Paradoxical (REM) Sleep Deprivation Causes a Large and Rapidly Reversible Decrease in Long-Term Potentiation, Synaptic Transmission, Glutamate Receptor Protein Levels, and ERK/MAPK Activation in the Dorsal Hippocampus

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Study Objectives: It has been shown that wake (W) and slow wave sleep (SWS) modulate synaptic transmission in neocortical projections. However the impact of paradoxical sleep (PS) quantities on synaptic transmission remains unknown. We examined whether PS modulated the excitatory transmission and expression of glutamate receptor subtypes and phosphorylated extracellular signal-regulated kinases (p-ERK1/2).

Design: PS deprivation (PSD) was carried out with the multiple platforms method on adult male Sprague-Dawley rats. LTP, late-LTP, and synaptic transmission were studied in the dorsal and ventral hippocampus of controls, 75-h PSD and 150-min PS rebound (PSR). GluR1 and NR1 protein and mRNA expression were evaluated by western blot and real-time PCR. P-ERK1/2 level was quantified by western blot and immunohistochemistry.

Measurement and Results: PSD decreased synaptic transmission and LTP selectively in dorsal CA1 and PSR rescued these deficits. PSD-induced synaptic modifications in CA1 were associated with a decrease in GluR1, NR1, and p-ERK1/2 levels in dorsal CA1 without change in GluR1 and NR1 mRNA expression. Regression analysis shows that LTP is positively correlated with both PS quantities and SWS episodes duration, whereas synaptic transmission and late-LTP are positively correlated with PS quantities and negatively correlated with SWS quantities.

Conclusions: These findings unveil previously unrecognized roles of PSD on synaptic transmission and LTP in the dorsal, but not in the ventral, hippocampus. The fact that the decrease in protein expression of GluR1 and NR1 was not associated with a change in mRNA expression of these receptors suggests that a sleep-induced modulation of translational mechanisms occurs in dorsal CA1.

Keywords: Extracellular signal-regulated kinase (ERK), corticosterone, paradoxical sleep deprivation, excitatory synaptic transmission, Late-LTP

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SEVERAL STUDIES HAVE SHOWN THAT PARADOXICAL SLEEP (PS OR RAPID EYE MOVEMENT SLEEP) ENHANCES THE CONSOLIDATION OF SOME FORMS OF memory. In humans, PS facilitates long-term consolidation of visual discrimination tasks and emotional memory. The determination of the cellular mechanisms of PS-induced modulation of memory consolidation requires animal models; rodents are commonly used for understanding sleep-memory interactions.

In rats, PS deprivation (PSD) impaired spatial memory. PSD altered Morris water maze learning, but had no effect in the visible version of the task. Consolidation of contextual, but not cued, fear conditioning was decreased by PSD. Spatial memory and contextual fear conditioning are known to be mediated by the hippocampus. Interestingly, electrophysiological studies have shown that the threshold for inducing hippocampal long-term synaptic potentiation (LTP), a cellular mechanism of learning and memory, was increased by PSD. PSD might impair CA1 and dentate gyrus LTP by altering N-methyl-D-aspartate receptors NMDAR-mediated transmission and trafficking in pyramidal cells. In contrast, CA1 long-term synaptic depression (LTD), which has also been linked to learning process, was spared by PSD. These studies suggested that PSD selectively modulates long-term synaptic plasticity and glutamate receptor-mediated transmission in the hippocampus.

Major issues remain to be investigated to clarify cellular and molecular mechanisms of PSD on memory impairment. Late LTP (L-LTP), which is induced with repeated high frequency trains (4 tetani with an interval of 5 min), has never been tested in PSD, in contrast with LTP (or early LTP, evoked by one high frequency train). L-LTP is a cellular model of memory consolidation that, unlike LTP, requires protein synthesis. LTP impairment alone is not sufficient to explain decreased performances in memory consolidation. Thus, one goal of this paper is to determine whether L-LTP was also altered by PSD.

We also addressed 2 important controls to discard potential nonspecific effects of PSD on LTP and L-LTP. First, it has been shown that PSD not only modulates PS, but also slow wave sleep (SWS) quantities. Thus, we assessed the quantities of different vigilance states during the 27-h period that preceded...
LTP and L-LTP experiments. We sought to reveal whether there is a role for PS by correlating vigilance state quantities with LTP and L-LTP amplitudes. Second, given the deleterious effects of stress on synaptic plasticity, it was necessary to determine the stress level induced by the PSD procedure. Thus, we characterized these nonspecific effects by quantifying plasma corticosterone concentration, a well-established marker of stress. We also examined the presence of a correlation between the plasma corticosterone levels and LTP and L-LTP amplitudes.

Homeostatic synaptic scaling is another major form of synaptic plasticity that has not been tested in PSD. LTP and L-LTP are localized at individual synapses and are rapidly induced by specific patterns of synchronized synaptic activity. In contrast, synaptic scaling adjusts synaptic strength in a compensatory fashion in response to prolonged changes of the cell’s electrical activity. Synaptic scaling is critical to protect cortical networks against epileptogenic activity and to maintain optimal processing. Synaptic down-scaling can result after demanding tasks and has recently been suggested to be modulated by vigilance states. SWS decreases the synaptic strength of callosal connections in rat frontal cortex, whereas wake increases it. In this study, we tested the hypothesis that PS may also modulate synaptic transmission. We determined whether PSD induces a synaptic down-scaling of glutamatergic transmission by quantifying the excitory synaptic potentials (EPSPs) at the Schaffer collaterals (SC)-CA1 pyramidal cell synapses.

Another key point that remains to be elucidated is the sleep recovery duration necessary to rescue PSD-induced effects on synaptic plasticity. It has been shown that LTP was restored after 24 h of complete PS recovery following 72-h PSD. However, the rescue of synaptic plasticity after PSD might occur faster during the early stages of sleep homeostatic processes. Thus, we examined the LTD, L-LTP, and synaptic efficacy after a short-term (150 min) PS recovery (also called PS rebound, PSR).

A selective PSD.

PSD rats underwent 72-h PSD on multiple platforms (MP) in a standard container filled with water (2-3 cm depth). Three platforms (6.2 cm diameter, 7-12 cm height) were spaced 7-8 cm apart, so that rats could easily move between them. This constitutes a modification of the “flower pot” technique that resulted in a selective PSD. MP were added to prevent the stress induced in rats by immobilization. During PSD, food and water were available ad libitum, and the container was cleaned daily. During this period, the rats were placed in a dry cage for 30-45 min where they stayed awake (grooming and exploratory behavior).

To determine the possible cellular mechanisms responsible for PS-dependent modulations of synaptic function, we investigated by western blot and real-time PCR the expression of the GluR1 has been established as an important factor of synaptic scaling. Also we characterized the phosphorylation level of GluR1 at Ser-845 site (p-GluR1), given its role in long-term synaptic plasticity. Finally, the activation of extracellular signal-regulated kinase, ERK-MAPK, cascade is also required for L-LTP. Thus, we determined whether the level of ERK phosphorylation (p-ERK1/2, p44/42 ERK-MAPK at Thr202/Tyr204 sites) is modulated by PSD.

MATERIALS AND METHODS

Polygraphic Recording of Vigilance States

Experiments were performed on male Sprague Dawley rats (7-8 weeks old, 250-350 g, Charles River Laboratories, France). Animals were implanted under chloral hydrate anesthesia (400 mg/kg), as described previously, to monitor electroencephalogram (EEG) and electromyogram (EMG). EEG and EMG bipolar signals were amplified, digitized at 250 Hz, and collected with CED using Spike-2 interface software (Cambridge Electronic Design). Hypnograms were scored off-line by using cortical EEG and nuchal EMG. When sleep stages were difficult to identify, hypnograms were blindly scored by 2 trained polysomnographers and ambiguous cases were removed from analysis (n = 3 of 34). The episodes of each vigilance state were scored by using a 10-sec sliding time window according to the following criteria: wake (W) was characterized by desynchronized low-voltage and high-frequency activity EEG and by sustained neck muscle tone at the EMG; SWS was characterized by high-voltage slow waves (1.5–4 Hz) and spindles (10–14 Hz) combined with low muscle tone; PS episodes were identified by a decrease in the EEG amplitude and a prominent theta rhythm (5–9 Hz) associated with muscle atonia at the EMG.

Paradoxical Sleep Deprivation

Animals spent 3-5 days in recovery from surgery in individual standard Plexiglas containers under standard laboratory conditions (i.e., 12/12-hour light-dark cycle with light-on at 07:00, 22–24°C ambient temperature, food and water ad libitum). After a 5-d habituation period to the recording environment, the protocol was performed over a 6-d period during which most of the rats were recorded. Baseline sleep was recorded during 48 h for the 4 groups that were used in this study.

PSD rats underwent the same deprivation protocol as PSD rats and were maintained on MP until the end of PSD, at which point the rats were placed in a standard container at 07:00 day 6. After ≈ 40 min of grooming and exploration, the recovery of the PS debt induced a sustained and selective increase in PS quantities (PS rebound) during several hours. PSR rats were euthanized 150 min after the first PS episode (i.e., approximately at 10:00, day 6). Basal sleep was assessed with the control (Ctl) group, which remained in a standard container during the protocol. We also had another control group, namely the large platform (LP) group, to discard potential contextual-related effects on hippocampal synaptic plasticity in PSR rats, since the PSR protocol was associated with an environmental change. The LP rats spent 72 h on a large platform, after which they were placed in a standard container. LP rats were euthanized 150 min after the first PS episode. LP condition differed from PSR by the size of the platform (15.2 cm diameter, 8 cm height). All animals were euthanized by pentobarbital (i.p., 150 mg/kg, Sigma) on the same schedule to prevent circadian effects.

The polygraphic results shown in Table 1 and in Figure S1 (see supplementary figures at www.journalsleep.org) were collected from rats that were used for slice recordings (n = 31). Figure S1 indicates the vigilance state quantities for the groups during the last 27 h before the slicing experiments. Two parameters were used to quantify PS in the sleep-wake cycle: PS quantities

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an ACSF-filled glass microelectrode (~1 MΩ). Stimulations were delivered by bipolar tungsten electrodes, and field evoked potentials (fEPSPs) were recorded with an ACSF-filled glass microelectrode (~1 MΩ). Stimulation (100-µs duration) were applied at a frequency of 0.033 Hz via a stimulus isolator (World Precision Instruments). The electrodes for stimulation and recording were placed in the CA1 stratum radiatum 200 µm from the stratum pyramidale and were separated by 100-200 µm. The fEPSP slopes were carefully adjusted to obtain a similar size for each hippocampal slice (Ctl in mV/ms: 0.11 ± 0.02; PSD: 0.11 ± 0.03; PSR: 0.10 ± 0.01; LP: 0.12 ± 0.04; n = 10 in each group). LTP induction protocol consisted in one high-frequency train (tetanus of 100-Hz train of 1 sec). L-LTP was induced with repeated high-frequency trains (4 tetani with an interval of 5 min). The LTP and L-LTP amplitude (initial fEPSP slope) was normalized with the average fEPSPs during the baseline period (~10 to 0 min before tetanus). To assess synaptic transmission, we carried out the input/output (I/O) relation by progressively raising the stimulation intensity from 40 to 200 µA. The input is given by the amplitude of the fiber volley (FV, presynaptic action potentials) that precedes the fiber volley (FV, presynaptic action potentials) at the different stimulation intensities (Figure S2; available at www.journalsleep.org). For the 4 groups of rats, no significant difference was observed in the slope of the FV amplitude at the different stimulation intensities (Figure S2; available at www.journalsleep.org). For the 4 groups of rats, no significant difference was observed in the slope of the FV amplitude at the different stimulation intensities (Figure S2; available at www.journalsleep.org). For the 4 groups of rats, no significant difference was observed in the slope of the FV amplitude at the different stimulation intensities (Figure S2; available at www.journalsleep.org). For the 4 groups of rats, no significant difference was observed in the slope of the FV amplitude at the different stimulation intensities (Figure S2; available at www.journalsleep.org).

Methods were previously described.22 Briefly, slices (400 µm thick, n = 67 rats) containing hippocampus were cut with a Vibratome (Leica VT1000S) in ice-cold artificial cerebrospinal fluid (ACSF, in mM: 124 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 1.3 MgCl₂, 2.5 CaCl₂, 26 NaHCO₃, and 10 Glucose). Dorsal (i.e., up to −5.0 mm to Bregma according to Paxinos Atlas coordinates) and ventral slices (Figure 4) recovered for 1 h at room temperature. Recordings were performed in a submerged chamber perfused with ACSF maintained at 32°C and constantly gassed with 95% O₂−5% CO₂. In each experiment, CA3 was cut away from the slice and picrotinox (GABA-A receptor antagonist, PTX, 100 µM) was added to the ACSF. Orthodromic stimulations were delivered by bipolar tungsten electrodes, and field excitatory postsynaptic potentials (fEPSPs) were recorded with an ACSF-filled glass microelectrode (~1 MΩ). Stimulation

Table 1—Vigilance States Quantification During the Last Recording 27 Hours

<table>
<thead>
<tr>
<th></th>
<th>PS (%)</th>
<th>PS Nb</th>
<th>PS Dur</th>
<th>SWS (%)</th>
<th>SWS Nb</th>
<th>SWS Dur</th>
<th>PS/TS (%)</th>
<th>W (%)</th>
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<tbody>
<tr>
<td><strong>Last 24 h</strong></td>
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<tr>
<td>Ctl (6)</td>
<td>7.8 ± 0.5</td>
<td>81.5 ± 8.4</td>
<td>83.6 ± 4.5</td>
<td>41.3 ± 3.0</td>
<td>381.5 ± 30.3</td>
<td>95.2 ± 10.3</td>
<td>15.9 ± 0.6</td>
<td>50.9 ± 3.4</td>
</tr>
<tr>
<td>PSD (8)</td>
<td>1.9 ± 0.4***</td>
<td>75.8 ± 15.4</td>
<td>21.8 ± 2.7***</td>
<td>40.6 ± 1.8</td>
<td>634.0 ± 24.2***</td>
<td>55.6 ± 3.9**</td>
<td>4.4 ± 0.8***</td>
<td>57.5 ± 2.0</td>
</tr>
<tr>
<td>PSR (7)</td>
<td>1.8 ± 0.4***</td>
<td>78.4 ± 20.6</td>
<td>20.6 ± 1.8***</td>
<td>33.3 ± 2.4</td>
<td>557.0 ± 25.1***</td>
<td>52.1 ± 4.1**</td>
<td>4.9 ± 1.1***</td>
<td>64.9 ± 2.6**</td>
</tr>
<tr>
<td>LP (3)</td>
<td>4.6 ± 0.3**</td>
<td>70.6 ± 16.4</td>
<td>58.3 ± 13.5</td>
<td>30.0 ± 4.4</td>
<td>456.3 ± 77.9</td>
<td>59.2 ± 9.6</td>
<td>13.6 ± 1.0</td>
<td>65.3 ± 4.7**</td>
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<tr>
<td>Light-off</td>
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<tr>
<td>Ctl (6)</td>
<td>10.7 ± 0.7</td>
<td>51.0 ± 2.3</td>
<td>89.4 ± 6.4</td>
<td>52.5 ± 3.8</td>
<td>224.8 ± 26.1</td>
<td>103.1 ± 10.8</td>
<td>17.0 ± 0.8</td>
<td>36.8 ± 4.4</td>
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<tr>
<td>PSD (8)</td>
<td>2.5 ± 0.5***</td>
<td>48.0 ± 9.4</td>
<td>23.7 ± 3.7***</td>
<td>47.8 ± 1.3</td>
<td>348.0 ± 17.1**</td>
<td>59.6 ± 4.1**</td>
<td>4.9 ± 0.9***</td>
<td>49.7 ± 1.5**</td>
</tr>
<tr>
<td>PSR (7)</td>
<td>2.0 ± 0.5***</td>
<td>46.3 ± 12.6</td>
<td>20.0 ± 2.0***</td>
<td>40.3 ± 2.9*</td>
<td>326.6 ± 20.3**</td>
<td>53.8 ± 4.0***</td>
<td>4.7 ± 1.1***</td>
<td>57.7 ± 3.1**</td>
</tr>
<tr>
<td>LP (3)</td>
<td>7.2 ± 0.7**</td>
<td>44.7 ± 6.0</td>
<td>71.4 ± 9.1</td>
<td>33.6 ± 4.9*</td>
<td>220.0 ± 12.5</td>
<td>65.8 ± 9.0</td>
<td>18.0 ± 1.9</td>
<td>59.2 ± 5.3*</td>
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<td><strong>Last 150 min</strong></td>
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<tr>
<td>Ctl (6)</td>
<td>12.3 ± 2.1</td>
<td>123.0 ± 7.8</td>
<td>90.5 ± 11.1</td>
<td>30.3 ± 4.8</td>
<td>157.0 ± 13.3</td>
<td>82.6 ± 11.2</td>
<td>13.5 ± 1.3</td>
<td>64.8 ± 5.8</td>
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<tr>
<td>PSD (9)</td>
<td>1.2 ± 0.3**</td>
<td>29.4 ± 5.9</td>
<td>16.7 ± 2.1***</td>
<td>23.9 ± 2.4</td>
<td>288.9 ± 13.3***</td>
<td>49.7 ± 3.9**</td>
<td>3.4 ± 0.7***</td>
<td>65.9 ± 2.6</td>
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<tr>
<td>PSR (10)</td>
<td>1.5 ± 0.4*</td>
<td>32.3 ± 8.4</td>
<td>21.1 ± 1.9***</td>
<td>26.3 ± 2.4</td>
<td>230.4 ± 7.7***</td>
<td>49.7 ± 4.6*</td>
<td>5.2 ± 1.0***</td>
<td>72.1 ± 2.7</td>
</tr>
<tr>
<td>LP (5)</td>
<td>2.1 ± 0.1</td>
<td>31.3 ± 10.5</td>
<td>41.1 ± 18.5</td>
<td>26.5 ± 7.0</td>
<td>236.7 ± 69.3</td>
<td>51.9 ± 8.1</td>
<td>8.4 ± 2.2</td>
<td>71.4 ± 7.0</td>
</tr>
</tbody>
</table>

(% refers to the vigilance state quantities expressed as a percentage of the recording period; Nb, the number of bouts; Dur, the mean duration (in sec) of each bout. PS/TS (%) represents the percentage of paradoxical sleep quantity related to the total sleep quantities.* indicates a statistically significant difference between values obtained in Ctl group and other experimental groups (*P < 0.05. **P < 0.01. ***P < 0.001). In case of significance, the test was followed by a post hoc test to identify significant pairwise differences. The statistical test was the one-way analyses of variance (ANOVA). Values are expressed as mean ± SEM. Number of animals is indicated in italic.

PS: paradoxical sleep; SWS: slow wave sleep; PS/TS: paradoxical sleep efficacy; W: wakefulness.

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PSD-Induced Synaptic Impairments—Ravassard et al
Tissue Dissection and Sample Preparation for Western Blot and qPCR

Slices (1-mm thick) from the dorsal and ventral poles of hippocampus (n = 27 rats), containing CA1-CA3, were rapidly dissected on ice. For western blotting, specimens were homogenized in 20 mM Tris Base-HCl pH 7.4, 10% (wt/vol) sucrose, 5 mM EGTA, 1 mM EDTA, and an anti-protease inhibitor cocktail (Complete, Roche). For qPCR experiments, hippocampal tissues were directly introduced in dry aliquots and immersed into liquid N2 and stored at −80°C until use. For each rat (Ctl, PSD, and PSR groups), dissected hippocampi were randomly distributed for further western blot and qPCR experiments.

Protein Expression Level Determined by Western Blot

Methods were previously described.29 Western blotting was performed with 7.5 µg per line of homogenized tissue for phospho-Ser-845-GluR1 and NR2B and 10 µg for ERK1/2, p-ERK1/2, GluR1, and NR1. Molecular weight was controlled with a prestained protein ladder (Fermentas, Life Science). Protein samples were separated by 9% (wt/vol) and 6% (for NR1 and NR2B) SDS-polyacrylamide gel electrophoresis (SDS PAGE) and electroblotted onto PVDF membranes. Blots were blocked in Tris buffer saline: (in mM) 50 Trizma base, 150 NaCl, pH 7.4, 0.1% (v/v) Tween-20, 5% (wt/vol) dry skimmed milk, and then incubated overnight at 4°C with the rabbit polyclonal primary antibodies against ERK1/2 (1:1000), p-ERK1/2 (1:1000, PhosphoPlus p44/42 MAPK (Thr202/Tyr204), from Cell Signaling), GluR1 (1:1000), phospho-Ser-845-GluR1 (1:1000, p-GluR1), NR2B (1:1000), or NR1 (1:500) (from Chemicon). Mouse monoclonal primary antibody anti-GAPDH (1:20000) was purchased from Chemicon. GAPDH was used as a loading control and was processed on the previously used membranes. After rinses, the blots were incubated for 1 h at room temperature with alkaline-phosphatase-conjugated secondary antibody anti-rabbit or anti-mouse IgG (1:10000, Amersham Pharmacia Biotech). The antigen/antibody complex was detected with enzyme catalyzed fluorescence (ECF Amersham western blotting reagent packs) and visualized using a Fluorimager (Molecular Dynamics). The antigen/antibody complex was detected using an anti-rabbit or anti-mouse IgG (1:10000, Amersham Pharmacia Biotech). The antigen/antibody complex was detected with enzyme catalyzed fluorescence (ECF Amersham western blotting reagent packs) and visualized using a Fluorimager (Molecular Dynamics). The fluorescence intensity was quantified by ImageQuant (Molecular Dynamics). The ANOVA Fisher test was performed to compare differences between expression levels in the different groups for each protein.

Real-Time PCR (qPCR).

Total RNA was extracted from hippocampus using the RNeasy Lipid Tissue mini kit (Qiagen) according to the manufacturer’s protocol; qPCR was performed with a Light Cycler Faststart DNA master SYBR green I kit (Roche). RNA samples were treated with RNase-free DNase Set (Qiagen) to eliminate DNA contamination. Total RNA quality and yield were assessed using a bioanalyzer (Agilent2100, Agilent Technologies). Primer pairs were generated to amplify specific 100-200 bp fragments from rat GluR1 (Genebank accession number: NM 031608, Forward, 5’ to 3’: CGCCCTGAGAAATCCGATTA) and NR1 (NM 017010, TTCACAGAAGTGCGATCTGG). Expression levels were normalized with the following housekeeping genes: beta-actin (GCATTGCTGACAGGATGCAG), HPRT (Hypoxanthine guanine phosphoribosyl transferase: CAGGCCAGTTTGGGAT) and YWHAE (Tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein, GTTTGCGGTAATGGTTTTGC), which were used as internal controls for quantitative analysis. Similar results were obtained with these 3 housekeeping genes. The Tukey-Kramer multiple comparisons test was performed to compare differences between groups.

Immunohistochemistry

Animals (n = 12) were perfused with a Ringer lactate solution followed by a fixative buffer (4% paraformaldehyde in 0.1M phosphate buffer). Coronal sections containing hippocampus (25-µm thick) were incubated with phospho-ERK1/2 (1:1000, Cell Signaling) rabbit polyclonal antibody for 72 h at 4°C. After 2-h incubation in biotinylated anti-rabbit secondary IgG (1:1000, Rockland, Tebu-bio), sections were processed with avidin-biotin horseradish peroxidase complex (ABC, Elite Kit from Vector Laboratories), and the reaction was visualized with diaminobenzidine (DAB, Sigma). Optical density was quantified on 3-5 sections of hippocampus by using a microscope equipped with a digital camera and an image analyzing system (Mercator, Explora Nova). One-way ANOVA and unpaired 2-tailed Student t-test were performed to compare the differences between groups.

Corticosterone Radioimmunoassay

After decapitation under anesthetic, the cardiac blood was collected in rats of the 4 groups used for LTP/L-LTP experiments. Samples were centrifuged at 2000 rpm for 15 min at 4°C, and the plasma fraction was isolated and stored at −80°C. Samples were blindly analyzed by radioimmunoassay at the Centre de Médecine Nucléaire (HCL, Lyon) directed by Pr. Bruno Claustrat. One-way ANOVA and unpaired 2-tailed Student t-test were performed to compare the differences between groups.

RESULTS

PSD Resulted in a Decrease in PS Quantities and Elicited a Large PS Rebound During Recovery

We first characterized sleep quantities in the different groups of rats (Table 1 and Figure S1). During the day before slicing, PS was characterized by a significant decrease in bout quantity and duration for the deprived groups (PSD and PSR) compared to the Ctl group. By contrast, SWS quantities did not differ between groups. In these groups, the increase in W quantity was mainly related to the large decrease in PS quantity. Similar circadian changes of vigilance states were observed between different groups during the lights-on and lights-off periods. During the last 150 min before slicing experiments, PSD rats showed a highly significant decrease in PS quantity. A 28% reduction in SWS quantity also characterized the PSD group compared to Ctl, due to a decrease in SWS bout duration. However, PS efficacy (PS duration/total sleep duration) was significantly re-
LTP and L-LTP (%): percentage of LTP and L-LTP amplitude; Cort.: plasma corticosterone level; I/O curve: mean I/O curve slope; I/O curve dors./ventr.: mean I/O curve slope in dorsal and ventral hippocampus. * indicates a significant difference between values obtained in Ctl group and other experimental groups (**P < 0.05, ***P < 0.005). The statistical test was the one-way analyses of variance (ANOVA). In case of significance, the test was followed by a post hoc test to identify significant pairwise differences. Values are expressed as mean ± SEM. Number of slices is indicated in italic.

**Table 2**—SC-CA1 Pyramidal Cell Long-Term Potentiation and Synaptic Transmission

|          | LTP (%)   | L-LTP (%) | Cort. (ng/ml) | I/O curve | I/O curve dors. | I/O curve ventr. |
|----------|-----------|-----------|---------------|-----------|-----------------|-----------------
| Ctl      | 201.3 ± 17.6 (19) | 227.9 ± 29.5 (14) | 53.6 ± 13.7 (11) | 1.14 ± 0.11 (28) | 1.20 ± 0.14 (15) | 1.02 ± 0.16 (13) |
| PSD      | 127.3 ± 7.4 (23)** | 143.5 ± 14.0 (14)* | 85.4 ± 22 (9) | 0.74 ± 0.17 (19)* | 0.71 ± 0.13 (13)* | 0.95 ± 0.31 (9) |
| PSR      | 185.8 ± 19.6 (25) | 239.6 ± 31.0 (17) | 19.9 ± 8 (10)* | 1.62 ± 0.24 (22)* | 1.63 ± 0.39 (11) | 1.61 ± 0.30 (11) |
| LP       | 205.8 ± 15.5 (14) | 241.0 ± 22.7 (13) | 57.2 ± 16.5 (8) | 1.09 ± 0.20 (14) | 1.13 ± 0.28 (10) | 1.01 ± 0.20 (6) |

produced by 80% in PSD compared with Ctl, indicating that the PSD procedure decreased PS quantity more than SWS quantity. During the last 150 min, the PSR rats showed a highly significant increase in PS quantity compared to Ctl and PSD. This was caused by a rise in the number and duration of PS bouts. PSR and PSD rats had similar SWS quantities. Thus, PS efficacy was highly increased in the PSR group compared to the other groups. Finally, sleep quantity in the LP group was intermediate between Ctl and PSR groups.

**LTP and L-LTP Were Impaired by PSD and Restored After 150 Min of PSR**

The dorsal (septal), but not the ventral, hippocampus plays an important role in spatial memory. Given the sensitivity of spatial memory to PSD procedures, we collected the following data from dorsal slices. We first compared the amount of LTP induced by one train of high-frequency stimulation (100 Hz during 1 s) at the SC-CA1 pyramidal cell synapse in the 4 groups. LTP amplitude significantly decreased by 37% in PSD compared to Ctl, whereas PSR had LTP amplitude similar to Ctl and LP (Figure 1A-B and Table 2). We then examined the relationship between LTP amplitude and the vigilance state quantities by using a linear regression analysis (Figure S3; available at www.journalsleep.org). As summarized in Figure 1C-D, the PS quantity, SWS quantity, and PS efficacy during the last 150 min were not correlated with LTP amplitude; whereas W quantity showed a weak but significant negative correlation. We also correlated the LTP amplitude with vigilance state quantity of the previous day (Figure 1C). We found a highly significant positive correlation between PS quantity or efficacy, and LTP when the last lights-off period was considered. By contrast, no correlation was found with the other vigilance states. Because SWS bout duration was also reduced in PSD, we correlated the episode duration of each vigilance state with LTP amplitude. A positive correlation was found between LTP and the SWS and PS bout duration for the last 150 min (r = 0.59, P = 0.003 for SWS episodes and r = 0.46, P = 0.03 for PS episodes; data not shown).

We then studied if L-LTP was altered by PSD in dorsal hippocampus. Four 100-Hz trains in PSD slices failed to produce a strong and stable L-LTP, which showed a 37% decrease compared to Ctl (120 min after the first high-frequency train, Figure 2A-B and Table 2). L-LTP amplitude in the PSR group was not significantly different from controls, indicating that L-LTP, like LTP, was entirely restored after 150 min of recovery. We then assessed the effect of vigilance state quantity on L-LTP amplitude. When the last 150 min period was considered, there was a significant positive correlation between L-LTP amplitude and PS quantity and efficacy (Figure 2C-D). No correlation was observed with W and SWS quantities. When the last 24 h were considered, we found that L-LTP amplitude was positively correlated with W quantity but negatively correlated with SWS quantity (Figure 2C). During this period, no correlation was found between L-LTP and PS quantity. Finally, a positive correlation was observed between L-LTP and PS bout duration for the last 150 min (r = 0.46, P = 0.03, data not shown), whereas no correlation was found for SWS bout duration (r = 0.22, P = 0.3; data not shown).

Next, we considered that LTP and L-LTP impairment after PSD might result from the stress related to the deprivation method. In order to assess this nonspecific effect, the plasma corticosterone levels were quantified in rats used for LTP and L-LTP experiments. The results revealed that the corticosterone level was not significantly increased in PSD as compared to Ctl (Table 2). In PSR, a decrease in corticosterone level was observed that was significant compared to Ctl, but not to LP. In addition, no correlation was observed between corticosterone level and vigilance state quantity (n = 20; W: r = 0.359, P > 0.05; SWS: r = -0.299, P > 0.05; PS: r = -0.147, P > 0.05). Finally, corticosterone level was not correlated with LTP (n = 16, r = -0.003, P > 0.05) or L-LTP amplitude (n = 18, r = -0.014, P > 0.05). Altogether, these results suggest that PSD alteration of LTP and L-LTP was not related to the plasma corticosterone level.

**PSD and PSR Modulated SC-CA1 Pyramidal Cell Synaptic Transmission**

We then quantified the basal synaptic transmission at SC-CA1 pyramidal cell synapses in the 4 groups by examining the input/output relationship (see Methods and Figure 3A). To that end, we computed the slope of the input/output curves (I/O slope). Our results showed a significant 35% decrease in the I/O slopes in PSD as compared to Ctl slices (Figure 3B and Table 2). Furthermore, the I/O slopes were also significantly increased by 42% in PSR compared to Ctl slices. As with LTP/L-LTP experiments, we correlated the vigilance state quantity with the I/O slope. We found a significant positive correlation for PS quantity and PS efficacy during the last 150-min period (Figure 3C-D). By contrast, no correlation was observed between the I/O slope and the vigilance state quantity of the previous day.

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PSD Decreased SC-CA1 Pyramidal Cell Synapse Transmission and LTP in the Dorsal, but Not in the Ventral, Hippocampus

Next, we considered the possibility of a differential effect of PSD on the SC-CA1 pyramidal cell synapse in the ventral hippocampus. We first reexamined the input/output curve experiments shown in Figure 3B in relationship with the localization of the recording site (i.e., dorsal vs ventral hippocampal slices). In the dorsal hippocampus, PSD showed a 41% decrease in the I/O slopes as compared with Ctl slices (Figure 4A, Table 2). In contrast, no difference was found in the ventral hippocampus between PSD and Ctl (Figure 4B, Table 2). A differential effect on basal synaptic transmission in the hippocampus was discarded by confirming that I/O slopes were similar in its dorsal and ventral poles for Ctl slices, as previously shown.\(^3\) When the last 150 min were considered, we observed a positive correlation between PS quantity and efficacy and the I/O slope in the dorsal (Figure 4A) but not in the ventral (Figure 4B) hippocampus. In contrast, a negative correlation with SWS quantity was found during the last 150 min and the last 24 h. Finally, we found that PS bout duration was positively correlated with the I/O slope \((r = 0.64, P = 0.004; \text{data not shown})\). SWS bout duration was not correlated with the I/O slope \((r = 0.31, P = 0.2)\).

Given these results which suggest differential effects in dorsal and ventral CA1, we compared the amount of LTP induced by one high-frequency train (100 Hz during 1s) at the SC-CA1 pathway in the ventral hippocampus (Figure 4B4). In contrast with the dorsal hippocampus (Figure 4A4), our results showed that LTP in the ventral hippocampus had similar amplitudes in PSD and Ctl slices. Consistent with the study mentioned above,\(^3\) LTP in Ctl slices was smaller in ventral than in dorsal CA1.

PSD Decreased the Expression of Glutamate Receptor Subtypes in the Dorsal, but Not in the Ventral, Hippocampus

Then, we sought to identify which underlying mechanisms might affect LTP, L-LTP, and the I/O slopes in the dorsal hippocampus. It has been shown that the AMPAR subunit GluR1, its phosphorylated form at Ser-845 site (Phospho-Ser-845-GluR1, p-GluR1), and the NMDAR subunit NR1 are required for CA1 LTP and L-LTP.\(^10,19,22\) We observed a 59% decrease in p-GluR1 level in PSD compared to Ctl in the dorsal hippocampus (Figure 5A1). GluR1 protein expression was reduced by 64% and NR1 by 42% after PSD compared to Ctl group. The protein level of another NMDA receptor subtype, NR2B, was not significantly decreased in PSD (data not shown). We also found that 150 min PSR entirely restored p-GluR1, GluR1, and NR1 expression. In contrast, the ventral hippocampus did not reveal any significant change between the different groups for the protein expression (Figure 5B1). Furthermore, the LP group did not differ from the Ctl group in the dorsal and ventral hippocampus (Figure S4; available at www.journalsleep.org). In order to test a nonspecific effect of PSD on the overall protein expression, we characterized the housekeeping GAPDH protein level along the dorsoventral axis of the hippocampus (Figure 5A1-B1). GAPDH expression was not modified by PSD compared to Ctl group in the dorsal.
condition, a dense labeling was observed in the CA1 stratum of the immunohistochemical staining between groups. In Ctl of ERK1/2 (p-ERK1/2) in CA1, by comparing the intensity

We first examined the localization of the phosphorylated form with an impaired ERK1/2 activation in the dorsal hippocampus. Thus, we sought to determine whether the decrease in glutamate receptor expression levels during PSD was related to chemistry. Thus, we looked to see if the decrease in p-ERK1/2 level by PSD in the dorsal hippocampus.

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The extracellular signal-regulated kinases (ERK1/2), key enzymes of the ERK/MAPK cascade, are required in LTP and L-LTP. ERK1/2 activation occurs through phosphorylation of Thr202/Tyr204 sites, which can be recognized by immunohistochemistry. Thus, we sought to determine whether the decrease in glutamate receptor expression levels during PSD was related with an impaired ERK1/2 activation in the dorsal hippocampus. We first examined the localization of the phosphorylated form of ERK1/2 (p-ERK1/2) in CA1, by comparing the intensity of the immunohistochemical staining between groups. In Ctl condition, a dense labeling was observed in the CA1 stratum radiatum, the region of pyramidal cell apical dendrites, in both the dorsal and ventral hippocampus (Figure 6A1-B1). Consistent with the representative PSD rat shown in Figure 6A1, optical density analysis of the stratum radiatum labeling revealed an almost 2-fold decrease of p-ERK1/2 expression in PSD compared to Ctl and PSR in dorsal CA1 (Figure 6A2). Such a decrease was not found in the ventral stratum radiatum (Figure 6B2).

We then quantified by western blot the protein expression of the 2 isoforms of ERK (ERK1, P44 and ERK2, P42) to compare their total and active (i.e., phosphorylated) forms between conditions. In the dorsal hippocampus of PSD rats, we found a 50% decrease in the phosphorylated isoforms p-ERK1 and p-ERK2 as compared to Ctl rats (Figure 6A3). However, the expression of the total form of ERK1/2 was not modified. Thus, the p-ERK/ERK ratio showed a significant 2-fold decrease in PSD compared to Ctl. In addition, the p-ERK1/2 level was fully restored by 150 min PSR. In contrast, no modification of p-ERK1/2 level and p-ERK/ERK ratio was observed in the ventral hippocampus of PSD rats compared to Ctl (Figure 6B3). Ctl and LP groups showed similar levels of expression in both dorsal and ventral hippocampi (Figure S4). These results suggest a straightforward relationship between the altered expression level of p-GluR1, GluR1, and NR1 and the decrease in p-ERK1/2 level by PSD in the dorsal hippocampus.

**DISCUSSION**

We have shown that a 75-h PSD not only impaired LTP, but also L-LTP at the SC-CA1 pathway. Furthermore our results re-
Methodological Considerations

Our experiments were designed to minimize the induction of stress. For this reason, to perform PSD, we did not use the classical one platform “flower pot” (disk over water) technique that largely increases the corticosterone level. Instead, we suspect a modulation in protein degradation or mRNA translation localized at the dendritic level. The most convincing argument is provided by a recent in vitro study demonstrating that the I/O slopes at the SC-CA1 pyramidal cell synapses, unlike LTP, are not modulated by stress procedures nor corticosterone application along the dorsoventral axis of CA1. This indicates that the selective decrease in synaptic efficacy caused by PSD in dorsal CA1 was not related to stress in the present work. Our data suggest that PSD-induced modulation of LTP, L-LTP and synaptic efficacy is mainly a consequence of a modulation of the vigilance states quantities.

**PSD Causes a Rapidly Reversible Decrease in LTP and L-LTP Amplitude**

We have shown that the maintenance of L-LTP, as assessed by 4 repeated tetani, is impaired by PSD. In addition, the results also indicate that the alteration of LTP by PSD was selectively observed in dorsal but not in ventral CA1. Thus this study adds two important results on the previously known rat immobilization, a major source of chronic stress. We quantified the plasma corticosterone levels and found a nonsignificant increase in PSD compared to controls, confirming a previous study that used a similar MP technique. Furthermore, although it has been shown that an increase in corticosterone level caused by major stress is negatively correlated with LTP, we did not observe any correlation between plasma corticosterone concentration and LTP/L-LTP amplitude. The most convincing argument is provided by a recent in vitro study demonstrating that the I/O slopes at the SC-CA1 pyramidal cell synapses, unlike LTP, are not modulated by stress procedures nor corticosterone application along the dorsoventral axis of CA1. This indicates that the selective decrease in synaptic efficacy caused by PSD in dorsal CA1 was not related to stress in the present work. Our data suggest that PSD-induced modulation of LTP, L-LTP and synaptic efficacy is mainly a consequence of a modulation of the vigilance states quantities.

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Figure 4— PSD selectively impaired LTP and excitatory synaptic transmission in the dorsal, but not the ventral, CA1. **Left panel:** Experiments performed in the dorsal hippocampus as shown by the schematic illustration of the recording site. (A1) PSD altered CA1 synaptic efficacy in the dorsal hippocampus. The summary bar graphs illustrate the mean I/O slopes (± SEM) for each condition. The I/O slopes in the dorsal hippocampus was significantly decreased in PSD (n = 7, 13 slices) compared to Ctl (n = 8, 15 slices, P < 0.02) and PSR (n = 7, 12 slices P < 0.01). (A2) Relationship between the vigilance state quantity and the I/O slope. The correlation coefficient r is represented for each vigilance state during the previous recording day, light-on and light-off periods, and the final 150 min before sacrifice. (*P < 0.05, ***P < 0.001). (A3) Regression analysis for the last 150 min period illustrated in A2. The scatter plots show a strong correlation between I/O slopes and PS quantity or PS efficacy in the dorsal hippocampus. SWS is negatively correlated with I/O slopes (n = 18; empty circle: Ctl, up arrow: PSR, down arrow: PSD, grey square: LP). (A4) Early phase of CA1 LTP is impaired by PSD in the dorsal hippocampus (illustrated in Figure 1). **Right panel:** Experiments performed in the ventral hippocampus as shown by the schematic illustration of the recording site. (B1) In contrast to the dorsal hippocampus, there was no significant difference between conditions in the ventral hippocampus. (B2-3) No correlation was obtained between the vigilance state quantity and the I/O slopes in the ventral hippocampus. (B4) Same amount of CA1 LTP in the ventral hippocampus of PSD (n = 4) and Ctl rats (n = 4).
p-ERK1/2 expression was also decreased by PSD. The GluR1 PKA-dependent site of phosphorylation, Ser-845 (ie. p-GluR1), is required for LTP and L-LTP and we observed an important decrease in p-GluR1 expression in the dorsal hippocampus. However, this result is more difficult to interpret since this decrease was associated with a reduction of the total form of GluR1. Finally, the restoration of LTP and L-LTP by 150 min of PSR was concomitant with the rescue of p-GluR1, GluR1, NR1, and p-ERK1/2 protein expression. Our results thus showed a possible relationship between the PS-dependent regulation of LTP and L-LTP, and the modulation of NR1, GluR1, p-GluR1 and p-ERK1/2 protein expression.

CA1 L-LTP is considered as an important cellular mechanism for memory consolidation. Memory consolidation requires activation of the MAPK/ERK cascade, protein synthesis, and structural reorganization of dendritic spines in CA1 pyramidal cells. Importantly, spatial memory tasks enhance p-ERK1/2 level in dorsal, but not ventral, CA1; and the blockade of the MAPK/ERK cascade in dorsal CA1 selectively impairs spatial memory consolidation. Altogether, these results suggest the potential cellular and molecular mechanisms by which PSD inhibits certain hippocampal-dependent forms of memory consolidation.

Before discussing the putative mechanisms responsible for this LTP and L-LTP disruption, we should discard a potential caveat which might be caused by a PSD-induced decrease in synaptic transmission. A decrease in synaptic transmission might lead to a modulation of cooperativity, a well-known property that controls CA1 LTP. This property relates to the need of the activation of a sufficient number of afferent fibers that cooperate to induce LTP during the high frequency train. This is unlikely since, as indicated in the Methods section, we took great care in adjusting the baseline fEPSPs for the LTP and L-LTP experiments in order to obtain the same amount of cooperativity in the four groups. Moreover, a study showed that altered synaptic transmission does not always result in a hippocampal LTP impairment.

Among other glutamate receptor subtypes, NR1 and GluR1 play crucial roles in LTP and L-LTP and L-LTP. Therefore, it is likely that the decrease in protein level for these glutamate receptor subtypes explains, at least in part, the alteration of LTP and L-LTP. Beside the decrease in GluR1 and NR1 expression, western blotting of dorsal CA1-CA3 homogenates revealed one other candidate for the PSD-induced impairment of L-LTP. ERK1/2 activation is involved in L-LTP maintenance and p-ERK1/2 expression was also decreased by PSD. The GluR1 PKA-dependent site of phosphorylation, Ser-845 (ie. p-GluR1), is required for LTP and L-LTP and we observed an important decrease in p-GluR1 expression in the dorsal hippocampus. However, this result is more difficult to interpret since this decrease was associated with a reduction of the total form of GluR1. Finally, the restoration of LTP and L-LTP by 150 min of PSR was concomitant with the rescue of p-GluR1, GluR1, NR1, and p-ERK1/2 protein expression. Our results thus showed a possible relationship between the PS-dependent regulation of LTP and L-LTP, and the modulation of NR1, GluR1, p-GluR1 and p-ERK1/2 protein expression.

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Figure 6—PSD selectively altered p-ERK1/2 expression in the dorsal, but not the ventral, hippocampus. (A1) Photomicrographs of the dorsal hippocampus from Ctl, PSD and PSR rats show p-ERK1/2 immunostained sections (brown cytoplasmic and dendritic staining). PSD was characterized by a clear decrease in the intensity of labeling in the dorsal hippocampus. CA1: so: stratum oriens, sp: stratum pyramidale, sr: stratum radiatum, DG-iml: inner layer of Dentate Gyrus, CA3-MF: Mossy Fibers. (A2) Optical density quantification of the p-ERK1/2 staining in the CA1 stratum radiatum. The mean optical density ± SEM was assessed through the whole stratum radiatum on 2-3 sections for Ctl, PSD and PSR rats (n = 4 for each condition) in the dorsal hippocampus. The p-ERK1/2 density was significantly decreased for PSD condition compared to Ctl (*P < 0.04) and PSR group (P < 0.05). (A3) Representative immunoblots (top) illustrate the two isoforms ERK1 (■, P44) and ERK2 (□, P42) level of expression and their phosphorylated forms (p-ERK1 and 2) in the dorsal (C1) and the ventral (C2) hippocampus (2 animals for each condition, C: Ctl, D: PSD, R: PSR). The bar graphs (bottom) represent the average expression levels in percentage (± SEM) of the Ctl group (set as 100%) for total ERK1 and ERK2 (left), their phosphorylated fractions (middle) and the p-ERK/ERK ratio (right). Total ERK1/2 expression showed no change between groups in the dorsal hippocampus, whereas p-ERK1/2 level was strongly decreased in PSD (n = 5) compared to Ctl (n = 7, †††: P < 0.001 for the 2 isoforms) and PSR group (n = 7, *P < 0.03 for ERK1). The p-ERK/ERK ratio was also significantly decreased in PSD compared to Ctl (***P < 0.001 for the 2 isoforms) and PSR group (**P < 0.05 for ERK1, P < 0.02 for ERK2). (B1) In contrast with the dorsal hippocampus, immunostained sections of the ventral hippocampus showed no difference. (B2) Optical density quantification revealed no significant change in the ventral hippocampus. (B3) Total ERK1/2, p-ERK1/2 levels and the p-ERK/ERK ratio were not changed by PSD in the ventral hippocampus (Ctl : n = 7, PSD : n = 6, PSR : n = 7).
PSD and PSR-Induced Modulation of Synaptic Transmission

We found that basal synaptic transmission was decreased by PSD. This was not the consequence of previous LTD during PSD since LTD was normal in PSD slices (unpublished results and see^4). In our experimental conditions (presence of extracellular magnesium and GABA-A receptor antagonist), fEPSPs are mostly mediated by AMPAR activation, since NMDAR and GABA-A receptor mediated synaptic transmission are inactive. These functional PSD-induced alterations were only localized in the dorsal hippocampus, shown by a decrease in the expression level of the AMPAR subtype GluR1. Interestingly, GluR1 is an important component of the SC-CA1 AMPAR mediated synaptic response in the adult rodent. Thus, our results suggest that the modulation of GluR1 protein and its phosphorylation level may be, at least in part, responsible for the PSD-induced decrease in synaptic efficacy. However, given that western blot analysis was done on whole homogenates, the issue of postsynaptic membrane insertion of GluR1 is not resolved. Thus, it is important to be cautious before explaining the PSD-induced decrease in synaptic transmission as the resulting effect of reduced GluR1 expression.

In contrast with LTP and L-LTP, we find that synaptic transmission was increased by PSR. This increase in synaptic transmission cannot be entirely explained by the rescue of GluR1 (or p-GluR1) expression, since the GluR1 level is the same in PSR and Ctl groups. This PSR-induced enhancement of synaptic transmission could occur through presynaptic and postsynaptic changes acting synergistically as observed in other conditions where synaptic transmission is increased at SC-CA1 pyramidal cell synapses.29

Mechanisms of PSD-Induced Decrease and Fast Rescue of GluR1 and NR1 Protein Levels

PSD decreased the level of NR1 and GluR1 protein expression in the dorsal hippocampus. First, this result suggests a global decrease in these receptors protein levels, as we used whole homogenates of CA1-CA3 regions. However, we can exclude overall effects of PSD on protein synthesis, as the expression of NMDAR receptor subtype NR2B, the total form of ERK1/2, and the housekeeping protein GAPDH in PSD was identical to Ctl. It has been previously shown that PSD induced a significant increase of the intracellular pool of NR1 and NR2A subunits. This result suggests that PSD also impairs NMDAR cellular trafficking in the hippocampus.11 Interestingly, this study showed a non-significant decrease in the total expression of NR1 and NR2A subunits. In contrast with this study where the location of the tissue extracts in the hippocampus was not specified, we analyzed the protein level in specific hippocampal regions (dorsal vs ventral CA1-CA3). We suggest that the authors may thus have underestimated the decrease of NMDAR subtypes expression by mixing dorsal and ventral hippocampal areas.

We also observed that GluR1 and NR1 mRNA expression was not modified by PSD compared with Ctl. This suggests that the modulation of GluR1 and NR1 protein levels was due to downstream transcription mechanisms, i.e., a regulation of protein degradation and/or translation. It has been shown in cultured hippocampal neurons that synaptic activity regulates the turnover of postsynaptic proteins, including glutamate receptors.44 Proteasome inhibitors prevented the degradation of NR1 and other postsynaptic proteins suggesting the implication of the ubiquitin proteasome system (UPS). Reduced GluR1 and NR1 protein levels might be explained by such a mechanism during PSD. However, the fast rescue of GluR1 and NR1 expression during PSR might not be compatible with UPS activity, according to the slow time-course of its modulation in neurons.44

Alternatively, the fast rescue of GluR1/NR1 protein expression could result from a modulation of mRNA translation. Recently, several studies have revealed the importance of dendritic protein synthesis in hippocampal pyramidal cells as well as the modulation of translational factors and ribosomal proteins by phosphorylation cascades. This local dendritic protein synthesis leads to functional synaptic glutamate receptors, such as GluR1, within the time window of 90 min.21 This time-course is in accordance with the 150 min period of PSR which allowed the rescue of GluR1 and NR1 levels. Interestingly, it has been shown in the hippocampus that changes in neuronal activity may regulate dendritic protein synthesis.21 An enhancement in spontaneous synaptic activity may thus induce decreased dendritic protein synthesis. This homeostatic translational regulation may constitute a major mechanism for synaptic scaling. In addition, these studies indicate that p-ERK1/2 expression may play a key role in regulation of dendritic protein synthesis in CA1 pyramidal cells.45 It was also previously shown that the ERK/MAPK cascade may control the phosphorylation of translational factors and ribosomal proteins.23 Therefore, we hypothesize that PSD-dependent modulation of GluR1/NR1 protein expression might result from a regulation in dendritic protein synthesis under the control of ERK1/2 phosphorylation through the activation of translational factors.

In the hippocampus, ERK1/2 activation is regulated by several neuromodulators, such as trophic factors, monoamines, and neuropeptides. Cellular signaling through β-adrenergic receptor activation and the tyrosine kinase receptor B/ brain-derived neurotrophic factor (BDNF) axis plays an important role in the activation of the ERK/MAPK cascade. The decrease in β-adrenergic receptor binding and in BDNF expression in the hippocampus during PSD46-48 may reduce p-ERK1/2 levels. On the other hand, the rescue in ERK1/2 phosphorylation during PSR may imply dopaminergic and cholinergic neuromodulations. First, dopamine, which is released during PS,49 increases protein synthesis and activates ERK1/2 via D1/D5 receptors.50 Second, hippocampal acetylcholine, which reaches a higher concentration during PS than during W and SWS,51 is also known to activate ERK1/2 and to increase protein synthesis in CA1 pyramidal cells.52-54 Thus, one key issue is now to determine which factor might modulate the level of p-ERK1/2 during PS.

PS as a Positive Regulator of Hippocampal L-LTP and Synaptic Transmission

The regression analysis of vigilance state quantities and synaptic parameters suggests a differential involvement of PS and SWS in LTP, L-LTP, and synaptic transmission. First for LTP, we observe that potentiation amplitude is positively correlated with total sleep quantities (i.e., negatively correlated to W quantities). Interestingly, it has been shown that a short-term total sleep...
deprivation (TSD) also decreases CA1 LTP.54 Furthermore this study indicates that sleep decreases the threshold for LTP induction, possibly by regulating NMDAR activation through the post-synaptic reorganization of NR2 subunits.54 Both sleep states seem to be involved since we find a positive correlation between the duration of both SWS and PS episodes, and LTP during the light-off period and the last 150 min. The duration and number of SWS episodes is considered as an index of SWS fragmentation, and it has been shown that protocols inducing sleep fragmentation (i.e., protocols that decrease the duration of SWS episodes) impair LTP.53 However, PSD also caused a strong PS fragmentation that may contribute to LTP alteration. Thus, both PS and SWS fragmentation may play important roles in the regulation of the threshold for LTP induction.

In contrast with LTP, the regression analysis of L-LTP uncouples the effects of PS and SWS. First, when the last 150 min period is considered, we find that L-LTP was positively correlated with PS quantity and efficacy. However, our results unmask a role of SWS on L-LTP during the last 24-h period. During this period, SWS quantities are negatively correlated with L-LTP amplitude. Assessment of the duration of SWS episodes suggests that this effect is not related to sleep fragmentation. These results suggest that PS and SWS have opposite effects on L-LTP potentially through a differential regulation of ERK1/2 phosphorylation.

Finally, our results suggest that PS quantities might have an important impact on hippocampal synaptic transmission. For the last 150 min period, we find that the synaptic transmission is highly correlated with PS quantity and efficacy. A positive correlation is also observed between synaptic transmission and the duration of PS episodes but not with the duration of SWS episodes. It has recently been found that the strength of synaptic transmission of callosal connections in the frontal cortex is regulated by SWS and W.18 This article reports a negative correlation between synaptic strength and SWS quantities. Although these authors used a different protocol, we observe a similar result in Schaffer collateral synaptic transmission when we consider the 24-h period. This suggests that SWS is an important regulator of synaptic transmission in different cortical networks. Altogether these results suggest that PS and SWS stages might respectively induce synaptic up- or down-scaling, depending on the recent history of vigilance states in the animal.

What is the physiological relevance of these findings? During SWS episodes, synaptic transmission in the hippocampus and frontal cortex are progressively down-scaled. This SWS-induced down-scaling may protect memory traces from interferences during the hippocampo-frontal cortex transfer. After SWS, PS episodes could induce a fast synaptic up-scaling and a facilitation in LTP maintenance in hippocampal networks. Both effects may optimize learning and memory formation during subsequent waking. Studies in humans have demonstrated that sleep plays an important role for memory formation during the next day.56 Given our results on L-LTP and synaptic transmission, we hypothesize that PS might be involved in this process.

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DISCLOSURE STATEMENT

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Figure S1—PSD resulted in a decrease in PS quantities and elicits a large rebound during PSR. Time course of the vigilance states quantities for Ctl (blue line, n = 6), PSD (green, n = 9), PSR (purple, n = 10) and LP (orange, n = 5) groups during the last 27 hours before the slice experiments. Right, the last 150-min period (sacrifice at time = 00:00) and left the last recording day (from −27:00 to −03:00). Vigilance state quantities are expressed as mean percentage ± SEM for Wake (top), SWS (middle), and PS (bottom). The different vigilance states of the LP, PSD, and PSR groups are compared with those of the Ctl group. The level of significance of the Student t-test are shown hour per hour and follow a color code (see upper panel).
Figure S2—Pharmacological characterization of the Schaffer collateral fiber volley. Above: Paired-pulse stimulation of Schaffer collaterals evoked EPSPs (average of 10 traces) in the presence of picrotoxin (left). The EPSPs were entirely blocked by 10 mM kynurenate (middle). The application of tetrodotoxin (1µM) blocked the fiber volley (shown by small straight lines). The stimulus artefacts (shown by filled circles) were partially removed. Below: The figure (left) which shows the subtraction of the trace in picrotoxin by the trace in picrotoxin, kynurenate and tetrodotoxin illustrates the trace after removal of the stimulus artefact. Right, the figure shows the isolation of the fiber volley after subtraction of the trace in kynurenate and picrotoxin by the trace in picrotoxin, kynurenate and tetrodotoxin. PTX: picrotoxin, KYNU: kynurenate (AMPAR&NMDAR antagonist), TTX: tetrodotoxin.
Figure S3—Time course of the $r$ and $p$ coefficients for the regression analysis between the vigilance states quantities and electrophysiological parameters. Linear regression analysis of the vigilance states quantities associated with the synaptic function (LTP, L-LTP and synaptic transmission) was computed hour per hour with data from each animal. The $r$ correlation coefficients for each vigilance state were calculated for LTP (A), L-LTP (B), I/O slopes (C), I/O slopes in the dorsal hippocampus (D), and IO slopes in the ventral hippocampus (E). The $r$ values are given hour per hour for Wk (orange line), SWS (blue), PS (purple) and PS efficacy (black). Left: the last 150-min period before slice experiments (sacrifice at time = 00:00). The $p$ values for Fisher F-test values were given hour per hour and followed a color code (upper panel).
Figure S4—Large platform protocol did not affect the glutamate receptors and p-ERK expression in the hippocampus compared with controls. (A1) Glutamate receptor expression in the dorsal hippocampus. Immunoblots (top) illustrate (from left to right) GluR1, phospho-Ser-845 GluR1 (p-GluR1), NR1 and GAPDH proteins detected in the dorsal hippocampus for representative animals (2 animals for each condition, C: Ctl, L: LP). The summary bar graphs (bottom) represent the average expression levels in percentage (± SEM) of the Ctl group (set as 100%) for GluR1 (black bar), p-GluR1 (white bar), NR1 (grey bar) and GAPDH (black bar). No significative difference was found (Ctl: n = 4, LP: n = 4). The expression level of the housekeeping protein GAPDH was the same in LP and Ctl groups. (A2) As illustrated in A1, Typical immunoblots of ERK1 (■, P44) and ERK2 (□, P42) level of expression and their phosphorylated forms (p-ERK1 and 2). The bar graphs (bottom) represent the average expression levels for total ERK1 and ERK2 (left) and their phosphorylated fractions (right). Total ERK1/2 and p-ERK1/2 expression showed no change between Ctl (n = 4) and LP (n = 4) in the dorsal hippocampus (B1) In the ventral hippocampus, the levels of GluR1, p-GluR1, NR1 and GAPDH were the same in LP and Ctl. (B2) Total ERK1/2 and p-ERK1/2 were not changed in the ventral hippocampus of LP rats.