ADInstruments, Australia) and then stored on the computer hard disk for further off-line analysis. The records were collected from 400 milliseconds before CS onset and to 200 milliseconds after UCS onset (sampling rate of 1000 Hz). The digitized EMG signals were rectified and low-pass filtered using a 5-point moving average method.

During data analysis, responses in each trial were inspected for the presence of a conditioned response (CR). For that purpose, the mean value (BLmean) and standard deviation (BLSD) of the baseline EMG amplitude (the first 365 milliseconds: Figure 1) were calculated for each trial. In the next step, the mean value of the CR EMG amplitude (CRmean) was estimated. The time window for the detection of the CR was from 250 milliseconds after the onset of the CS to the onset of the UCS. The CRs were determined when the CRmean was greater than the BLmean plus 2.5 times the BLSD of a particular trial. If the algorithm...
detected spontaneous eyeblinks during the baseline, these BLmeans were excluded from the calculation. In this case, the average of the BLmeans of the preceding and following trials was taken. A response occurring within 250 milliseconds after the onset of a CS was classified as an alpha-response to the CS (Fig. 1). The percentage of CRs out of the total valid trials per block was calculated for consecutive blocks of 8 trials. The CR incidence data were pooled within each experimental group, and group CR incidence plots were then constructed.

The rate of appearance of the alpha-response was compared among the three groups. The alpha-response was detected during 150 milliseconds periods after the onset of CS in the CS-only phase, by a similar method to that in which the CR was detected in each trial. The EMG amplitude of the UCR calculated for the mean value of the UCR amplitude was divided by the mean value plus 2.5 times the SD of the baseline amplitude for each trial. The UCR mean was calculated for 100 milliseconds after the onset of UCS in the UCS-only phase, and the baseline mean was calculated during the first 365 milliseconds as in the detection of the CR.

The grouped data were statistically analyzed using the StatView (Abacus Concepts, Inc., CA) software program. The data from the classical conditioning experiments were analyzed using analysis of variance (ANOVA) for repeated measures implementing factors of the experimental group and the blocks of trials. The results of the sleep data were analyzed using ANOVA among the three groups. The same type of data analysis was performed for the results relative to the alpha-response, UCR amplitude, and UCS threshold. When significant differences were found for the groups, Fisher posthoc analyses were conducted. The hypothesis rejection level for all tests was p<0.05.

RESULTS

Sleep

The total sleep time was not significantly different among the three groups (RD: 345.3±26.7, SD: 420.2±10.6, control: 387.2±89.6 min; F=1.950, p=0.177, df=2,15). As compared with the control group, the RD group had a shorter and the SD group had a longer sleep time. But there were no significant differences (RD vs control: F=0.855, p=0.377, df=1,10; SD vs control: F=0.750, p=0.407, df=1,10). Figure 2 shows the sleep-stage distribution for each group. The REM sleep time in the RD group and the SWS time in the SD group were nearly completely inhibited, as expected (REMS: F=19.528, p<0.01, df=2,15; SWS: F=19.733, p<0.01, df=2,15). The RD group had significantly longer waking periods than did the SD group (p<0.05). The subjective sleepiness, evaluated just before the CEC, had no significant difference among the three groups (RD: 4.53±0.49, SD: 4.22±0.45 points, F=1.089, p=0.362, df=2,15). The RD group and SD group had a higher subjective sleepiness than did the control group, but the differences were not significant (RD vs control: F=1.498, p=0.249, df=1,10; SD vs control: F=1.139, p=0.311, df=1,10)

Classical eyeblink conditioning

Figure 3 displays the percentage of CRs for each group across the 64 acquisition trials, with the mean percentage of CRs broken down into 8-trial blocks to illustrate group differences in CR acquisition. The RD group had a significantly lower percentage of CRs than did the control group during the eyeblink conditioning phase (F=7.320, p<0.05, df=1,10). The SD group had no difference in CRs compared with the control groups (F=0.447, p=0.519, df=1,10) and had a higher percentage of CRs than did the RD group, although it did not reach a significant level (F=2.601, p=0.137, df=1,10). The RD group had a lower total average of 28.9±3.8% CRs compared to 60.4±4.8% CRs for the control group and 50.3±5.2% CRs for the SD group. The three groups ANOVA did not reach a significant level (F=2.864, p=0.088, df=2,15).

Figure 4 shows the effects of sleep deprivation on the sensory and motor processes, as these relate to eyeblink conditioning. The UCR (the reflex eyeblink to the UCS) amplitude was compared among the three groups to inspect the motor processes associated with eyeblink (Figure 4A). There was no significant difference among the RD groups ANOVA: F=0.024, p=0.977, df=2,15; RD vs control: F=0.051, p=0.825, df=1,10; SD vs control: F=0.023, p=0.882, df=1,10). The UCR (the reflex eyeblink to the UCS) amplitude was compared among the three groups to inspect the sensory processes associated with eyeblink (Figure 4B). There was no significant difference among the three groups (sensory; Figure 4C; three groups ANOVA: F=2.196, p=0.146, df=2,15; RD vs control: F=3.433, p=0.094, df=1,10; SD vs control: F=0.310, p=0.590, df=1,10). Furthermore, the appearance of the alpha-response (unconditioned eyeblink to CS) also showed no significant difference among the three groups (control: F=0.935, p=0.414, df=2,15; RD vs control: F=0.233, p=0.640, df=1,10; SD vs control: F=0.723, p=0.415, df=1,10).

DISCUSSION

In the present study, RD subjects were deficient in their capacity to acquire conditioned eyeblinks to the tone CS. This contrasted with the rapid acquisition in the case of control subjects. In previous studies dealing with RD and learning, it has been reported that RD produces a slower acquisition in Y-maze discrimination in cats,1 and in the passive...
avoidance, active avoidance, and an appetite alternation discrimination in rats. The findings in the present study suggest that, in humans, RD is deleterious to the acquisition of a CEC, one of the implicit learned tasks. This is the first report showing that REM sleep is closely related to not only consolidation, but also to the acquisition of implicit learning in humans.

No difference among the three groups was observed in the subjective sleepiness questionnaire administered before performing CEC. We, therefore, conclude that the present results were not caused by a change in consciousness level. Although no significant differences in sleep time were observed among the three groups, the SD group acquired 22% more sleep than did the RD group. In order to determine the effect of sleep length on the acquisition of CR, coefficients of correlation were calculated between total sleep time and the percent CRs. No correlation was found for either the initial RD group with non-learners or the final RD group without non-learners (initial RD group: R^2=0.178, p=0.297, n=8; final RD group: R^2=0.106, p=0.529, n=6). Furthermore, the SD group that spent the longest sleep time had almost the same subjective sleepiness as did the RD group. We, therefore, conclude that the difference in total sleep time does not have a significant effect on the consciousness level and on the acquisition of CEC. The UCS threshold and the appearance of alpha-response (unconditioned eyelink to CS) was essentially the same among the three groups, indicating that RD had no effect on the sensory processes of UCS and CS. The UCR amplitude (reflex eyelink to the UCS) also did not differ significantly among the three groups, indicating that RD had no effect on the motor processes of the eyelink. It is, therefore, considered that the suppressed acquisition of the conditioned eyelink is due not to an impairment in the performance of the afferent or efferent processes of the eyelinking conditioning but, rather, to a suppressed cerebellar function associated with eyelinking conditioning.

Extensive animal and human studies indicate that cerebellar involvement is essential to eyelinking conditioning, and mechanisms for this have been proposed, as follows. Cells of the deep nuclei provide the sole output of the cerebellum. The sole output of the cerebellar cortex is the inhibitory Purkinje cell, which synapses in the cerebellar nuclei. Moreover, this output is influenced by two input types, climbing fibers and mossy fibers synapse in both the deep nuclei and Purkinje cells, which display quite different characteristics. The CS reaches the cerebellum via mossy fibers and the UCS via climbing fibers, contacting both the deep nuclei and Purkinje cells. The output of the cerebellar deep nuclei is required for CR expression. The formation of a CR is mediated by long-term depression (LTD) at the site of the mossy fiber-Purkinje cell synapse and by long-term potentiation (LTP) at the mossy fiber-deep nuclei synapse. A paired presentation of the CS and UCS is considered to induce an LTD at the mossy fiber-Purkinje cell synapse by the conjunctive activation of mossy fibers and climbing fibers. The LTD induces a decrease in Purkinje cell activity, disinhibiting the deep nucleus cells and then eliciting the conditioned eyelink response. With further training, the mossy fiber-deep nuclei synapse would undergo LTD due to the decreased Purkinje cell input during the tone. The LTP at a mossy fiber-deep nuclei synapse increases the deep nucleus cell activity, and encodes a robust eyelink response.

The LTD at the mossy fiber-Purkinje cell synapse can be generated by various bioactive substances. It is hypothesized that RD may alter the metabolism of such substances that are involved in the synaptic plasticity, thus affecting eyelink conditionining. Brain-derived neurotrophic factor (BDNF) has been reported to be closely related to eyelink conditionining in the cerebellum. Mutant mice with a cerebellar BDNF deficiency are greatly impaired in CEC. On the other hand, the protein level in the cerebellum may be decreased by selective RD in rat. Therefore, it is possible that the decrease of the cerebellar BDNF by the selective RD inhibits eyelink conditionining.

On the other hand, Mallick’s group reported, in rats, that RD altered monoamine oxidase-A (MAOA) activity, hexokinase activity and calcium concentrations in the cerebellum. First, after 4 days of RD, a significant decrease in MAOA activity in the cerebellum was detected. However, it has been reported that MAOA-deficient mice are able to acquire CEC at normal levels. Therefore, it is unlikely that a decrease in MAOA activity by RD would effect the results. Second, hexokinase activity, a key enzyme involved in glucose metabolism, increased after 4 days of RD. The increase in hexokinase activity induced by RD indicates an increased expenditure of energy during RD. There is a possibility that the increased energy expenditure during RD may be related to the lower acquisition of CEC in the RD group. Finally, total calcium levels decreased after 4 days of RD. Modulation of neuronal excitability, firing rate, and nerve-impulse conduction due to changes in calcium are well documented. Moreover, it has been reported that intracellular calcium plays an important role in memory processing. Therefore, it is possible that a decrease in calcium level may also affect the mechanism of eyelink conditioning in the cerebellum.

Three previous studies in rats suggest a close relationship between the neural development of the cerebellum, eyelink conditionining, and REM sleep. First, percent duration of REM sleep dramatically decreases at 15 to 25 days after birth, from 70% to 20%, which is equal to the normal adult level. Second, BDNF protein and its mRNA level reach the adult level 14 to 21 days after birth. Third, eyelink conditionining can be acquired 17 to 24 days after birth. The ontogenetic changes in REM sleep duration, the BDNF protein and its mRNA level, and the acquisition of the CEC occur at almost the same time. Further studies on the metabolism of the substances such as BDNF in the cerebellum during normal REM sleep periods or after RD will be required to fully understand the relationship between REM sleep and cerebellar function in learning.

Although it was not statistically significant, the amplitude of UCR following RD was half that of the control UCR (Figure 4B). The suppressed amplitude had no effect on the detection of the CRs, because the amplitude of the UCR in the RD group was higher by 2.5-fold of the BLmean of the UCR amplitude had no effect on the detection of the CRs, because the amplitude of the UCR in the RD group was higher by 2.5-fold of the BLmean. It is, however, possible that RD may suppress the activity of the motor system and then affect the appearance of the CR. Additional research will be needed to clarify the relationship between RD and motor function.

In conclusion, RD suppresses the acquisition of the conditioned eyelink. This finding suggests that RD suppresses the cerebellar function in CEC and that REM sleep is closely linked with the learning function in the cerebellum.

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REFERENCES

2. Stern WC. Acquisition impairments following rapid eye movement sleep deprivation in rats. Physiol Behav 1971; 7: 345-52.


15. Bracha V, Zhao L, Wunderlich DA, Morrissy SJ, Bloedel JR. Patients with cerebellar lesions cannot acquire but are able to retain conditioned eyelid reflexes. Brain 1997; 120: 1401-13.


32. Kim JJ, Shih JC, Chen K, et al. Selective enhancement of emotion-