

Phase-locking of hippocampal interneurons' membrane potential to neocortical up-down states

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During quiet wakefulness and sleep, and under anesthesia, the membrane potentials of neocortical pyramidal neurons show synchronous, slow oscillations, so-called up-down states (UDS), that can be detected in the local field potential (LFP). The influence of this synchronized, spontaneous neocortical activity on the hippocampus is largely unknown. We performed the first *in vivo* whole-cell recordings from hippocampal dorsal CA1 interneurons and found that their membrane potentials were phase-locked to neocortical up-down states with a small delay. These results provide strong evidence for cortico-hippocampal interaction and suggest that neocortical activity drives hippocampal interneurons during UDS.

Cortico-hippocampal interaction during sleep is thought to be important for learning¹. During quiet wakefulness² and sleep³, and under the influence of a wide range of anesthetics such as urethane and isoflurane, the predominant neocortical spontaneous activity pattern is indicative of slow-wave sleep (SWS), that is characterized by 0.1- to 2-Hz oscillations of the membrane potential between the up and down states²⁻⁴. The UDS of cortical pyramidal neurons are highly synchronized and can be clearly detected in neocortical LFPs (refs. 2,3). In contrast, the hippocampal LFP predominantly shows large irregular activity (LIA) that is thought to be mostly uncorrelated with neocortical SWS oscillations^{1,5}. Recent studies have described correlated activity between cortical and hippocampal pyramidal neurons during various stages of sleep⁶⁻⁹. However, the contribution of hippocampal interneurons to the cortico-hippocampal dynamics has received little attention.

The R-LM interneurons, located at the border of the stratum radiatum and the stratum lacunosum moleculare, of the dorsal CA1 region of the hippocampus exert feedforward inhibition on other CA1 neurons, and may thus have a powerful inhibitory influence on hippocampal dynamics and sculpt cortico-hippocampal interactions^{10,11}. The primary inputs to these R-LM interneurons come from the neocortex via the entorhinal cortex and CA3 (ref. 12). Hence, we hypothesized that the synchronous neocortical up-down states would influence these neurons. To test this hypothesis, we recorded the activity of seven R-LM interneurons from the dorsal hippocampal CA1 region using whole-cell recordings in urethane-anesthetized mice.

Simultaneously, using LFP measurements, we monitored the synchronous up-down states from layer 2/3 of parietal cortex, which strongly projects to the hippocampal formation. All experimental procedures were carried out according to the animal welfare guidelines of the Max Planck Society (**Supplementary Methods** online).

We observed that the neocortical LFP showed pronounced UDS, and the R-LM interneurons' membrane potential also showed UDS modulation (example shown for a single R-LM interneuron in **Fig. 1a**; micrograph of the cell in **Fig. 1b**). In each experiment, for these and all subsequent computations, we used data acquired contiguously over at least 900 s. The average value of interneurons' membrane potential was -57.3 ± 1 mV (mean \pm s.e.m.). The average membrane potential during the down states was -64.4 ± 0.8 mV, whereas the up-state amplitude was highly variable. Autocorrelations of all neocortical LFPs (**Fig. 1c**) and all the interneurons' membrane potentials (**Fig. 1d**) showed slow, ~ 0.5 -Hz up-down state oscillations. Thus, the activity of R-LM interneurons of the hippocampus exhibited up-down states.

The hippocampal interneurons' UDS could either be independent of the neocortical UDS or related to it. To discriminate between these two possibilities, we computed the cross-correlation coefficient, which is bounded between +1 and -1, with +1 indicating the highest possible covariation, between the neocortical LFP and the interneuron's membrane potential (**Fig. 2a**). The cross-correlation was large and positive for all the interneurons (0.44 ± 0.06) and highly significant ($P = 0.0004$ *t*-test, $P = 0.008$ binomial test) at zero latency. This strong and positive correlation ruled out the possibility that the interneuronal up-down states were independent of the neocortical ones.

The positive correlation between neocortical and interneuronal up-down states could arise due to three general mechanisms: hippocampal up-down states could contribute to generating the neocortical up-down states, or vice versa, or the two could be driven by a common source. To differentiate between these three possibilities, we did three independent computations.

First we computed the latency at which the membrane potential-LFP (MP-LFP) cross-correlation attained a maximum. This maximal correlation (0.59 ± 0.03) occurred at positive latencies for all the interneurons (average latency 162 ± 50 ms, $P = 0.008$ binomial test), indicating that the up-down states of the hippocampal interneurons' membrane potentials occurred after the up-down states in the neocortex. This negated the first of the three mechanisms listed above.

Second, we computed the average of the interneuron's membrane potential, triggered at the time when the neocortical LFP made the transition from the down to the up state. If the interneuronal up-down states were a result of the neocortical ones, the LFP-triggered average of the interneurons' membrane potential should also show down-to-up transitions, which should occur shortly after those in the neocortical LFP. The results of our analyses were consistent with this prediction (**Fig. 2b**). The LFP-triggered average of the interneuron's membrane

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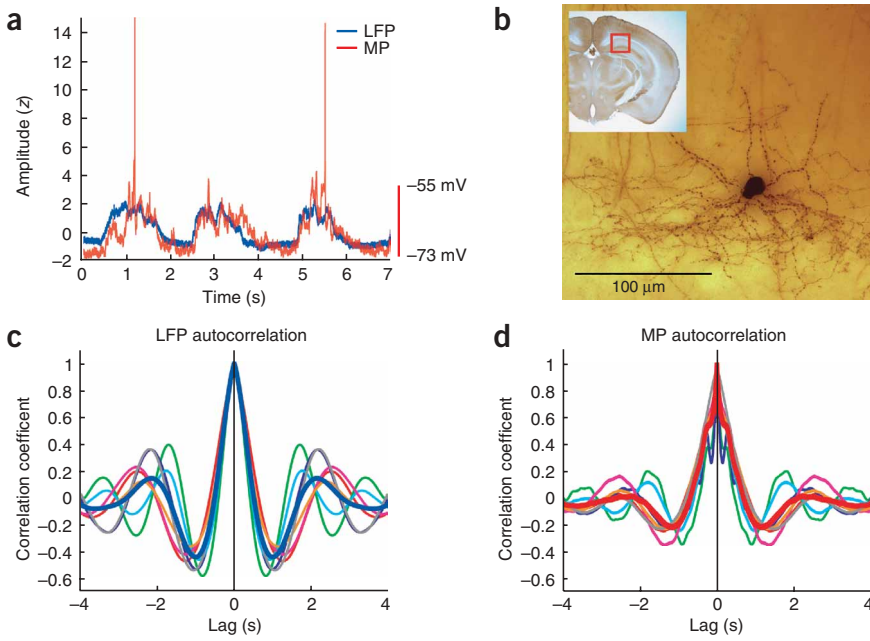


Figure 1 Up-down states in neocortex and hippocampus. **(a)** Up-down state modulation of simultaneously recorded neocortical layer 2/3 LFP (blue) and a CA1 interneuron's membrane potential (MP) (red). The membrane potential (mV, vertical red bar) was converted to Z-score to allow comparison between the LFP and MP (**Supplementary Methods**). **(b)** A micrograph of the inhibitory interneuron from the molecular layer of CA1, from which the membrane potential shown in **a** was recorded. Inset, location of the shown cell. **(c)** Autocorrelation of neocortical LFP. Thick blue line, average LFP autocorrelation for all seven experiments; thinner colored lines, autocorrelation of individual LFPs. **(d)** Autocorrelation of interneuronal membrane potential. Thick red line, average membrane potential autocorrelation for all seven neurons; thinner colored lines, autocorrelations of the individual interneuron's membrane potentials. The same color scheme is used in **c** and **d**, and in **Figures 2a,b** and **Supplementary Fig. 1**. Vertical black line at the center of this and all the subsequent figures indicates zero latency. The autocorrelations of all the neocortical LFP and the CA1 interneuron's membrane potential showed a ~ 0.5 -Hz up-down state modulation.

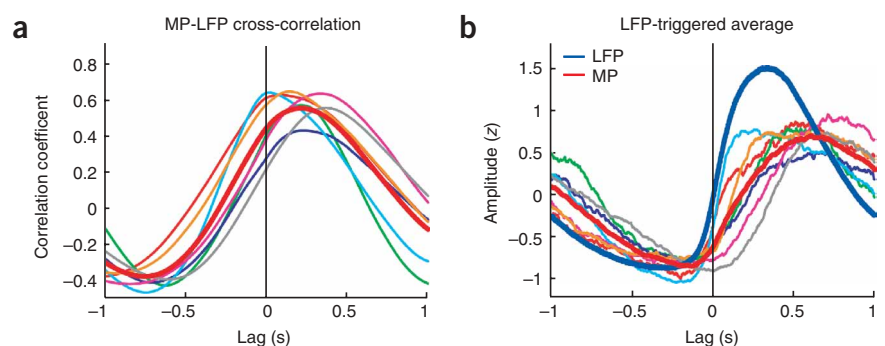
potential was large and significantly different from 0 (0.79 ± 0.05 , $P = 0.000009$ *t*-test, $P = 0.008$ binomial test), demonstrating that the interneuron's membrane potential was phase-locked to the neocortical up-down state. Moreover, the LFP-triggered average membrane potential reached a maximum 184 ± 69 ms ($P = 0.04$) after the neocortical LFP reached its maximum value. This latency was not significantly different ($P = 0.76$) from the phase lag described above (163 ± 50 ms), obtained using the MP-LFP cross-correlation.

Third, we computed the correlation between the interneuronal action potentials and the neocortical LFP (**Supplementary Fig. 1** online). The mean firing rate of the interneurons was 0.41 ± 0.23 Hz. We used 2,893 action potentials to compute the action potential-LFP (AP-LFP) correlation. This correlation also showed a significant maximum (0.0097 ± 0.0025) at positive latencies (402.6 ± 215.8 ms). These results are consistent with the results of the sub-threshold MP-LFP correlation noted above, but are considerably more variable, perhaps because a much smaller amount of data contributed

to the computations of AP-LFP correlations than to that of the MP-LFP correlations. These results demonstrated that the UDS modulation of the interneurons' subthreshold membrane potential was also reflected in their spiking activity, which could influence the dynamics of other neurons in the hippocampus.

Although it is beyond the scope of this study to estimate the up-down state modulation of the diverse hippocampal interneuron groups or types^{10,11}, it is notable that all the recorded R-LM interneurons from the dorsal CA1 behaved in a similar fashion. Specifically, all the interneurons showed clear UDS modulation (**Fig. 1d**, $P = 0.008$, binomial test), the depth of their UDS modulation was weaker than that of neocortical LFP (**Fig. 2b**, $P = 0.008$, binomial test), the maximum MP-LFP correlation occurred at positive latencies (**Fig. 2a**, 162 ± 50 ms, $P = 0.008$, binomial test), and the membrane potential made a transition to the up state after the LFP did (**Fig. 2b**, 184 ± 69 ms, $P = 0.008$, binomial test). This suggests that these results may hold for most R-LM interneuron types.

Figure 2 CA1 interneurons' membrane potential is phase-locked to neocortical LFP with a short delay. **(a)** Correlation coefficient between neocortical LFP and interneuronal membrane potential. Thick red line, average of all experiments; thinner colored lines, individual experiments. The correlation was positive at zero latency for all the MP-LFP pairs ($P = 0.008$ binomial test), and it reached a maximum of 0.56 ± 0.04 at 162 ± 50 ms. Thus, all the CA1 interneurons' membrane potentials were significantly phase-locked with and phase-lagged with respect to the neocortical LFP ($P = 0.006$ *t*-test, $P = 0.008$ binomial test). **(b)** Both the average neocortical LFP (thick blue line) and the interneuronal membrane potential (thick red line), triggered around down-to-up transitions of the LFP, showed clear down-to-up transition modulations. Thin colored lines, LFP-triggered membrane potential for individual interneurons. The maximum cortical LFP occurred on average 345 ± 19 ms after the down-to-up transition trigger. The maximum of interneuronal membrane potential occurred 529 ± 64 ms after the transition trigger. The LFP-triggered membrane potential of the interneurons showed clear up-down state modulation, with the maximum interneuronal membrane potential occurring significantly after the maximum neocortical LFP ($P = 0.04$).



Phase-locking of the interneuron's membrane potential with neocortical UDS, the smaller UDS depth of modulation of the interneuron's membrane potential compared to that of neocortical LFP, and a small phase lag between the two are all consistent with the hypothesis that the neocortical UDS propagate to the hippocampus and generate UDS in the R-LM interneurons. This hypothesis is consistent with the observation that a primary source of inputs to R-LM interneurons comes from the neocortex, which shows highly synchronized UDS. The results support neither the hypothesis that the hippocampal interneuronal activity is independent of neocortical activity during SWS, nor the hypothesis that the hippocampal activity drives the neocortex during SWS. However, our findings can neither rule out other mechanisms, such as a common source of UDS inputs to the neocortex and hippocampus, nor establish a clear causal relationship between neocortical and hippocampal activities during slow-wave sleep.

These results provide strong evidence for a cortico-hippocampal interaction. They also provide the first evidence for the involvement of hippocampal interneurons in this interaction. Given the similarities between the up-down states in anesthetized and sleeping animals, it is conceivable that similar results would hold in sleeping animals. The phase-locking of hippocampal interneuronal and neocortical activities would have a strong impact on the activity of CA1 pyramidal neurons activity, and might be relevant for consolidating recently learned information¹³ to the neocortex by means of mechanisms of plasticity^{1,14,15}.

Note: Supplementary information is available on the Nature Neuroscience website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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