

Sleep Deprivation Impairs Long-Term Potentiation in Rat Hippocampal Slices

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Received 23 October 2001; accepted in final form 24 April 2002

Campbell, I. G., M. J. Guinan, and J. M. Horowitz. Sleep deprivation impairs long-term potentiation in rat hippocampal slices. *J Neurophysiol* 88: 1073–1076, 2002; 10.1152/jn.00873.2001. To determine if 12-h sleep deprivation disrupts neural plasticity, we compared long-term potentiation (LTP) in five sleep-deprived and five control rats. Thirty minutes after tetanus population spike amplitude increased $101 \pm 15\%$ in 16 slices from sleep deprived rats and $139 \pm 14\%$ in 14 slices from control rats. This significant ($P < 0.05$) reduction of LTP, the first demonstration that the sleep deprivation protocol impairs plasticity in adult rats, may be due to several factors. Reduced LTP may indicate that sleep provides a period of recuperation for cellular processes underlying neural plasticity. Alternatively, the stress of sleep deprivation, as indicated by elevated blood corticosterone levels, or other non-sleep-specific factors of deprivation may contribute to the LTP reduction.

end of the light period (prior to lights off) and decapitated within 5 min of removal. Control rats were also decapitated at the end of a 12-h light period.

Slice preparation

The brain was removed and chilled for 2 min in 2°C artificial cerebral spinal fluid (ACSF) containing the following (in mM): 125 NaCl; 3.5 KCl; 2.0 CaCl₂; 1.25 NaH₂PO₄; 2 MgSO₄; 26 NaHCO₃; 10 dextrose. Hippocampi were sectioned (450- μ m slices) and incubated in ACSF aerated with 95% O₂-5% CO₂ for a minimum of 90 min before being transferred to a recording chamber perfused with 95% O₂-5% CO₂ gassed ACSF at $28 \pm 0.2^\circ\text{C}$ for 30-min equilibration prior to recording.

Population spike recording

To assess overall changes in hippocampal plasticity (synaptic changes and changes in coupling between synaptic events and action potential generation), population spikes from CA1, evoked by stimulation of Schaffer collaterals, were recorded and averaged. The test stimulus intensity was adjusted to evoke a 1/3 maximal response. Subsequent records of population spike amplitude were made by averaging five evoked responses with an inter-stimulus interval of 5 s (a trial). After obtaining a stable response level, LTP was induced by giving three tetanic stimulus trains (0.1 ms, 100 pulses/s for 1 s) at twice the test stimulus intensity at 1-min intervals. The stimulus was then returned to the pretetanus test level and averaging trials every 5 min were resumed until 45 min after tetanus.

Statistical analysis

For each slice, the response to a stimulus 30-min posttetanus (T_2 on Fig. 1A) was expressed as percentage increase in population spike amplitude above the mean pretetanus response (T_1 on Fig. 1A). An animal mean was determined for each rat by averaging percentage increase for all slices (3 slices in 8 rats, 2 slices in 1 rat, and 4 slices in 1 rat). The treatments were on individual animals rather than individual slices; therefore, differences between control and sleep-deprivation treatments were evaluated with Mann-Whitney *U*-tests conducted on animal means. However, we also present slice data as are common for LTP experiments.

Corticosterone assay

In six animals, three control and three sleep deprived, serum obtained from trunk blood collected at the time of decapitation was

INTRODUCTION

Homeostatic models of slow wave sleep propose that sleep serves a recuperative function for the brain (Borbely 1982; Feinberg 1974). Moruzzi (1966) suggested that the restorative processes of sleep provide recovery specifically from the plastic activities of waking. One prediction based on Moruzzi's proposal is that sleep deprivation should impair plasticity. Long-term potentiation (LTP) of hippocampal synapses is a form of plasticity that has been implicated as a cellular mechanism of memory (Malenka and Nicoll 1999). As a test of whether sleep deprivation disrupts plasticity, we determined if LTP is impaired in hippocampal slices from sleep-deprived rats.

METHODS

Ten male Sprague-Dawley rats, 8 to 9 mo old, were individually housed in a controlled environment (20–22°C; 12-h light:dark on a reversed light cycle) for ≥ 2 wk prior to recording. The UC Davis Animal Use and Care Administrative Advisory Committee approved all protocols and procedures.

Sleep deprivation

Rats were deprived of sleep via forced locomotion in a slowly (1.333 rpm) rotating drum (Tobler and Borbely 1986). Rats had free access to food and water while in the drum. All 10 rats were trained to the sleep deprivation device in successive sessions of 30-, 45-, and 60-min duration. Five of the 10 rats were individually placed in the deprivation device for the entire 12-h light period, the typical rest period for rats. Rats were removed from the deprivation device at the

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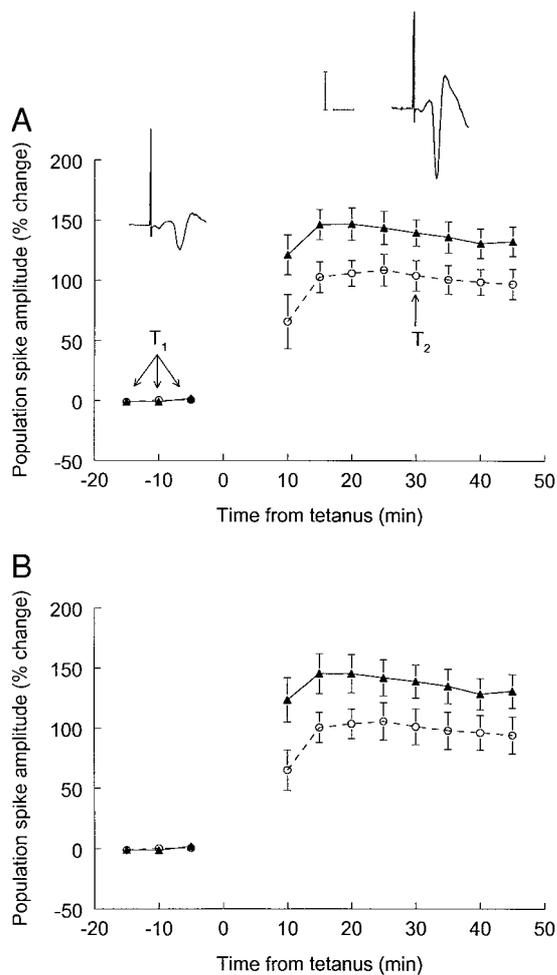


FIG. 1. Mean population spike amplitudes (standardized as a percentage change from mean pretetanus amplitude, T_1) are plotted against time relative to tetanizing stimulus. For both animal (A) and slice (B) means, the magnitude of the potentiation of population spike amplitude was reduced in sleep-deprived (open circles, dotted line) vs. control (filled triangles, solid line) animals. Insets at top show typical pretetanus and 30-min posttetanus (T_2) population spikes from a single slice. Vertical and horizontal calibration bars on insets indicate 1 mV and 1 ms, respectively.

shipped to Vanderbilt University DRTC Hormone Assay Core Lab for determination of corticosterone levels with radioimmunoassay.

RESULTS

LTP of population spike amplitude was impaired in sleep-deprived rats (Fig. 1A). At 30-min posttetanus the average (mean \pm SE) potentiation in control rats ($140 \pm 11\%$) significantly ($U = 21$, one-tailed $P = 0.038$) exceeded that in sleep-deprived rats ($104 \pm 13\%$). Treating each slice as an independent trial yielded similar results (Fig. 1B). Mean potentiation in slices from control rats ($139 \pm 14\%$, $n = 14$) significantly ($U = 162$, one-tailed $P = 0.019$) exceeded that in slices from sleep-deprived rats ($101 \pm 15\%$, $n = 16$).

The pretetanus population spike amplitude relative to maximum response amplitude can affect the magnitude of potentiation. The similarity of pretetanus response amplitude in slices from control rats ($37 \pm 2\%$ of maximum response amplitude) and sleep-deprived rats ($35 \pm 2\%$) confirms that this parameter was adequately controlled.

Corticosterone levels in sleep-deprived (37.5 ± 1.2 mg/dL) significantly ($t = 3.69$, one-sided $P = 0.03$) exceeded that in control (19.1 ± 4.9 mg/dL) animals.

DISCUSSION

Sleep deprivation, which has been one of the major tools in the study of sleep, impacts many physiologic processes. LTP is the major cellular model for plasticity and may be related to learning and memory (Malenka and Nicoll 1999). We show here that the sleep-deprivation protocol reduces plasticity as measured by LTP in rat hippocampal slices. Sleep deprivation may prevent sleep-dependent recuperation of plasticity. Alternatively the plasticity reduction we recorded may be related to a nonspecific effect of sleep deprivation such as stress.

Among the numerous physiological processes impaired by sleep deprivation, memory consolidation, cognitive performance, and learning may be related to our finding of reduced plasticity. Numerous studies have found that memory consolidation is disrupted following either rapid eye movement (REM) or nonrapid eye movement (NREM) sleep deprivation (Smith 1995; Gais et al. 2000); however, our results may more directly bear on effects of sleep deprivation on subsequent learning. Horne (1988) found that divergent thinking tasks that require creativity and flexibility are particularly sensitive to sleep deprivation. Sleep deprivation diminishes performance on neuropsychological tests of prefrontal cortex function, including tests that involve working memory (Harrison et al. 2000; Thomas et al. 2000). In rats, REM sleep deprivation impairs spatial reference memory, which is associated with the hippocampus (Youngblood et al. 1997). Reduced plasticity, apparent in our studies as diminished LTP, may be at the root of these sleep deprivation induced cognitive impairments.

The most dramatic effect of sleep deprivation is the alteration of subsequent sleep and EEG within subsequent sleep. The large increase in slow wave intensity following sleep deprivation has led to homeostatic models of slow wave sleep which propose that sleep serves a recuperative function for the brain and that the intensity of the recuperation process is reflected in the intensity of NREM delta electroencephalograph (EEG) (Borbely 1982; Feinberg 1974). Rats deprived of sleep with forced locomotion even for a 12-h dark period show a large increase in slow wave EEG during NREM sleep (Tobler and Borbely 1986), indicating that the 12-h sleep deprivation used here was sufficient to increase the need for recuperation.

One homeostatic model proposes that sleep provides recuperation specifically to plastic areas of the brain. This hypothesis regarding restoration of plasticity was until recently supported only by indirect evidence such as parallel ontogenic changes in sleep EEG and plasticity. Total sleep time and the intensity of NREM slow wave EEG decrease dramatically across late childhood and adolescence (Feinberg et al. 1990) as do many aspects of brain plasticity. Further indirect evidence is provided by the huge increase in NREM delta intensity following elevation of hippocampal metabolism by MK-801 or ketamine (Campbell and Feinberg 1996). The current finding on sleep deprivation impairment of LTP and Frank et al.'s (2001) finding on the sleep-related enhancement of plasticity related to monocular deprivation during visual system development begin to add direct evidence for a role for sleep in recuperation of plasticity. It should be noted that specific REM

sleep deprivation has been shown to exacerbate rather than block plastic changes resulting from monocular deprivation during development (Oksenberg et al. 1996).

Our data showing that sleep deprivation reduces hippocampal LTP in adult rats complement the study by Frank et al. (2001), which proposed that sleep enhances plasticity in the developing visual cortex of young rats. Both studies are consistent with the proposal that molecules critical to plasticity may be exhausted during waking and replenished during sleep. NREM sleep favors restoration of cerebral proteins (Nakanishi et al. 1997; Ramm and Smith 1990), some of which may be crucial in preparing the brain for plasticity during waking. Cirelli and Tononi's (2000a) recent finding, that genes related to plasticity (P-CREB, Arc, and BDNF) are expressed in waking and not sleep, may be related to how LTP can be induced during waking but not NREM sleep (Bramham and Srebo 1989). The unidentified genes that are up-regulated during sleep relative to waking (Cirelli and Tononi 2000b) may be critical to restoration of plasticity for subsequent waking and may provide a molecular explanation of the mechanism by which sleep deprivation impairs LTP.

The method of sleep deprivation used in this experiment raises the possibility that the LTP impairment we recorded resulted from an effect of forced locomotion not specific to sleep deprivation. Stress is the most prominent of these nonspecific effects. Because of the extensive work on stress and hippocampal plasticity (reviewed in McEwen 2000), as a preliminary estimate of the stress response to 12-h sleep deprivation, we measured corticosterone levels at the time of decapitation. The serum corticosterone levels at this one time point in only six animals must be considered preliminary, but corticosterone in sleep-deprived animals was twice as high as in control animals. All animals were decapitated at the end of the light period when the corticosterone diurnal rhythm is at its peak. Sleep deprivation raised corticosterone to levels (38 $\mu\text{g}/\text{dl}$) that can impair LTP (Diamond et al. 1992). Stress can reduce LTP, but the nature of the stressor is critical. Restraint and tail shock impaired LTP (Foy et al. 1987), whereas acute cold, which caused a four-fold increase (7 to 29 $\mu\text{g}/\text{dl}$) in corticosterone, did not affect LTP (Bramham et al. 1998). Inescapable and escapable electric shock both raise corticosterone levels (63 and 59 $\mu\text{g}/\text{dl}$, respectively) above the levels in the sleep-deprived rats, but the inescapable shock produces a far greater decrement in LTP (Shors et al. 1989). Although it is unclear whether sleep deprivation is a type of stressor that can impair LTP, the elevated corticosterone levels suggest that the stress response to sleep deprivation may have contributed to the reduction of LTP.

Other nonspecific effects of the deprivation such as exposure to a novel environment and exercise may impair or enhance LTP. Although rats were trained to the deprivation device, exposure to this environment for 12 h, as compared with rats staying in their home cage for 12 h, may have altered LTP in the sleep-deprived rats. Similarly the acute exercise of forced locomotion may have affected LTP independently of sleep-deprivation effects. Opportunity for voluntary exercise over an extended period has been shown to enhance LTP and measures of learning in mice (Anderson et al. 2000). It is unclear how acute forced exercise would affect LTP.

The current study is the first demonstration that the sleep-deprivation protocol reduces plasticity as measured by LTP. Further experiments are planned to establish the mechanism of this reduction. Protocols that evaluate or control the possible roles of corticosterone, novel environment, or exercise will help determine if sleep itself is responsible for restoration of neural plasticity. If further studies establish that sleep restores plasticity, LTP provides a well-established model for testing, at a cellular level, further hypotheses regarding the function of sleep relative to plasticity.

This work was supported by National Institute of Mental Health Grants R01MH-50741 and R01MH-57928 and the Research Service of the Department of Veterans Affairs.

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