

Impaired Long-Term Potentiation Reverses After 21-day Recovery from Sleep Deprivation in Rats

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Abstract

Purpose: Although total sleep loss has been repeatedly reported to impair both the acquisition and the consolidation stages of hippocampus-dependent memories, little is known about the effect of recovery period on impaired long-term potentiation (LTP) by sleep deprivation remains unclear, especially in chronic animal models. In the present study, we investigated alterations of LTP immediately after 1 days of sleep deprivation or after 21 days of sleep recovery.

Methods: Twenty-four adult male Wistar rats were randomly divided into four groups: a sleep deprivation group, control for sleep deprivation group, recovery group, and a control for recovery group (sham). Field potentials, as superimposed form of the field excitatory postsynaptic potential (fEPSP) and the population spike (pSpike), were recorded from the dentate gyrus, stimulating of perforant pathways in urethane anesthetized rats. LTP was induced by four sets of tetanic trains (HFS), separated by 5 min intervals. Input-Output (I/O) curves of field potentials were treated in order to verify whether exposure to 21-day SD paradigm and/or 21-day recovery period influenced basal circuitry properties of the DG.

Results: There was no significant difference among groups in the baseline characteristics and input-output function in the dentate gyrus. HFS-induced potentiation of fEPSP gradually decreased and returned to baseline approximately 20 min after the last HFS, and no maintained LTP was observed ($98 \pm 5.2\%$ of baseline) in sleep deprived rats. In contrast, LTP was maintained for 60 min at least in control, sham and recovery groups.

Conclusion: These data indicate that a 21-day recovery period restores the impairment of maintained LTP by sleep deprivation at the dentate gyrus synapses.

Keywords: Long-Term Potentiation; Sleep Deprivation; Stress, Psychological; Hippocampus

Introduction

Mammalian brain activity during sleep is characterized by the alternation of 2 distinct states, rapid-eye-movement (REM), or paradoxical sleep, and non-REM (NREM), or slow-wave sleep. Animal and human studies suggest that the quantity and quality of sleep have a profound impact on learning and memory, although the relationship is not entirely understood. Learning and memory requires intact hippocampus that is involved in a circuit that is required for several types of memory in both humans [1] and rodents [2], and includes three functions, namely acquisition, consolidation, and recall.

Consolidation of explicit memory in the mammalian hippocampus is associated with any changes in the synaptic connections between neurons, including strengthening and weakening of synapses, changes in the distribution of receptor proteins and postsynaptic

signal transduction mechanisms, and even changes in the number and distribution of synapses formed between pairs of neurons [3]. In experimental preparations, synaptic responses can be potentiated for short or long term lasting milliseconds to hours, depending on the pattern of stimulated neuronal activity. Long-term potentiation (LTP) is a long-lasting enhancement of synaptic transmission induced by high-frequency afferent stimulation. LTP is considered to be involved in the mechanisms of some forms of memory and learning [4]. The cAMP-response element binding protein (CREB) are critical for long-lasting forms of synaptic plasticity and associated memory consolidation [5]. Hippocampal cAMP levels are elevated during REM sleep [6] and cAMP signaling is impaired by sleep deprivation [7]. Although total sleep loss has been repeatedly reported to impair both the acquisition [8,9] and the consolidation stages of hippocampus-dependent memories [10-12], little is known about the specific contribution of REM sleep in the long-term potentiation.

Earlier studies showed that (SD) depressed LTP in area CA1 of the hippocampus *in vivo* [13] and *in vitro* [14,15] in rats. Impaired LTP in the dentate gyrus (DG) was also shown in 3-h [16], 4-h [16, 17] or 21-d [18] REM sleep deprived (REM-SD) rats. If the changes in synaptic plasticity induced by SD were indeed related to the absence of sleep, then a period of recovery sleep after SD should reverse these effects. It was reported that 1-, 2-, and 3-day schedules of SD significantly impaired LTP induction in medial perforant path-granule cell synapses and that LTP recovered fully by the end of 24h [16]. Although previous investigations demonstrated that disruption of LTP in the hippocampus following REM sleep deprivation could be restored by a recovery period, prolonged recovery period *in vivo* may be affected differently, because the amount of REM sleep (REM rebound) increased in the recovery nights compared to normal levels of REM sleep [19]. The increase in sleep duration during recovery from sleep deprivation are usually only a fraction of the sleep time that was lost [20]. Therefore we choose a recovery period of 21 days and compare the LTP with those reported in sleep deprived rats with shorter recovery period.

Methods

REM-Sleep deprivation: In the experiments we used two types of Plexiglas tanks surrounded by water. Plexiglas tanks with multiple small platforms (6 cm in diameter; WTSP) was used to prevent animals from sleeping because if they felled asleep, they would consequently fall into the water due to muscular atonia. This manipulation has been previously shown to deprive the paradoxical sleep and also of an amount of slow-wave sleep [21]. Plexiglas tanks with multiple large platforms (14 cm in diameter; WTLP) allowed rats to sleep without falling into the water. The protocol consisted of submitting the rats to the flower-pot technique for 18 h (beginning at 02:00 p.m.) and returning to the vivarium for 6 h (beginning at 08:00 a.m) for 21 days. Thus, there is a partial compensation for the sleep loss [21].

Experimental animals and groups: Twenty-four adult male Wistar rats were used to test effects of chronic SD and recovery of SD. Rats were randomly divided into four groups: Six rats subjected as control group and were housed in vivarium cage for 42 days. Six rats subjected as sham group and were housed in WTLP for 42 days. Remaining rats were deprived from sleep for 21 days by placing them into WTSP either prior to (n = 6; Recovery group) or after (n = 6; Deprivation group) WTLP for 21 days. All rats were aged 120 - 150 days at the start of experiment and LTP were recorded after a further 42 days. Rats fed with tap water and Purina rodent chow ad lib. Experiments were carried out after receiving approval from Erciyes University’s Committee on Ethics in Animal Experimentation, and financially supported by the Erciyes University Research Foundation (TSA-2013-4003). Experimental schedule is shown in figure 1.

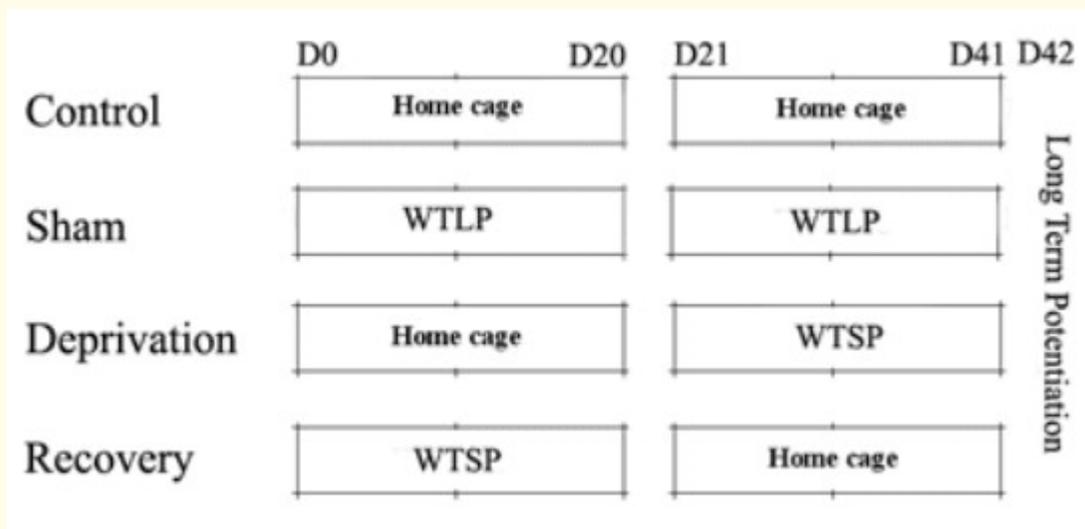


Figure 1: Experimental schedule. Six rats subjected as control group and were housed in home cage for 42 days. Six rats subjected as sham group and were housed in a water tank with large platform (WTLP) for 42 days. Remaining rats were deprived from sleep by placing them into water tank with small platform (WTSP) either prior to (n = 6; Recovery group) or after (n = 6; Deprivation group) home cage for 21 days. Long-term potentiation was recorded on day (D) 42.

Electrophysiological Recording: Field potentials were recorded from the DG on the day 42 in all rats. Rats were anesthetized with urethane (1.5 g/kg, ip) and placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). A bipolar tungsten electrode (stainless steel, Teflon coated, 127- μ m in diameter; insulated except at its tips) was used to stimulate the medial perforant path (from bregma, in mm: AP: -8.0, ML: 4.2, DV: 2-2.5 below dura) of the right hemisphere. A stimulating electrode was connected to the output of a stimulus isolator (World Precision Instruments, USA). A vertical puller (P30, Sutter Instrument Co, USA) was used to make microelectrodes. A glass micropipette (Borosilicate, o.d.:1.5 mm, 10 cm length, World Precision Instruments) was filled with 3-M NaCl (tip resistance: 2-10-M) and inserted into the granule cell layer of the ipsilateral DG (in mm: AP: -3.5, ML: 2.15, DV: 2.5-3 mm below dura) to record field potential. An Ag-AgCl disc electrode was positioned under the neck skin and served as a reference electrode. The recording and reference electrodes were connected to an amplifier (VCC600 single channel epithelial voltage/current clamp system, Physiological Instruments) using a head-stage (Physiological Instruments, VCC600). The entire system was shielded using a Faraday cage. The depth of recording and stimulating electrodes was adjusted to obtain a large positive fEPSP followed by a negative-going PS in response to perforant path stimulation.

The Scope program (ADInstruments, Colorado Springs, CO, USA) was used to control stimulation and recording. Monophasic 10 V and 0.175-ms pulses were generated by the A/D board (Powerlab/8SP, ADInstruments, Colorado Springs, CO, USA) of a computer and triggered to a stimulus isolator (World Precision Instruments, USA, A385). Biological signals were amplified (1000x) on a pre-amplifier at a bandwidth of 0.110 kHz. Waveforms were digitized on-line at a rate of 40 kHz for 20 msec, displayed on a computer monitor, and stored using Scope for off-line analysis.

Input Output (I/O) Curve: Fifteen minutes after electrode placement, stimulation consisting of 175 s duration monophasic constant current pulses was delivered once every 20 sec to obtain an I/O curve. The stimulation current ranged from 0.1 to 1.5-mA. For each current value, there was an average of three evoked responses. The raw values of the fEPSP slope and PS amplitude of the averaged responses were used. The relation between stimulus intensity and the fEPSP slope or PS amplitude was described by a sigmoidal curve; the half maximal stimulus intensity was determined from this curve. Stimulus intensity that produced 50% of maximum response (i.e. test pulse) was used in subsequent experiments. We also plotted I/O curves for EPSP onset time (t₁), PS onset time (t₂) and PS peak time (t₃) to see the effect of sleep deprivation on time parameters of field potentials.

Long-term potentiation: Then, after a 15 min baseline recording of fEPSPs, four sets of tetanic trains (high frequency stimulation: HFS, 100-Hz, 1-s), separated by 5 min intervals, were administered to induce LTP. Following delivery of the last tetanic train, test stimuli were repeated every 30s for up to 60 min.

Data analysis and statistics: The slope of the field EPSP was calculated as the amplitude change at 20 - 80% of the voltage difference between the start of the waveform and the field EPSP amplitude at the onset of the pSpoke. The pSpoke amplitude was measured from the first positive peak to negative peak. The mean value of the fEPSP slope or pSpoke amplitude before the first HFS was evaluated as 100 percent and was defined as the baseline. Each EPSP and each pSpoke was expressed as the percentage of the baseline value. Increases in the fEPSP slope and pSpoke amplitude 55-60 min after the last HFS were considered as a measure of LTP magnitude. The means of fEPSP slope and pSpoke amplitude in input-output curves and in three time windows of LTP were separately subjected to a repeated measures ANOVAs, with group as fixed factors. Body weight and basal synaptic characteristic (slope of pSpoke-I/O curve and EPSP-I/O curve, maximum pSpoke amplitude, maximum EPSP slope and test stimulus intensity) were separately subjected to univariate ANOVAs, with group as fixed factors. A two-tailed significance level was held at 0.05; data are reported as mean \pm standard error.

Results

Body Weight: Rats were weighted on the day 0 and day 41, and the weight increase was calculated. The univariate ANOVA showed significant main effects on the weight increase for group ($F_{3,24} = 8.367$, $p = 0.0001$). Post-hoc analysis revealed that body weight increase in the SD group (25.68 ± 0.64 g) was lower than those in the control group (29.62 ± 0.94 g; $p = .018$), the sham group (29.68 ± 0.51 ; $p = .017$) and the recovery group (33.38 ± 1.53 ; $p < 0.001$) groups.

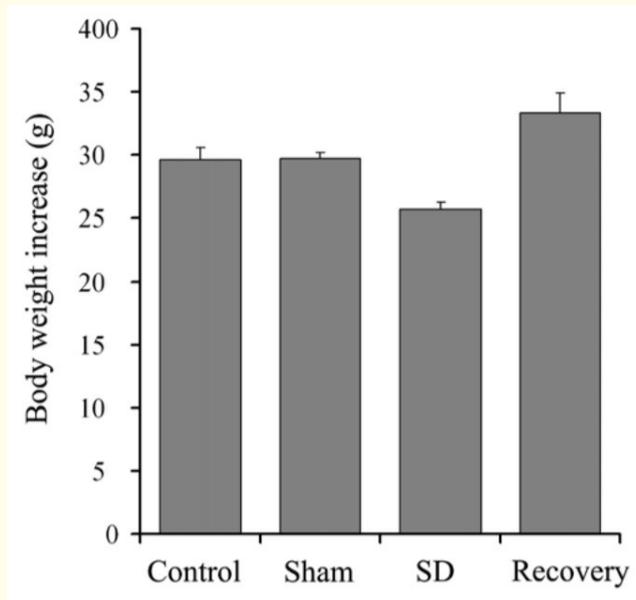


Figure 2: Body weight increase after 21-day sleep deprivation (SD).

I/O Curves: Input-Output Curves of pSpike amplitude and EPSP slope were treated in order to verify whether exposure to 21-day SD paradigm and/or 21-day recovery period influenced basal circuitry properties of the DG. Parameters of the input-output curves of the DG are shown in table 1. One-way ANOVAs showed no significant main effect for slope of pSpike-I/O curve, slope of EPSP-I/O curve, maximum pSpike amplitude, and maximum EPSP slope ($P_s > 0.05$).

	n	slope of pSpike-I/O curve	slope of EPSP-I/O curve	maximum pSpike amplitude (mV)	Maximum EPSP slope (mV/ms)
Control	6	5.04 ± .59	1.28 ± .34	8.51 ± .58	5.33 ± .64
Sham	6	4.24 ± .19	1.59 ± .24	7.61 ± .71	6.34 ± .82
SD	6	5.16 ± .76	1.56 ± .23	8.42 ± .83	5.92 ± .11
REC	6	5.94 ± .82	1.54 ± .25	8.60 ± .90	5.09 ± .32

Table 1: Basal synaptic characteristics for the Dentate Gyrus.

Data are mean ± S.E. No differences in basal characteristics were found in the DG between the different groups.

pSpike amplitudes and EPSP slopes evoked by perforant pathway stimulating ranging from 0.1 mA to 1.5 mA are shown in Figure 3 as current value as well as percentage of maximum amplitude or slope. Repeated measures ANOVA showed that the EPSP slope ($F_{7,140} = 93.093$; $p < 0.001$) and PS amplitude ($F_{7,140} = 166.88$; $p < 0.001$) increased as stimulus intensity increased. Group effect for PS amplitude ($F_{1,20} = 0.426$; $p = 0.521$) and EPSP slope ($F_{1,20} = .483$; $p = 0.495$) did not reach to a significant p values. When data were analyzed using percentage values similar statistical results were obtained.

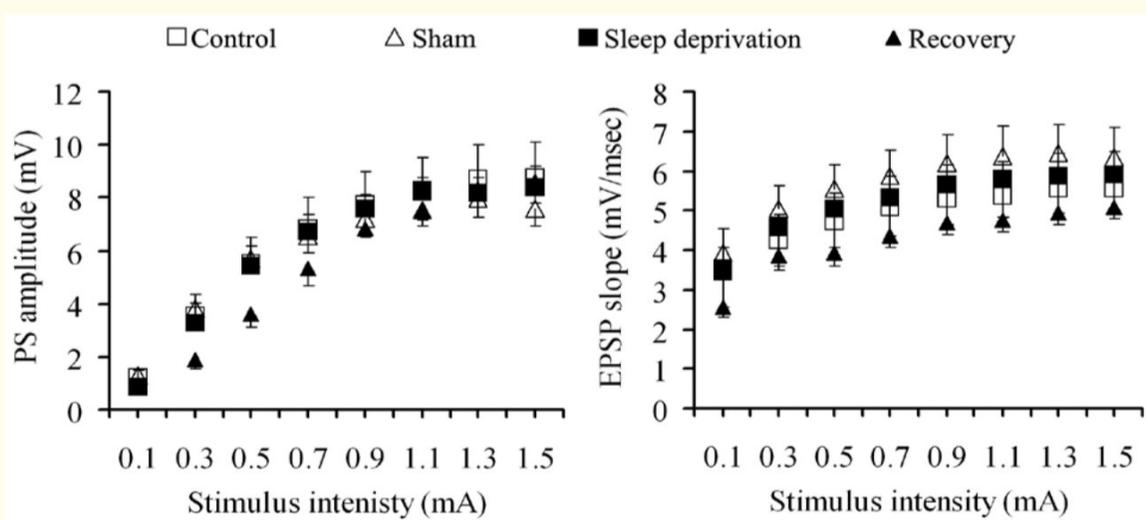


Figure 3: The effect of sleep deprivation on baseline transmission in perforant pathway–dentate gyrus synapses. Absolute input/output curves of the population spike (PS) amplitude (left) and excitatory postsynaptic potential (EPSP) slope (right) in the dentate gyrus (DG) area according to the increasing intensity of stimulation of the perforant pathway (horizontal axis) before the induction of long-term potentiation. Error bars denote the standard errors of the means. Note that neither deprivation nor recovery has effect on the baseline synaptic transmission and that there is no gross difference in baseline synaptic transmission before LTP is induced between groups.

Long-term potentiation

The potentiation of the fEPSP is associated with the formation and enlargement of dendritic spines, whereas the PS potentiation reflects the somatic response, the amplitude of which is dependent on the number of granule cells that discharge in synchrony. The product of the synaptic component of LTP (fEPSP) together with the non-synaptic component (PS) has a much stronger effect on the neuronal output in comparison to cases when only one of these two components is potentiated. As shown in Figure 4, four tetanus trains of 100 pulses at 100 Hz induced LTP of both PS (Figure 4A) and fEPSP (Figure 4B) in the dentate gyrus neurons of four groups.

The magnitude of somatic potentiation (PS amplitude) was different amongst groups ($F_{3,20} = 6.24$; $P = 0.004$) in the 5-min period after the last HFS, with the SD group having the lowest potentiation ($225 \pm 8.9\%$ of baseline) and the sham group having the largest potentiation ($300 \pm 13.4\%$ of baseline). The Post-hoc LSD test showed significant differences between the SD group and the REC group ($P = 0.029$) and between the SD group and the sham group ($P = 0.001$) and between the sham group and the control group ($P = 0.008$). In the same period, the magnitude of synaptic potentiation (fEPSP slope) was also different amongst groups, with the SD group having the lowest potentiation ($114 \pm 5.6\%$ of baseline) and the control group having the largest potentiation ($165 \pm 6.6\%$ of baseline). The Post-hoc LSD test showed significant differences between the SD group and the control group ($P = 0.002$) and between the SD group and the sham group ($P = 0.006$). These data indicate that a 21-day recovery period restores the impairment of LTP induction by sleep deprivation at the dentate gyrus synapses.

As shown in Figure 4, HFS-induced potentiation of somatic response gradually decreases but lasted at least 60 min, reaching to a level of $201 \pm 12.5\%$, $183 \pm 22.9\%$, $227 \pm 22.8\%$ and $131 \pm 9.4\%$ of baseline in the last 5 min period (55 - 60 min after the last HFS) in the groups of control, sham, recovery and sleep deprivation, respectively, indicating maintenance of LTP. According to the fEPSP slope, LTP was maintained reaching to a level of $113 \pm 8.7\%$, $119 \pm 4.6\%$, and $118 \pm 14.5\%$ of baseline in the control group, sham group and recovery group, respectively. In sleep deprived rats, however, the increase in the slope of fEPSPs returned to baseline approximately 20 min after the last HFS, and no maintained LTP was observed ($98 \pm 5.2\%$ of baseline). Statistical analysis showed that 1-h maintained LTP was also impaired in sleep deprived rats, indicated by significant differences between SD group and control group (PS-LTP: $P = 0.021$; fEPSP-LTP: $P = 0.021$) and between SD group and recovery group (PS-LTP: $P = 0.003$). These data indicate that a 21-day recovery period restores the impairment of maintained LTP by sleep deprivation at the dentate gyrus synapses.

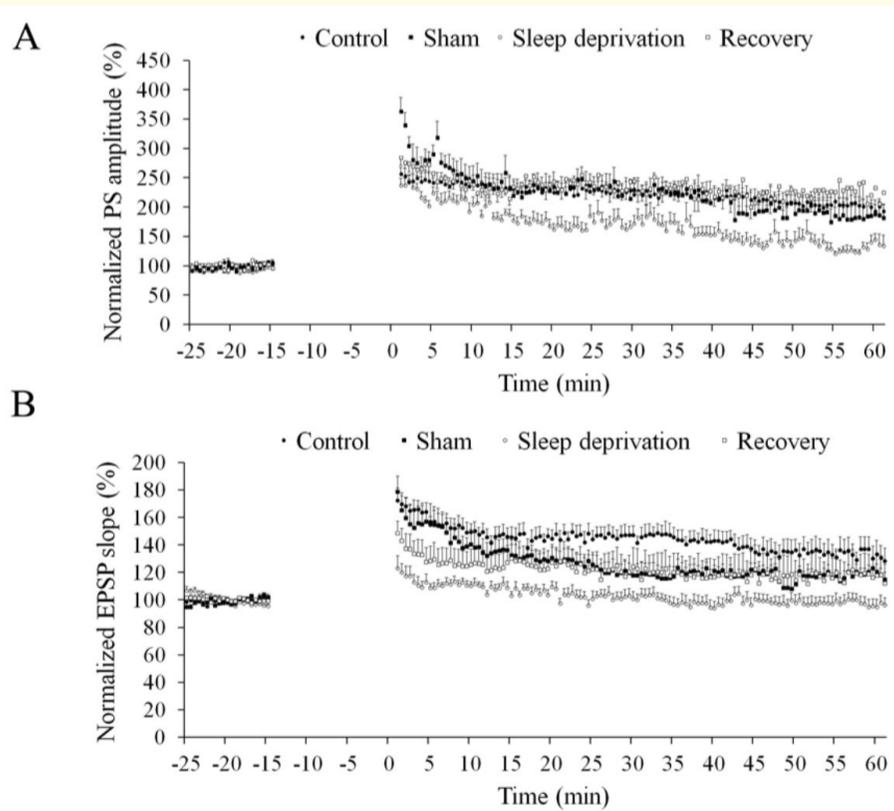


Figure 4: A 21 day recovery period rescues the impairment of long-term potentiation of both the population spike (PS) and dendritic EPSP by sleep deprivation in hippocampal dentate gyrus. A and B: Time course of population spike (PS; A) amplitude and excitatory postsynaptic potential (EPSP; B) slope changes from 0 to 60 minutes following high-frequency stimulation. After a 15-minute baseline recording, LTP was induced by means of high-frequency stimulation (arrows; 100 Hz, 1 sec, 4 times), which was applied beginning at time -15. Error bars denote the standard errors of the means, $n = 6$ for each group. Note that the LTP magnitudes in the sleep deprived rats were significantly lower as compared to the other groups.

Discussion

In this study we have shown that a 21 day SD severely impairs the induction and maintenance of LTP in the PP-DG pathway. Furthermore our results reveal that this impairment completely abolished after 21 day recovery period.

Sleep deprivation, which has been one of the major tools in the study of sleep, impacts many physiologic processes. Our experiments were designed to minimize the induction of stress. It has been shown that this type of deprivation has an effect on paradoxal sleep stages. In contrast to the one platform “flower pot” (disk over water) technique that largely increases the corticosterone level [22], multiple platform technique has been reported not to induce an increase in corticosterone levels. Moreover, adrenalectomized rodents still show memory deficits after sleep deprivation [23] further emphasizing that while sleep deprivation can be stressful, it is sleep loss itself, rather than stress induced by sleep loss, that perturbs synaptic plasticity underlying memory consolidation. Importantly, the deficits in LTP were unrelated to the mild increase in corticosterone levels observed after sleep deprivation indicating that sleep loss, rather than accrued stress, were underlying these LTP impairments [7]. In a previous study we showed stress response of the 21 day SD group was not different from the pedestal or cage control groups, showed by the corticosterone levels and elevated T maze test [18]. Therefore it may mention that the selective decrease in synaptic efficacy caused by 21-day PSD in the DG is not related to stress in the present work.

LTP is the major cellular model for plasticity and may be related to learning and memory [24]. Numerous studies have found that REM sleep deprivation impairs spatial reference memory, which is associated with the Hippocampus [25]. Therefore deficits in learning and memory may be related to reduced plasticity. Three prior studies examined sleep deprivation for effects on hippocampal LTP [15,26,27]. In each of these studies, LTP was assessed *in vitro* using the brain slice preparation. In one study, 12 hr of total sleep deprivation was produced by forced locomotion. The remaining two studies used selective REM sleep deprivation procedures (using the ‘inverted flower pot’ method) for up to 72 hr. Despite the variations in duration of SD, the present *in vivo* study and previous *in vitro* studies are in general agreement: sleep deprivation inhibits LTP and selective REM sleep deprivation affects LTP persistence with little or no change in the maximal initial potentiation immediately following tetanization.

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