Cortical Up states induce the selective weakening of subthreshold synaptic inputs

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Slow-wave sleep is thought to be important for retuning cortical synapses, but the cellular mechanisms remain unresolved. During slow-wave activity, cortical neurons display synchronized transitions between depolarized Up states and hyperpolarized Down states. Here, using recordings from LIII pyramidal neurons from acute slices of mouse medial entorhinal cortex, we find that subthreshold inputs arriving during the Up state undergo synaptic weakening. This does not reflect a process of global synaptic downscaling, as it is dependent on presynaptic spiking, with network state encoded in the synaptically evoked spine Ca2+ responses. Our data indicate that the induction of synaptic weakening is under postsynaptic control, as it can be prevented by correlated postsynaptic spiking activity, and depends on postsynaptic NMDA receptors and GSK3β activity. This provides a mechanism by which slow-wave activity might bias synapses towards weakening, while preserving the synaptic connections within active neuronal assemblies.
uring non-rapid eye movement sleep (NREM), local populations of cortical neurons exhibit synchronized membrane potential oscillations between depolarized ‘Up states’ and hyperpolarized ‘Down states’. Up states are intrinsically generated in the cortex via recurrent excitation, with network activity stabilized through balanced increases in inhibition. These patterns of synchronized activity are reflected as slow waves in macroscopic extracellular field potentials, and can propagate across the cerebral cortex as travelling waves, recruiting the majority of neurons in neocortex and paleocortex. The power and incidence of slow-wave activity (0.5–4 Hz in the electroencephalogram) change across development, and are under global and local homeostatic control, suggesting that slow waves may serve an important role in cortical circuit function.

Slow-wave activity increases during infancy and preadolescence, and subsequently decreases over the course of natural maturation. This inverted U-shaped profile has correlates with the density and strength of synaptic connectivity in cortical circuits, paralleling consecutive periods of synaptic genesis, pruning, and synaptic reorganization. It may be that slow waves not only reflect the functional architecture of cortical circuits, but also provide a self-generated substrate for offline retuning following experience. Indeed, even after cortical maturation is complete, there remains a global homeostatic modulation of slow-wave activity, which is high after sustained wakefulness, and dissipates across NREM sleep episodes. There are also region-specific increases in cortical slow-wave activity following experience and learning, and both natural and induced increases in slow-wave oscillations can improve subsequent performance in learned tasks.

While there is strong evidence that slow-wave activity promotes memory consolidation, particularly for declarative memories involving the temporal lobe system, the underlying cellular and circuit mechanisms have yet to be resolved. One prominent model suggests that slow-wave activity drives the reactivation of memory traces across hippocampal-para-hippocampal-neocortical networks, and thus supports the redistribution and transformation of new memories for long-term storage. An alternative hypothesis is that slow-wave activity induces a global downscaling of synaptic weights across cortical
networks, which is necessary to compensate for the net build-up of synaptic weight as a result of experience and learning during the preceding waking period\(^\text{22}\). It has been suggested that this synaptic homeostasis could subsequently promote memory recall by increasing the signal-to-noise ratio of synaptic communication.

A role for slow-wave activity in neuronal reactivation and memory consolidation is not mutually exclusive with a more global process of synaptic homeostasis. Indeed, these hypotheses have been difficult to tease apart, with numerous studies investigating the effect of sleep on synaptic plasticity producing mixed results, with evidence for synaptic depression, but also indications of synaptic potentiation\(^\text{23–25}\). In order to resolve this, it will be necessary to elucidate the specific rules of synaptic plasticity operating during cortical slow-wave activity. Here, we studied these mechanisms of plasticity in acute-slice preparations in vitro, which preserve Up/Down states\(^\text{26, 27}\), while enabling exquisite control over state transitions and synaptic activation\(^\text{28–30}\). In rodents, slow-wave activity emerges in the second postnatal week\(^\text{10}\) and already begins to decline by the end of the third postnatal week\(^\text{31}\). We studied synaptic plasticity during this preadolescent period of high slow-wave activity (P14-21), focusing on the mouse medial entorhinal cortex (mEC), which is a key interface between the hippocampus and neocortex. We demonstrate that the depolarized Up state phase can induce synaptic weakening of subthreshold inputs, which is counteracted by correlated pre- and postsynaptic spiking activity.

**Results**

**Synaptic weakening and maintenance during mEC Up states.** We performed whole-cell current-clamp recordings from mEC layer III principal cells and controlled the precise timing of network Up states and synaptic activation by local electrical stimulation (Fig. 1a). Spontaneous Up states were sparse or absent, while controlled Up state induction was reliable (Fig. 1b). The coincident membrane depolarization, increase in membrane potential variability and activity in the simultaneously recorded local field potential from layer III confirmed that the observed Up states are in fact a cellular manifestation of a network-driven event (Fig. 1c).

Since membrane depolarization and background synaptic activity are well-known factors influencing synaptic plasticity, we sought to determine the effect of pairing synaptic inputs with network Up states (Fig. 1d). To monitor synaptic efficacy, excitatory postsynaptic potentials (EPSPs) were evoked every 15 s during Down states. As evoked waveforms often contain disynaptic inhibitory components, the slope of the EPSP was used as a measure of excitatory synaptic strength. Following the recording of a baseline period, we applied 5 Hz stimulus trains, to approximate the Up state spike rates of mEC layer III principal cells in vivo\(^\text{28}\). The 5 Hz trains were applied in three different plasticity protocols, which had significantly different effects on the EPSP slope measured 10–20 min post-pairing (F\(_{2, 18}\) = 9.63, P < 0.01, \(\eta^2 = 0.52\); One-way analysis of variance (ANOVA)):

(i) In control experiments, 5 Hz stimulus trains were applied during the Down state, for a total of 50 pairings. Following this synaptic activation, the EPSP slope changed only marginally to 108.4 ± 6.5% of the baseline value (n = 6).

(ii) When 5 Hz synaptic stimulation trains were paired with Up states, the slope was significantly reduced to 72.9 ± 7.9% (n = 9, P < 0.01 cf Down state pairing, One-way ANOVA followed by Tukey’s post hoc test; Cohen’s d = 1.95). Despite the Up states being associated with membrane depolarization (9.2 ± 1.3 mV, n = 9; measured prior to train stimulation) and spiking activity (mean: 1.3 ± 0.6 Hz, range: 0.0–4.4 Hz, n = 9), the pairings themselves typically evoked subthreshold responses, consistent with the in vivo observation that sensory-evoked responses are less effective in driving spiking during Up states compared to Down states\(^\text{29}\). Indeed, the pairing during the Up state induced a post-stimulus suppression of spontaneous spiking for up to 45 ms (Supplementary Fig. 1), most likely due to synchronized disynaptic inhibition. Synaptic weakening following pairing did not strongly correlate with either the background Up state spike rate (R\(^2\) = 0.08, P = 0.46, n = 9) or the strength of the evoked response (measured as baseline EPSP slope; R\(^2\) = 0.13, P = 0.35, n = 9; Pearson linear correlation test).

(iii) To determine whether suprathreshold spike pairings could influence plasticity during Up states, 5 Hz trains were again paired with evoked Up states (pre-stimulus amplitude: 9.0 ± 1.6 mV, n = 6), in combination with brief depolarizing current steps to trigger typically one, occasionally two, postsynaptic spikes within the 15 ms period following synaptic stimulation. Correlated pre- and postsynaptic spiking was found to prevent Up-state-induced synaptic weakening, with the change of the EPSP slope of 108.0 ± 3.0% comparable to that observed after Down state pairings (n = 6; n.s. cf Down state pairing, Cohen’s d = 0.02; P < 0.001 cf subthreshold Up state pairing; Cohen’s d = 1.92; One-way ANOVA followed by Tukey’s post hoc test).

The synaptic weakening induced by subthreshold Up state pairings was not specific to 5 Hz stimulation trains, and could also be induced by pairing at 2.5 Hz (72.2 ± 5.2% of baseline EPSP slope, n = 6, t\(_{(5)}\) = 5.35, P < 0.01, one-sample t-test) and 7.5 Hz (72.0 ± 6.0% of baseline EPSP slope, n = 7, t\(_{(6)}\) = 4.63, P < 0.01, one-sample t-test). This suggests that synaptic weakening can occur across a range of stimulation frequencies that approximates the range of Up state spike rates observed in vivo\(^\text{28}\).

High frequency bursting following synaptic inputs can be particularly effective in inducing synaptic plasticity\(^\text{30, 31}\), and might thus enable synaptic potentiation during Up state periods. In 4 of the subthreshold pairing experiments, input-burst pairings during Up state periods were applied following the stabilization of synaptic weakening, and were found to reliably reverse depression, with a trend towards potentiation (85.3 ± 4.9% following subthreshold pairing compared to 124.0 ± 9.3% following burst pairing, measured relative to the initial baseline EPSP slope; t\(_{(3)}\) = 4.00, P < 0.05, paired t-test; Supplementary Fig. 2). We next wanted to test whether input-burst pairings during Up state periods could induce potentiation at naïve synapses, and whether this in turn could be reversed by subsequent subthreshold pairing.

Pairing 5 Hz stimulus trains with spike bursts during the Up state, for a total of 100 pairings, led to an increase in synaptic strength that did not stabilize within the 20 min post-pairing period (Fig. 2). We therefore performed two sets of interleaved experiments—in the first group synaptic responses were measured for >40 min after input-burst pairing (n = 7), and in the second group an additional subthreshold Up state pairing was applied 20 min after input-burst pairing (n = 6). These experiments revealed a significant decrease in normalized EPSP slope measured at 10–20 min and 30–40 min post input-burst pairing (F\(_{1,11}\) = 8.57, P < 0.05; \(\eta^2 = 0.48\), but no significant differences between the pairing paradigms (F\(_{1,11}\) = 0.01, P = 0.96; \(\eta^2 = 0.001\), or interaction between time and pairing paradigm (F\(_{1,11}\) = 0.02, P = 0.90; \(\eta^2 = 0.001\); mixed ANOVA). Across the groups, the normalized EPSP slope was significantly potentiated at 10–20 min (158.5 ± 10.5%, n = 13, t\(_{(12)}\) = 5.47, P < 0.001) and 30–40 min post-input burst pairing (133.5 ± 12.8%, n = 13, t\(_{(12)}\) = 2.62, P < 0.05; one sample t-tests, with Bonferroni correction for multiple comparisons; Fig. 2).

These results suggest that inputs that consistently evoke subthreshold responses during Up states undergo synaptic weakening, while suprathreshold inputs are preserved, protected, and sometimes strengthened. However, with our extracellular stimulation protocol, we were not able to determine the source of
Controlled in either group. Unitary postsynaptic EPSPs were evoked throughout the recording every 15 s by presynaptic spikes during Down states, and the response amplitudes were measured. After a baseline period, Up states were triggered once every 15 s for ~15 min. In the presynaptic suppression group (Fig. 3b), the EPSP amplitude changed on average only marginally to 96.5 ± 3.8% (n = 5) of the baseline value. In the group of pairs with spontaneous presynaptic Up state spiking, however, a gradual reduction in synaptic strength started shortly after Up state induction, and the inputs remained weakened until the recording end (Fig. 3c). This synaptic weakening to 71.1 ± 7.0% of the baseline value (n = 6) was significantly different from the presynaptic suppression group (t(10) = 3.00, P < 0.05, unpaired t-test; Fig. 3d). The results confirm that Up state-dependent weakening of synaptic inputs occurs at recurrent connections within mEC layer III, and demonstrate that the induction can be achieved by spontaneous presynaptic spike patterns. The results also demonstrate that Up state activity alone is not sufficient to modulate synaptic weights, and are consistent with this form of plasticity being input-specific.

As we aimed to explore the effects of naturally evolving spike patterns during Up states, the precise number of presynaptic spike pairings was not controlled. In order to explore whether this might explain some of the variations in the degree of synaptic weakening across experiments, the EPSP amplitude after Up state induction (average of the last 10 min period) was plotted against the mean presynaptic Up state spike rate (Fig. 3e). For the presynaptic suppression group, in which presynaptic spiking was low or absent, there appeared to be a small trend towards synaptic weakening in the experiments showing some presynaptic spikes. However, in the group with spontaneous presynaptic spiking, synaptic weakening became less prominent with higher presynaptic spike rates (R² = 0.76, n = 6, P < 0.05; Pearson linear correlation test). This at first appeared inconsistent with the results of the experiments with extracellular stimulation, in which synaptic weakening could be induced by stimulation at 2.5, 5, and 7.5 Hz. However, pre- and postsynaptic Up state spike rates were correlated (R² = 0.88, n = 6, P < 0.01; Pearson linear correlation test; Fig. 3f), and hence synaptic weakening also became less prominent with higher postsynaptic Up state spike rates (R² = 0.68, P < 0.05; Pearson linear correlation test). The timing of postsynaptic spikes displayed a relatively flat distribution around each presynaptic spike in the Up state (see inset in Fig. 3f), suggesting that increasing Up state spike rates promote spike pairings, and could thus counteract synaptic weakening. A role for spike timing is also consistent with the previous experiments showing that synaptic weakening was prevented by analogous induced suprathreshold pairings (Fig. 1d).

To explore the locus of expression of synaptic weakening, we performed a coefficient of variation (CV) analysis of the paired recording data. The CV of EPSP amplitude increased from 0.26 ± 0.04 (baseline) to 0.33 ± 0.05 (following Up state pairing; n = 6), and plots of the normalized CV−2 and the normalized mean EPSP amplitude showed that most points clustered around the line of unity (Supplementary Fig. 3a). We also applied the same analysis to the EPSP slope measurements recorded during subthreshold Up state pairing with extracellular stimulation (Fig. 1d), and found similar results (CV baseline: 0.26 ± 0.02; CV following Up state pairing: 0.34 ± 0.05; n = 9; Supplementary Fig. 3b). Changes in normalized CV−2 are indicative of altered presynaptic release (see also Supplementary Fig. 3c), but can also be produced by postsynaptic mechanisms, including the changes in the number of silent synapses. We therefore performed an additional series of experiments using paired pulse extracellular stimulation (inter-stimulus interval: 50 ms), and found that paired pulse facilitation increased the stimulated fibers, their spontaneous activity during Up states, or whether the plasticity reflects a global scaling of synaptic inputs. In order to overcome the inherent limitations of extracellular stimulation, and further understand how patterns of pre- and postsynaptic activity control plasticity during network Up states, it was necessary to perform paired-recordings.

**Synaptic weakening by spontaneous Up state spiking.** Extracellular stimulation at the border of the deep layers in mEC is likely to antidromically activate recurrent connections between LIII pyramidal neurons. In order to determine whether Up states could induce weakening at these specific synapses, we performed dual whole-cell patch-clamp recordings from synaptically coupled mEC LIII principal cells (Fig. 3a). Pairs were arbitrarily assigned to one of two groups: in the first group, hyperpolarizing current injection in the presynaptic cell was used to suppress presynaptic spikes during Up states (termed ‘presynaptic suppression group’, mean spike rate: 0.041 ± 0.028 Hz, n = 5; Fig. 3b). This manipulation was designed to test whether it might be possible for inactive synapses to undergo weakening during Up states, due to correlated changes in pre- and postsynaptic membrane potential, heterosynaptic interactions and/or a process of global down-scaling. In the second group, the presynaptic cell exhibited Up state-associated spiking at varying rates (mean spike rate: 4.1 ± 0.6 Hz, n = 6; Fig. 3c). Postsynaptic Up state spiking was not
Fig. 3 Spontaneous spike patterns generated during Up states (US) induce synaptic weakening in a frequency-dependent manner. **a** Two-photon reconstruction of two synaptically coupled mEC LIII principal cells labeled with biocytin. A putative synapse formed between a presynaptic axonal bouton (highlighted in red) and a postsynaptic basal dendrite (blue) is indicated in the magnified region below (white arrowhead). Scale bars: 40 μm/10 μm. **b** Presynaptic spikes were triggered by brief (3–5 ms) depolarizing current steps and the postsynaptic EPSP amplitudes were measured. Following the recording of a 10-min baseline, Up states were also induced once per sweep for a period of ~15 min. When necessary, a constant hyperpolarizing current injection was applied to the presynaptic neuron to keep Up state spiking activity low or absent. **Top:** representative traces of evoked presynaptic (upper) and postsynaptic (lower) activity, during the baseline period (left, averages of ten traces), Up state induction (middle; timing of Up state induction shown with white arrowhead), and at the recording end (right, averages of ten traces; baseline EPSP trace included in blue for comparison). **Bottom:** time course of changes in normalized EPSP amplitude (%). Period of Up state induction is highlighted in green, with numbers indicating the time points used to calculate the average traces. **c** As in **b**, except with presynaptic Up state depolarization sufficient to produce self-generated spike patterns of varying frequencies (n = 6). **d** Summary statistics. *P < 0.05, unpaired t-test. **e** Plot of the relationship between synaptic weakening and presynaptic Up state spike rate (circles: control group with subthreshold/sparsely spiking presynaptic Up states; triangles: group with presynaptic Up state spiking). For the group with spontaneous presynaptic Up state spiking, the degree of synaptic weakening correlated significantly with presynaptic spike rate (Pearson linear correlation test). **f** For the group with spontaneous presynaptic Up state spiking, there was a significant correlation between postsynaptic and presynaptic Up state spike rates (dotted line, Pearson linear correlation test). Bold line indicates unity correlation. Inset: distribution of presynaptic Up state spike timing relative to presynaptic activity from recording shown in **c**. Error bars show the SEM.

State dependence of synaptically evoked spine Ca\textsuperscript{2+} responses. Previous studies in quiescent slice preparations of neocortex and hippocampus have found that coincident pre- and postsynaptic activity can induce synaptic depression, with similar indicators of a presynaptic component of expression, and have shown that this plasticity is dependent on increases in postsynaptic Ca\textsuperscript{2+} concentration\textsuperscript{32–35}. Depolarization during network Up states might be expected to boost postsynaptic Ca\textsuperscript{2+} signaling, and thus enable state-dependent synaptic weakening. However, predicting...
the effect of Up states on synaptically evoked Ca\textsuperscript{2+} responses is not straightforward, as they involve the coordinated firing of excitatory and inhibitory neurons, and, for example, co-active spine-targeting interneurons could selectively inhibit local Ca\textsuperscript{2+} signaling\textsuperscript{36}. To investigate the influence of Up states on spine Ca\textsuperscript{2+} responses, we combined wide-field spine Ca\textsuperscript{2+} imaging with local electrical activation of synapses (Fig. 4a, b). mEC layer III principal cells were loaded with the calcium indicator OGB (200 μM) for at least 30 min via a patch-pipette. Another micro-electrode was positioned near a basal dendrite (~5–20 μm distance to imaged spine) for local synaptic activation (Fig. 4a). We targeted basal dendritic locations where we had previously observed LIIs-LIIs synapses (Fig. 3a). Cells were minimally hyperpolarized to minimize Up state spiking, and prevent contamination of the recorded postsynaptic Ca\textsuperscript{2+} transients by back-propagating action potentials. Evoking subthreshold Up states did not induce discernible Ca\textsuperscript{2+} transients in the target spine (Fig. 4b). In contrast, local synaptic stimulation during Down states resulted in EPSPs and Ca\textsuperscript{2+} transients in the spine head, while the adjacent dendritic shaft showed only a marginal change in fluorescence (Fig. 4b). Synaptic activation of the same input during Up states typically produced a smaller change in the somatic membrane potential, but also reliably evoked Ca\textsuperscript{2+} responses that were mostly restricted to the spine head (Fig. 4b). To quantify the state dependence of spine Ca\textsuperscript{2+} transients, synaptic activation was alternately triggered during Down state and Up state periods, and the mean signals evoked during successful synaptic transmissions were then compared (Fig. 4c). The average Ca\textsuperscript{2+} response evoked during Up states (ΔF/F, 45.3 ± 3.5%) was significantly increased compared to the average Down state response (ΔF/F, 34.6 ± 3.3%; n = 8 spines from 8 different cells, t\textsubscript{8} = 3.16, P < 0.05, paired t-test). Although we did not observe prominent Ca\textsuperscript{2+} responses in dendritic shafts during Up states, the wide-field imaging configuration is unable to eliminate scatter and out-of-focus interference. We therefore performed the same stimulation paradigm using two-photon spine Ca\textsuperscript{2+} imaging (Fig. 4a, d), in mEC layer III principal cells loaded with the calcium indicator Fluo-5F (400 μM) for at least 30 min via a patch-pipette. In some recordings, spontaneous spikes were recorded, which as expected, elicited Ca\textsuperscript{2+} responses in both the spine head and neighboring dendritic shaft. However, the synaptically evoked Ca\textsuperscript{2+} responses were mostly restricted to the spine head (Fig. 4d). Moreover, the average Ca\textsuperscript{2+} response evoked during Up states (ΔF/F, 46.6 ± 7.4%) was again found to be consistently and significantly increased compared to the average Down state response (ΔF/F, 31.4 ± 4.6%; n = 5 spines from 5 different cells, t\textsubscript{5} = 2.93, P < 0.05, paired t-test; Fig. 4e). Thus, both the wide-field and two-photon imaging data show that synaptically activated dendritic spines have information about the network state encoded in their mean Ca\textsuperscript{2+} response during the cortical Up states.
Fig. 5 Synaptically evoked spine Ca\(^{2+}\) transients depend on NMDAR. 

a Wide-field imaging experiments were used to pharmacologically examine the contribution of NMDAR to spine Ca\(^{2+}\) transients evoked by local synaptic stimulation, with the cells loaded with Alexa 594 (40 μM) and OGB (200 μM). For structural and Ca\(^{2+}\) imaging, respectively. a To mitigate the potential effects of run-down over the time course of drug application, synaptically evoked Ca\(^{2+}\) transients were imaged during a baseline period (B), and following treatment (T) with either control aCSF (left) or bath-applied D-AP5 (40 μM; right). Top: regions of interest for imaging Ca\(^{2+}\) response in the spine head. Middle: relative changes in OGB fluorescence (∆F/Φ) in the spine head following local synaptic stimulation during baseline and treatment conditions. Scale bars: 1 μm. Bottom: corresponding EPSPs evoked by local synaptic stimulation. Scale bars: 50 ms/1 mV (control) and 40 ms/2 mV (D-AP5). b Pooled data showing the mean spine ∆F/Φ responses (400 ms window) in the treatment condition relative to baseline. **P < 0.01, paired t-test. Error bars show the SEM.

Synaptically evoked Ca\(^{2+}\) transients in spine heads have previously been shown to depend on activation of N-methyl-D-aspartate receptors (NMDAR)\(^{37–40}\). We examined whether NMDAR also contribute to spine Ca\(^{2+}\) transients in mEC layer III principal cells using the wide-field imaging set-up (Fig. 5a). Blocking NMDAR with bath application of 40 μM D-AP5 was found to largely eliminate spine Ca\(^{2+}\) signals (mean ∆F/Φ measured relative to baseline period; control: 101.0 ± 25.8% vs. D-AP5: 7.2 ± 6.3%; n = 5 spines from 5 different cells in each group, t\(_{(8)}\) = 3.54, P < 0.05, unpaired t-test; Fig. 5b). Under our conditions, this also blocked evoked Up states, which is consistent with a role for NMDAR in Up state initiation in acute slices of mEC\(^{41}\). However, this data does suggest that NMDAR drive synaptically evoked spine Ca\(^{2+}\) transients, although other Ca\(^{2+}\) sources are likely to contribute to these spine transients, and their additional boost during Up states.

Postsynaptic mechanisms regulating synaptic weakening. As NMDAR provide a major source for synaptically evoked Ca\(^{2+}\) transients in spine heads\(^{37–40}\), and these transients are boosted in Up states relative to Down states (Figs. 4 and 5), we tested the role of postsynaptic NMDAR in Up state-dependent synaptic weakening using single-cell pharmacology. We repeated the subthreshold Up state pairing protocol (Fig. 1d) with two experimental groups: in the control group the patch-pipette was filled with standard internal solution, while in the second group the NMDAR antagonist MK-801 was additionally included (Fig. 6a). The inhibitor and control internal solutions were given ~30 min for equilibration after breakthrough, before recording baseline responses. Following Up state pairing, synaptic weakening to 68.8 ± 11.7% (n = 5) in the control group was significantly reduced to 94.42 ± 3.3% (n = 6) in the inhibitor group (t\(_{(9)}\) = 2.30, P < 0.05, unpaired t-test). This result supports a role for postsynaptic NMDAR in Up state-induced plasticity, but it is possible that presynaptic NMDAR could have been exposed to MK-801 during the patching procedure, and not recovered from use-dependent block over the approximately 40 min of equilibration and baseline recording. We used two approaches to explore this: (i) Glutamatergic terminals in the entorhinal cortex are thought to express GluN2B subunit-containing NMDAR, which tonically facilitate glutamate release\(^{42}\). We first confirmed that washing on the GluN2B subunit-selective antagonist Ro 25-6981 (0.5 μM) reduced the slope of evoked EPSPs in layer III pyramidal neurons (64.3 ± 9.6% of baseline, n = 6, t\(_{(5)}\) = 3.72, P < 0.05, one sample t-test; Supplementary Fig. 4a). In the presence of 0.5 μM Ro 25-6981, it was still possible to evoke Up states, and the subthreshold Up state pairing protocol (Fig. 1d) was found to induce synaptic weakening to 78.2 ± 4.9% (n = 4, t\(_{(3)}\) = 4.48, P < 0.05; one sample t-test), which was not significantly different from that obtained during interleaved vehicle controls (81.8 ± 4.7%, n = 4; t\(_{(6)}\) = 0.54, P = 0.69; unpaired t-test; Supplementary Fig. 4b). This suggests that inhibiting presynaptic NMDAR does not prevent Up state-induced synaptic weakening. (ii) As NMDAR are sensitive to voltage-dependent Mg\(^{2+}\) block, we also tested whether subthreshold postsynaptic depolarization would be sufficient to induce synaptic weakening in the preadolescent mEC layer III pyramidal neurons. Pairing 5 Hz trains with depolarizing current steps, to mimic the somatic depolarization observed during Up states, induced a significant reduction in synaptic strength to 88.9 ± 3.6% (n = 5, t\(_{(4)}\) = 3.10, P < 0.05, one sample t-test; Supplementary Fig. 4c). The changes in synaptic strength were not as large as those observed in any of the control subthreshold Up state pairing groups, but support the interpretation that synaptic weakening is under postsynaptic control. An important role for postsynaptic NMDAR in synaptic weakening following Up state pairing is consistent with many models of long-term depression (LTD)\(^{43}\), although they do not appear to be necessary for presynaptic LTD induced by spike timing protocols\(^{33–35}\).

Activity of glycogen synthase kinase-3β (GSK3β) can gate postsynaptic induction of NMDAR-dependent LTD\(^{44}\), and, interestingly, is more active during sleep in rats\(^{45}\). The same intracellular pharmacology approach was used to assess a possible role for GSK3β in the Up state-associated synaptic plasticity (Fig. 6b). Including the GSK3β inhibitor SB415286 in the patch-pipette, at a concentration that had previously been reported to block LTD\(^{44}\), also fully prevented synaptic weakening from 80.16 ± 4.1% (n = 6) in the vehicle control group (0.01% DMSO in internal solution) to 106.2 ± 5.2% (n = 6) in the inhibitor group (t\(_{(10)}\) = 3.91, P < 0.01, unpaired t-test). This suggests that synaptic weakening of subthreshold inputs during Up states depends on the postsynaptic activity of both NMDAR and GSK3β.

Discussion

During slow-wave sleep, anesthesia, and even quiet wakefulness, cortical neurons display a slow synchronized membrane potential oscillation between Up and Down states, reflected as slow waves in macroscopic extracellular field potentials. How this activity pattern regulates synaptic plasticity, a likely cellular substrate for memory, has remained largely unexplored. Here, we studied the rules and mechanisms of synaptic plasticity during the Up-Down oscillation as slow waves in the preadolescent mEC. We found that subthreshold inputs evoked by extracellular stimulation during Up states undergo synaptic weakening. Furthermore, we were able to demonstrate that spontaneous patterns of Up state spiking can induce synaptic weakening at identified recurrent connections between LIII pyramidal neurons. In classic spike-timing dependent plasticity, presynaptic activity that precedes postsynaptic spiking induces synaptic potentiation\(^{46}\). We found that pairing synaptic inputs with postsynaptic spiking during Up states affected synaptic strength with the same directionality: pairing inputs with single or double spikes maintained synaptic strength, while burst pairing
could enable either potentiation at naïve synapse or the reversal of synaptic weakening. Interestingly, potentiated synapses appeared to be protected against subsequent Up state-induced synaptic weakening. In summary, Up states seem to promote a reduction in synaptic efficacy that can be counteracted by coordinated postsynaptic spiking. As we typically recorded for less than thirty minutes after plasticity induction, we here referred to the observed depression as ‘synaptic weakening’. However, inputs typically remained weakened until the recording end at a stable level, and, thus, this synaptic weakening is likely to be a form of LTD.

The induction of synaptic plasticity is governed by multiple mutually-dependent factors, including firing rates of the pre- and postsynaptic cell, spike timing, and postsynaptic depolarization. Even for the same synapse type, the precise patterns of activity during plasticity induction can influence the molecular pathways recruited, and result in a pre- and/or postsynaptic form of plasticity. Here, we were able to induce synaptic weakening by extracellular synaptic stimulation paired with Up states, despite predominantly low or absent postsynaptic Up state spiking. This result is in agreement with the repeated observation in neocortex that presynaptic activity coinciding with moderate postsynaptic depolarization induces LTD. However, whereas many previous induction protocols involve an artificial modification of the membrane potential (e.g., via voltage-clamping or current injection), in our experiments, this depolarization was generated by network Up states. An independence of postsynaptic spiking for plasticity induction could be beneficial: while forms of plasticity that rely on postsynaptic spiking differentially affect synapses depending on their location, Up states occur throughout the dendritic tree, and, thus, properties of synaptic weakening could be more uniform within dendrites. We also showed that Up state-associated synaptic weakening induced by extracellular stimulation involves postsynaptic NMDA receptors and GSK3β. NMDAR-dependent LTD is well documented, and the NMDA-mediated Ca2+ influx in the postsynaptic cell is a critical element in the induction process. Interestingly, we find that synaptic activation during Up states leads to increased spine Ca2+ transients compared to Down states. Moreover, GSK3β, a kinase that was previously found to be important for the induction of NMDAR-dependent LTD in hippocampus, is also likely to be regulated by Ca2+ signaling pathways. Thus, our results are consistent with a model in which NMDA receptors, and possibly other Ca2+ sources such as voltage-gated Ca2+ channels, mediate a boost of synthetically evoked Ca2+ transients during Up states, which contributes to the induction of synaptic weakening by activating downstream signaling pathways that involve GSK3β.

We do not have conclusive evidence as to whether synaptic weakening is pre- and/or postsynaptically expressed, but there are some indications. Analyses of changes in the CV and paired pulse facilitation, based both on our paired-recording data and on the subthreshold Up state pairings experiments with extracellular synaptic stimulation, were consistent with a presynaptic component of expression. Similar changes in the statistics of synaptic transmission have been observed for postsynaptic depression induced by pairing inputs with a depolarizing current step in neocortical pyramidal cells. This protocol mimics the membrane potential trajectory observed during subthreshold Up states, and we were also able to partially reproduce Up state-induced synaptic weakening in mEC with postsynaptic membrane depolarization. In order for presynaptic expression to fully explain our results, retrograde signaling to the presynaptic terminal would have to be dependent on the activation of postsynaptic NMDAR. However, LTD that depends on postsynaptic NMDAR is often postsynaptically expressed, and it has been suggested that GSK3β regulates postsynaptic AMPA receptor function and trafficking. It may be that synaptic weakening induced during active network states involves a mixture of presynaptic and postsynaptic modifications. Indeed, the frequency and amplitude of miniature excitatory postsynaptic currents, measured in cortical slices of rats and mice, were found to be increased after prolonged wakefulness relative to recordings obtained after sleep, consistent with both presynaptic and postsynaptic changes. Further studies will be required to resolve the precise molecular mechanisms underlying the induction and expression of Up state-induced synaptic weakening, and their interaction with spike-related signaling.

Oscillations between cortical Up and Down states are a characteristic feature of slow-wave sleep. We studied state-
dependent synaptic plasticity in acute slices of mEC, which cannot mimic the complex distributed patterns of network activity that occur during natural sleep. However, our results do suggest that Up states could support elements of both synaptic homeostasis and the consolidation of synaptic memories. In our experiments, Up states promoted synaptic weakening, which could be prevented by correlated simple spiking activity. Such a plasticity mechanism is not suitable for simple synaptic scaling, but could be consistent with more selective renormalization, in which synapses are spared if they are sufficiently large or on crowded dendritic branches60, and thus potentially more likely to drive postsynaptic spiking. We also found that pairing synaptic inputs with burst firing during Up states induced synaptic strengthening, which could support an additional process of consolidation. A potential role for NREM activity patterns in supporting synaptic strengthening is consistent with a recent study in the hippocampal CA1 – this brain region shows sharp-wave–ripple activity during cortical slow-wave activity, and the study found that mimicking sharp waves was necessary to induce potentiation during the replay of spike patterns recorded in vivo21. It should be noted that when we examined the effects of Up state spike patterns generated spontaneously in vitro, we only observed variable degrees of synaptic weakening. Of course, even the preservation of synaptic weights within reactivated cell assemblies, on a background of more global synaptic weakening, could alone mediate a form of consolidation. A potential link between our results and sleep-related synaptic plasticity could be provided by the involvement of GSK3β activity. This kinase has been implicated in memory reconsolidation61, is more active during sleep45, and would be expected to promote synaptic weakening. Moreover, GSK3β activity can be locally inhibited following LTP44, which could explain why we were unable to induce Up state-induced weakening following input-burst induced potentiation, and offer a biochemical level of protection for recent synaptic memories. Interestingly, increased GSK3β activity has also been implicated in Alzheimer’s disease (AD) pathology, including the disruption of synaptic plasticity62, 63 and memory processing64. While there is likely to be a complex interaction between AD pathology, sleep, and circuit dysfunction65, it is possible that GSK3β over-activity in AD could preclude the fine tuning of synapses during slow-wave sleep.

In order to more fully understand the implications of our results, it will be important to determine whether the features of state-dependent synaptic plasticity observed here extrapolate to other brain regions and life stages, operate in vivo during slow-wave activity, and are capable of inducing persistent changes in synaptic structure and function. Slow-wave activity has also been suggested to provide privileged periods for homeostatic regulation of intrinsic excitability66, neuronal energy homeostasis, cellular repair, and the clearance of toxic substances and metabolites from the brain67, 68. To what extent each of these processes might contribute to the beneficial effects of sleep has also yet to be determined. However, this study characterizes the rules and mechanisms of synaptic plasticity that can operate during Up/Down states, and could thus provide a crucial mechanistic entry point for future research attempting to establish links between synaptic tuning and the higher-level functions of slow-wave sleep.

**Methods**

**Animals and acute-slice preparations.** All procedures involving animals were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986. C57BL/6 mice (P14–P21) were decapitated under deep isoflurane-induced anesthesia, and the brains placed in ice-cold artificial CSF (aCSF) containing (in mM): 126 NaCl, 3.5 KCl, 1.25 NaH₂PO₄, 1 MgSO₄, 2 CaCl₂, 26 NaHCO₃, 10 glucose, at pH 7.2–7.4 when bubbled with carbogen gas (95% O₂, 5% CO₂). Horizontal brain slices (350 μm) were prepared using a Leica Vibratome VT1200S, and transferred to an interface chamber filled with room temperature carbogated aCSF to recover for at least 1 h. For recordings, slices were transferred to submerged chamber, and superfused with carbogated aCSF heated to 32–34°C.

**Electrophysiology.** Whole-cell patch-clamp recordings from mEC layer III principal cells were obtained with standard borosilicate glass micropipettes (6–8 MΩ containing (in mM): 110 potassium-glucuronate, 40 HEPES, 2 ATP-Mg, 0.3 GTP, 4 NaCl (pH 7.2; 270–290 mOsM 1⁻). Biocytin (Sigma-Aldrich) was added at 4 mg ml⁻¹ to allow post hoc assessment of cell morphology. For local field potential (LFP) recordings, a micropipette (4–6 MΩ) was advanced with a calibrated 300 μm pipette placed in mEC layer III. In combined electrophysiology and multiphoton imaging experiments, current-clamp recordings were carried out using an Axon Multiclamp 700A amplifier (Molecular Devices) and digitized using an Axon Digidata 1440A, with data acquisition and stimulation protocols controlled using Axon pClamp. For all other experiments, recordings were obtained using an Axon Multiclamp 700B amplifier and digitized using an ITC-18 A/D board (Instrutech), with data acquisition and stimulation protocols controlled using custom-written procedures in IgorPro (WaveMetrics). Whole-cell recordings and local field potential (LFP) signals were low-pass filtered at 3 kHz, and acquired at 10 kHz. LFP signals were amplified (×1000), band-pass filtered (20–1000 Hz), and averaged using Axograph X (Neurosoft).

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5 or 10 stimuli per train, and with trains triggered every 15 s. Train stimulation was at 5 Hz, approximating typical Up state spike rates. In the subthreshold Up state pairings from Fig. 1d, whether the 50 total pairings were applied as 5 or 10 stimuli trains made virtually no difference on the degree of synaptic weakening achieved: 69±4.3% (n = 4) and 71±5.9% (n = 5), respectively, and these data were therefore combined. Local synaptic stimulation during spine Ca²⁺ imaging was achieved with a micropipette, filled with aCSF and Alexa 594 (40 μM) for placement under the fluorescence microscope. In spine Ca²⁺ imaging experiments, Up states were evoked 400–500 ms before synaptic activation was triggered – this was within the train stimulation period, while ensuring that phasic Ca²⁺ events could not be induced directly by the Up state stimulation electrode.

Pharmacology. In some patch-clamp experiments, MK-801 (200 μM; purchased from Sigma-Aldrich) and SB415286 (10 μM in internal solution + 0.1% of the vehicle DMSO; purchased from Abcam) were additionally included in the patch-pipette. The inhibitor and control internal solutions were given in an initial, ~30 min, equilibration time. Half of the experiments were blinded. Up state spike rates were relatively low (0.2±0.08, n = 23). It needs to be mentioned that there were two GSK isoforms (GSK3α and GSK3β) expressed in the mammalian brain. However, since the GSK3β isoform is generally considered to be the critical one in regulating synaptic plasticity, we refer throughout this study to GSK3β as the main target of SB415286.

Fluorescence imaging. An Olympus BX51WI microscope equipped with a CAIRN Research OptoLED Lite system, optiMOS xCMOS camera (QImaging), and a 40×/0.8 NA water-objective (Olympus) was employed for live-cell two-photon imaging, while a 40×/0.15 NA water-objective (Olympus) was used for high-resolution imaging of Ca²⁺-fluorescence microscope. In spine Ca²⁺ imaging experiments, Up states were bleach-corrected by fitting an exponential function to the original trace (i.e., correcting the original trace accordingly. The spine response was quantified as the mean ΔF/F value from the 200 ms period after synaptic stimulation (for the quantification of two-photon line-scans, a 20 ms offset was introduced due to the typically better temporal resolution). To examine the effect of the slow oscillation phase on synaptic evoked Ca²⁺ transients, raw data (in ΔF/F) of successful synaptic transmissions (peak Ca²⁺ response > 2.5 SD of baseline noise) from each group were determined and the means compared; using wide-field and two-photon microscopy, respectively, 4.0±0.5 and 3.6±0.4 responses in the Down state groups and 4.1±0.4 and 3.4±0.5 responses in the Up state groups were on average included. To investigate the dependence of spine Ca²⁺ transients on NMDAR, responses recorded ~5 min after D-AP5 or aCSF (control) bath application were normalized to the respective baseline response (mean of 5 ± 2.5 SD of baseline noise) from each group difference between group means divided by the estimate of the pooled standard deviation. Data are presented as mean ± SEM.

Data availability. Supporting data are available on request.

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Broadly, GABAergic interneurons play a crucial role in the control of neuronal excitability. The balance between excitatory and inhibitory synapses is finely regulated to maintain the proper functioning of the cerebral cortex. The present study aimed to investigate the role of GABAergic interneurons in the maturation of long-term potentiation (LTP) and depression (LTD) in the rodent hippocampus. The authors used a combination of electrophysiological and pharmacological approaches to address this question.

Key findings included the demonstration that GABAergic interneurons are essential for the normal development of LTP and LTD in the rodent hippocampus. The authors found that the activity of GABAergic interneurons is critical for the proper induction and expression of LTP and LTD. This finding highlights the importance of GABAergic interneurons in the regulation of synaptic plasticity and suggests that their dysfunction may lead to pathological changes in the brain.

The study contributes to our understanding of the cellular mechanisms underlying synaptic plasticity and provides insights into the role of GABAergic interneurons in brain function and disease. Further research is needed to elucidate the precise mechanisms by which GABAergic interneurons regulate synaptic plasticity and to explore the clinical implications of these findings.