Integration and Segregation of Activity in Entorhinal-Hippocampal Subregions by Neocortical Slow Oscillations

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Summary

Brain systems communicate by means of neuronal oscillations at multiple temporal and spatial scales. In anesthetized rats, we find that neocortical “slow” oscillation engages neurons in prefrontal, somatosensory, entorhinal, and subcortical cortices into synchronous transitions between UP and DOWN states, with a corresponding bimodal distribution of their membrane potential. The membrane potential of hippocampal granule cells and CA3 and CA1 pyramidal cells lacked bimodality, yet it was influenced by the slow oscillation in a region-specific manner. Furthermore, in both anesthetized and naturally sleeping rats, the cortical UP states resulted in increased activity of dentate and most CA1 neurons, as well as the highest probability of ripple events. Yet, the CA3-CA1 network could self-organize into gamma bursts and occasional ripples during the DOWN state. Thus, neo/paleocortical and hippocampal networks periodically reset, self-organize, and temporally coordinate their cell assemblies via the slow oscillation.

Introduction

The mammalian cortex is traditionally divided into neocortex (isocortex), paleocortex, and archicortex (hippocampus) and their subdivisions on the basis of cellular architectonics and connectivity patterns (MacLean, 1990). However, the physiological mechanisms that allow segregation and integration of neuronal information in the highly interconnected cortical-hippocampal networks (Sporns et al., 2000) are poorly understood. One hypothesis is that the spatial extent of neuronal recruitment and the direction of activity flow are controlled by a large family of neuronal oscillators (Buzsáki and Draguhn, 2004). Because the dominance of particular cortical oscillations is controlled by brain state and the animal’s behavior (Steriade and Buzsáki, 1990; McCormick, 1992; Buzsáki and Draguhn, 2004), it is expected that the functional boundaries of cooperative ensemble activity are determined by the particular constellations of oscillators at any given moment (Buzsáki, 1996; Friston, 2000; Sirota et al., 2003; Pelletier et al., 2004).

In the neocortex, deep slow wave sleep is characterized by widespread synchronized oscillatory patterns, defined primarily by spatially coherent delta waves (Achermann and Borbely, 1997). Neocortical delta waves represent transient (200–500 ms) cessation of synaptic and spiking activity of both principal cells and interneurons of all cortical layers, followed by episodes (0.3–1 s) of sustained activity (Steriade and Buzsáki, 1990; Battaglia et al., 2004). Similarly, under anesthesia, silent and active periods alternate regularly, giving rise to “slow” oscillation (0.5–1.5 Hz) (Steriade et al., 1993a, 1993b, 1993c; Amzica and Steriade, 1995; Destexhe et al., 1999). Intracellulary, the active and silent periods of network patterns, respectively, correspond to plateau depolarization, associated with subthreshold oscillations, intense synaptic barrage, and spiking activity (UP state) and hyperpolarized quiescence or disfacilitation (DOWN state), both under anesthesia (Steriade et al., 1993b; Cowan and Wilson, 1994) and natural slow wave sleep (Timofeev et al., 2001; Petersen et al., 2003). Although slow oscillations can also be observed in the thalamus (Hughes et al., 2002) and the basal ganglia (Wilson and Kawaguchi, 1996), it is regarded as an intrinsic neocortical pattern because it not only survives after surgical isolation of a slab of neocortex from its thalamocortical and subcortical inputs (Timofeev et al., 2000) but also persists in a small piece of neocortical tissue in vitro (Sanchez-Vives and McCormick, 2000; McCormick et al., 2003; Shu et al., 2003; Cunningham et al., 2006). The mechanisms that govern UP and DOWN states of neurons may include network-level excitatory-inhibitory mechanisms (Steriade et al., 1993b; McCormick et al., 2003) and intrinsic properties of the constituent neurons (Cowan and Wilson, 1994; Amzica and Steriade, 1995; Wilson and Kawaguchi, 1996; Sanchez-Vives and McCormick, 2000; Cunningham et al., 2006).

Here we utilize the alternation between synchronous activity and silent periods in the neocortex (Steriade et al., 1993c; Amzica and Steriade, 1995; Achermann and Borbely, 1997; Battaglia et al., 2004) as a unique temporal window to examine how neocortical, paleocortical (parahippocampal), and hippocampal networks integrate and segregate the activity of their neurons without the disadvantage of surgical or pharmacological manipulations. Do synchronous neocortical patterns associated with slow oscillations invade and affect all parahippocampal and hippocampal subregions equally...
Are these subregions controlled by neocortical inputs or can they support independent, self-organized gamma and ripple oscillations (Buzsaki et al., 1992; Bragin et al., 1995) in the absence of neocortical inputs? To address these questions, we simultaneously recorded and analyzed local field potentials and extracellular unit activity in one cortical area and intracellular activity in another cortical area in anesthetized rats, as well as local field potentials and extracellular unit activity in cortical and hippocampal regions in naturally sleeping rats.

Results

Bimodality of Membrane Potential in Neo- and Paleocortical Neurons

In 108 rats, we obtained stable intracellular recordings from neurons from the neocortex (prefrontal, n = 34; somatosensory, n = 22; visual n = 3) and paleocortex (entorhinal cortex, n = 27; subiculum, n = 26) with simultaneous local field potential (LFP) recordings from hippocampal CA1 pyramidal layer or from multiple sites in the CA1-dentate gyrus axis (n = 17) or, in some cases (n = 7), in the neocortex or entorhinal cortex. All the somatosensory neurons have been identified morphologically following intracellular injection of biocytin. In the absence of hippocampal theta oscillations (Buzsaki, 1996), neocortical and paleocortical neurons showed a prominent slow oscillation (0.5–1.5 Hz), consisting of alternating depolarized UP states with spikes and hyperpolarized DOWN states, resulting in a bimodal distribution of membrane potential (Figures 1A–1E and see Figure S1 in the Supplemental Data available online), confirming and extending previous observations both in vivo and in vitro (Steriade et al., 1993b, 1993c; Sanchez-Vives and McCormick, 2000; McCormick et al., 2003; Shu et al., 2003; Dickson et al., 2003; Cunningham et al., 2006). The duration of the UP and DOWN states alternated relatively regularly, giving rise to a peak power between 0.5 and 1.5 Hz (Figures 1A–1E, 1I, and Figure S1).

Similar to neocortical cells, principal neurons in both superficial (layer 2, n = 6; layer 3, n = 14) and deeper (layer 5, n = 8) layers of the entorhinal cortex (Figures 1B–1D) and the subicular pyramidal cells (Figure 1E;
n = 25) and one subicular interneuron displayed spontaneous alternations in their membrane potential between UP and DOWN state. During the UP state, neurons showed robust fast oscillations in the membrane voltage (20–70 Hz; gamma range), and one or several spikes per UP episode were triggered by the peaks of the fast oscillation. However, their firing rates during the slow oscillation periods (UP and DOWN states combined) were not, on average, significantly different from the firing rates during hippocampal theta periods (Table S1). Current injection-induced hyperpolarization of the membrane prevented the occurrence of the spikes but not gamma oscillations. During the DOWN states spiking was rarely observed, and fast oscillations were either absent or significantly less than in the UP state. The above observations suggest that, similar to the neocortex, UP states of entorhinal and subicular neurons during slow oscillations are determined primarily by network-generated synaptic mechanisms.

Quantitative assessment showed that the distribution of the membrane potential values was either bimodal or skewed in almost all neurons in the neocortex and paleocortex, with a large variance in the UP and small variance in the DOWN states (Figure 1G). The voltage difference between UP and DOWN states was smallest in layer 2 stellate/pyramidal cells of the entorhinal cortex, but, in pyramidal neurons of layers 3 and 5 and subiculum, the potential fluctuation was comparable to that of the neocortical cells (Figure 1F). The transition from DOWN to UP states was sharp and similar in all neurons. However, the UP to DOWN transition was significantly slower in entorhinal layer 3 neurons than in the other cell types (Figure 1H; p < 0.01; ANOVA and Duncan’s multiple range test). Similar to neocortical neurons UP and DOWN states alternated rhythmically (Figure 1I).

Coherent Slow Oscillations in the Neo- and Paleocortical Networks

The widespread nature of the slow oscillation was demonstrated by the robust synchrony of activity between structures. DOWN states could be recognized visually by the hyperpolarized membrane potential in intracellular recordings and by positive going local field potentials in deep layers associated with a reduction of fast activity and the absence or extreme paucity of extracellularly recorded spikes. In contrast, UP states were associated with fast activity in both intracellular and extracellular recordings. Simultaneous intracellular and extracellular recordings from various cortical locations showed that UP-DOWN fluctuations occurred coherently across virtually the entire neocortex, entorhinal cortex, and subiculum (Figure 2 and Figure S3). Prominent membrane potential fluctuations in a single neuron reliably correlated with the population patterns of the extracellularly recorded units and field potentials of a distant structure (Figure 2A). These observations were quantified by analyses in the frequency domain. Spectral power of both LFP and intracellular potential showed peaks between 0.5–1.5 Hz (Figure 2B). Coherence values were largest at the frequency of peak power of the slow oscillation (Figure 2C). The magnitude of phase difference between field and intracellular potential increased linearly with frequency for most neurons, indicating that fixed time delays determine the magnitude of phase shift between the respective oscillations. In
addition to coherence between the intracellular and extracellular signals, the strongest correlation between the power fluctuations of LFP and the intracellular signal was also confined to the slow oscillation band (Figure 2D). For group comparison, the extracellular recording site in the CA1 pyramidal layer was used for the assessment of neocortical slow oscillation (see justification below). The highest phase and power correlation with the LFP was observed in neocortical neurons and layer 5 entorhinal cells, followed by superficial entorhinal and subicular neurons (Figure 2E; see also Figure S2).

Absence of Membrane Potential Bimodality in Hippocampal Neurons

To compare the behavior of membrane potential of neocortical cells with that of hippocampal neurons, we examined intracellular activity of hippocampal neurons in an additional 106 rats. All recorded neurons were morphologically identified. In contrast to the robust bimodal distribution of the membrane potential in neocortical, entorhinal, and subicular neurons, prominent voltage fluctuation of the membrane potential was not present in hippocampal pyramidal neurons during cortical slow oscillations (Figure 3), and bimodality was present in only one CA3 neuron. Five of the eight granule cells, 28 of the 58 CA1, and 12 of the 41 CA3 pyramidal cells had skewed membrane potential distribution, whereas, in the remaining principal neurons and a single CA1 basket cell, the distribution of membrane potential values was unimodal and symmetric (Figures 3A–D). Fast activity, reflecting mostly synaptic inputs, was almost continuously present, in contrast to the regular alternation of “noisy” and “quiescent” epochs in neocortical, entorhinal, and subicular neurons.

Modulation of Hippocampal Circuits by Slow Oscillations

Although no bimodality of the membrane potential was observed in intracellular (somatic) recordings of hippocampal neurons during cortical slow oscillations, the slow oscillation-related synchronous discharge of entorhinal neurons may nevertheless impose a detectable effect on the hippocampal network. To examine this hypothesis, we monitored the laminar distribution of currents in the hippocampus, triggered by the DOWN-UP transitions of the intracellular membrane potential in entorhinal cortical neurons (n = 14 rats). The depth position of the recording electrodes in the CA1–dentate axis was calibrated by stimulus-evoked potentials in response to perforant path or commissural path stimulation and by the spontaneously occurring sharp waves. Current-source density (CSD) analyses of these events precisely identify the various cell body and dendritic layers in the hippocampus (Ylinen et al., 1995) (Figures 4A and 4B). The largest current sink, associated with the DOWN-UP shift, occurred in the str. lacunosum-moleculare in all rats (Wolansky et al., 2006; Kloosterman, 2003). In 8 of the 14 experiments, an additional sink was also present in the dentate molecular layer (e.g., Figure 4C), indicating excitatory synaptic activation of hippocampal neurons by the DOWN-UP shifts in entorhinal layer 2 and 3 cells (Amaral and Witter, 1989). The rhythmic nature of excitatory drive was quantified using coherence analysis between LFP at multiple hippocampal locations and the intracellular signal in each experiment (Figure 4D). For the representation of group data, principal component analysis was performed on the depth profiles of coherence at the frequency of slow oscillation across all animals (see Experimental Procedures). The depth profile of the first component likely reflected the contribution of volume-conducted currents produced by slow oscillation in neocortical circuits (see also Figure 2E) (Steriade et al., 1993a). The peak of the second component corresponded to the rhythmic sink locations in the molecular layer and str. lacunosum-moleculare, the target layers of the entorhinal inputs (Figure 4E).

Volume conduction of the potentials associated with neocortical slow oscillations to the hippocampus was exploited to relate the activity of intracellularly recorded neurons (n = 137) of different brain structures and separate animals to a common reference, i.e., the phase of slow oscillation derived from LFP in the CA1 pyramidal layer. LFP in the CA1 pyramidal layer reliably reflected volume-conducted slow oscillations generated in the neocortex as shown by the uniform phase (~180 degrees) of DOWN-UP transition of the intracellular membrane potential of neocortical cells (Figure S3). Superficial entorhinal cortical cells and subicular neurons, on
average, were phase shifted by approximately 60° relative to neocortical and layer 5 entorhinal neurons, corresponding to a 150–200 ms time delay (Figure S3). The phase shift was similar in layer 2 and layer 3 neurons. Although hippocampal pyramidal cells did not display bimodal membrane potential distribution, the CSD analysis suggested that pyramidal cells nevertheless were affected by the cortico slow oscillation.

To uncover the relationship between membrane potential of hippocampal neurons and the phasic modulation of slow oscillation, we analyzed the joint probability density function (JPDF) of the membrane potential of individual neurons and the phase of the slow oscillation (Figure S4). This analysis showed that the membrane potential of all neocortical, paleocortical, and most CA1 hippocampal cells is indeed biased by the phase of the slow oscillations (Figure 5A; see also Figure S4 for raw membrane potential). In contrast to neo/paleocortical neurons, the maximum relative depolarization of most CA3 cells coincided with the neocortical DOWN state. The phase relation of individual CA1 neurons varied across the entire cycle, suggesting that some may respond preferentially to the entorhinal input whereas others to the phase-shifted CA3 neurons. The slow oscillation-related intracellular activity of granule cells could not be examined because in those experiments the extracellular electrode was placed in the CA3-hilar region rather than the CA1 pyramidal layer.

To further investigate the phase relationship between slow oscillations and neuronal activity in the CA3-CA1 regions and dentate area, we examined the firing patterns of the extracellularly recorded and isolated principal cells and putative interneurons (n = 946 units in n = 137 rats). Analysis of individual units revealed three broad classes of CA1 units, preferentially active during UP, DOWN, or both states (Figure 5 and Figure S5). Although the proportions of neurons in each class may have been affected by the level of anesthesia, units representing different classes were observed on the same electrode (Figures 5B–5D). In-phase and anti-phase firing preferences were also observed in putative CA1 interneurons (Figure 5B). In the subset of animals implanted with silicon probes (n = 17), units were also recorded in the CA3 region and/or dentate gyrus. Neurons in the dentate region were preferentially active during the UP state of slow oscillation, whereas most CA3 units discharged preferentially during the DOWN state (Figures 5C–5E). In accordance with the membrane potential analysis of the intracellularly recorded cells, these findings are compatible with the hypothesis that, during the neocortical UP state, CA1 neurons are driven either directly by the layer 3 entorhinal input or by the dentate-CA3-CA1 trisynaptic pathway, whereas, in the DOWN state, the self-organized activity in the CA3 region is their main driving force.

**Periodic Reset of Neo-, Paleocortical, and Hippocampal Activity during Natural Sleep**

To examine to what extent the modulatory effects of slow oscillation on hippocampal activity is due to the effect of anesthesia, we reexamined many aspects of the above experiments in naturally sleeping rats (n = 9). DOWN states and concurrent delta waves in the neocortex were associated with cessation of neuronal activity in the neocortex, entorhinal cortex, and the dentate gyrus (Figure 6). Similar to the observations under anesthesia, the majority of CA3 neurons showed an enhanced discharge probability at times when the entorhinal input was silent (Figure 6A). The majority of CA1 neurons fired during the UP state, but a significant number of cells preferred to discharge during the DOWN state, likely driven by the synchronous activity of CA3 neurons. The similarity of the patterns between anesthesia and sleeping is further supported by the preserved phase relations of dentate and CA3 neurons in the same animal (Figure 6B).
Neo-, Paleocortex-Dependent, and -Independent Gamma Oscillations in the Hippocampus

The apparent slow oscillation-modulated depolarizations in single hippocampal neurons suggest that hippocampal network activity is also modulated by the slow oscillation. Gamma frequency power in the neocortex is known to be modulated by slow oscillations in all layers (Steriade et al., 1993b; Hasenstaub et al., 2005; Mukovski et al., 2006). In contrast, gamma power in the hippocampus varied as a function of both depth and phase of slow oscillation. Maximum gamma power in the dentate gyrus and CA1 str. radiatum sometimes alternated in phase (Figure 7A), suggesting that prominent gamma oscillation epochs in str. radiatum can emerge in the neocortex. Color-coded histogram (bottom), stacked cross-correlograms of 89 significantly correlated CA1 cells (n = 8 rats) sorted by time lag of their peaks. Blue histogram, distribution of the time lags of cross-correlogram peaks of CA1 neurons relative to the UP state. Note that the majority of CA1 cells fire preferentially in the UP state.
also during neocortical-entorhinal DOWN state. The average spectrograms of the LFP in str. radiatum, aligned to the onset of the UP state, showed a dominance of gamma power associated with the cortical DOWN state. In contrast, gamma activity in the dentate gyrus and str. lacunosum-moleculare was invariably larger during the UP state of entorhinal cortical cells (Figure 7B). To quantify the effect of slow oscillations on the intrahippocampal network activity, we computed the coherence between the membrane potential of entorhinal cells and the instantaneous gamma power (20–60 Hz) in the hippocampus. As in the behaving rat (Bragin et al., 1995), average gamma frequency power increased with depth in the CA1-dentate axis and also exhibited power fluctuations at slow oscillation frequency (Figures 7E and 7G). Coherence and phase shift between gamma power of the LFP and intracellular membrane potential varied differentially and significantly across hippocampal layers (Figures 7F, 7H, and 7I; n = 14 rats). Coherence values were highest in str. lacunosum-moleculare, the molecular layer, and the CA1 pyramidial layer and lowest in str. radiatum. In addition, the phase shift between gamma power and slow oscillation depended on the recording site and was larger in str. radiatum than in other layers (Figures 7F and 7I and Figure S7). These observations, combined with phase-shifted dominant activity of CA3 neurons (Figure 5), suggest that the CA3 circuit can generate self-organized gamma oscillations independent of cortico-entorhinal inputs, whereas gamma activity in the dentate gyrus is entorhinal input dependent.

Similar observations were made in naturally sleeping rats (n = 4 rats). Gamma power in the entorhinal cortex, dentate gyrus, and CA1 region correlated strongly with the UP state. In accordance with the enhanced activity of CA3 neurons during neocortical delta waves (Figure 6A), gamma oscillation trains confined to CA1 str. radiatum were often observed during the DOWN state (Figures 7C and 7D). The main difference between
sleeping and anesthetized animals was the higher frequency of gamma oscillation in the sleeping animal (compare Figures 7B and 7D).

Slow Oscillation Biases Ripple Generation
In addition to gamma oscillations, the CA3-CA1 region also gives rise to the most synchronous hippocampal pattern, the sharp wave-ripple complex (Buzsaki, 1996; Chrobak and Buzsaki, 1996), although at a significantly reduced rate in the anesthetized rat compared to the drug-free animal (Ylinen et al., 1995). Ripples occurred mostly in the UP state of the slow oscillation and were robustly correlated with the discharge of CA1 neurons. Ripples that occurred in the DOWN state evoked transient, large depolarizations and spiking in subicular and entorhinal neurons, after which the membrane potential reverted to the DOWN state (Figure 8A). Thus, ripple-associated synchronous hippocampal output was effective, however, in depolarizing target neurons, whereas the output associated with gamma episodes was not (n = 5; Figure S8). Analysis of the relationship of ripple occurrence relative to the time of DOWN-UP transition of the superficial entorhinal neurons (Figure 8B) and phase of the slow oscillation (Figures 8C and 8D) revealed that the lowest probability of ripple occurrence coincided with the onset of cortical DOWN state and increased to higher levels after the DOWN-UP transition, marked by the trough of the slow oscillation LFP. Observations in naturally sleeping animals corroborated these findings. At the group level, most ripples occurred during the UP state, particularly at ~100 ms after the entorhinal DOWN-UP transition and ~200 ms after the neocortical DOWN-UP shift (Figures 8E and 8F). Thus, although the sharp wave-ripple complex is a genuine intrahippocampal event (Buzsaki, 1996; Kubota et al., 2003), its occurrence can be temporally biased by the phase transition of neocortical networks from silence to activity (Siroti et al., 2003).

Discussion
During delta waves of sleep and slow oscillations of anesthesia, most neuronal activity in the neocortex becomes synchronously and periodically silent (Steriade et al., 1993c; Achermann and Borbely, 1997). Our findings extend these observations by showing that the paleocortical, entorhinal, and subicular structures and the dentate gyrus of the hippocampus are integral parts of this transient quiescence. Although the CA3 and CA1 network can sustain organized patterns independent of the neocortex (DOWN state), activity of their neurons is biased temporally by the slow oscillations.

Periodic Reset of Neocortical-Paleocortical-Hippocampal Activity
A remarkable feature of slow oscillation is the synchrony over large cortical areas (Amzica and Steriade, 1995; Destexhe et al., 1999; Battaglia et al., 2004). Our findings show that slow oscillation-related alternation of activity and silence expands from the prefrontal cortex to the hippocampal dentate gyrus and can also affect the CA3 and CA1 regions. The simplest explanation of synchronous activity over such a large cortical territory is to assume a common drive from a shared input. For example, inputs from the basal forebrain or brainstem...
whose neurons discharge in phase with the slow oscillation (Detari et al., 1997; Lestienne et al., 1997; Duque et al., 2000) can pace widely distributed areas of the cerebral cortex. An alternative common drive to the cortex involves the thalamus, because thalamic neurons also display UP-DOWN bistability (Hughes et al., 2002), timed by the corticothalamic inputs (Steriade et al., 1993a). However, the common drive hypothesis cannot fully account for our observations that CA3 and CA1 neurons were also active during the neocortical-paleocortical DOWN states. In addition, the sparsity of thalamic projection to the entorhinal cortex, subiculum, and hippocampus (Amaral and Witter, 1989) further limits the critical role of the thalamus in slow oscillations. Finally, the common drive hypothesis cannot simply explain the fronto-caudal spread of the slow oscillation, demonstrated in the human brain (Massimini et al., 2004) and the time shift between neocortical and dentate oscillations shown here. Alternatively, slow oscillations arise in the recurrent local circuits of deep cortical layers (Sanchez-Vives and McCormick, 2000; Huber et al., 2004; Luczak et al., 2006), and the widespread synchrony is brought about by long-range cortical connections originating in superficial cortical layers (Amzica and Steriade, 1995; Sporns et al., 2000; Shu et al., 2003). The importance of the superficial pathways is supported by our experiments. Both layer 2 and 3 entorhinal inputs to the hippocampal formation produced large sinks and gamma oscillations in the dentate molecular layer and str. lacunosum-moleculare and discharged a portion of the target neurons. Therefore, we hypothesize that in the intact brain the excitatory front of slow oscillation spreads from the neocortex to the hippocampus by way of the entorhinal cortex.

Defining features of the slow oscillation are the abrupt shifts of the membrane potential at the neuronal level and the periodic alternation of activity and silence at the network level (Steriade et al., 1993b; Shu et al., 2003). Extensive recurrent collaterals and balanced excitation and inhibition have been hypothesized to be the major requirements for the persistence of the UP state (Steriade et al., 1993b; Sanchez-Vives and McCormick, 2000; Hasenstaub et al., 2005), whereas the DOWN state is thought to be brought about by activity-induced intrinsic conductances and/or the metabolic constraints of the Na/K-ATPase pump (Cowan and Wilson, 1994; Wilson and Kawaguchi, 1996; Cunningham et al., 2006). Our findings imply that these necessary conditions for the UP state may not be sufficient for the maintenance of slow oscillations because recurrent collateral excitation and balanced inhibition and excitation are also present in the hippocampus, yet hippocampal neurons did not show bimodal distribution of their membrane potential. Instead, they were either driven by the excitatory entorhinal volleys or generated intrinsic gamma or ripple oscillations without robust bistable shifts in the membrane potential, indicating that hitherto not well-understood differences exist between neo/paleocortical and hippocampal neurons and networks. Recently, it has been suggested that the hippocampus gives rise to an independent slow oscillation because alternating sinks and associated unit discharges were observed in the hippocampus, concurrent with neocortical slow oscillations (Wolansky et al., 2006). Our findings are at odds with this interpretation and indicate instead that slow oscillations arise in neo- and paleocortical circuits, which impose their outputs on the hippocampal networks. From the perspective of dynamical systems, synchronization by slow oscillations within and between networks can be explained by coherence resonance. In this formulation, coupled linear (bistable) networks engage into synchronized oscillatory dynamics at an “optimum” level of noise. The different network size or connectivity of the hippocampus may prevent it from generating slow oscillations on its own ( Pikovsky et al., 2001), yet its activity can be paced by the neo/paleocortical inputs.

**Hippocampal Subregions Can Generate Independent Activity Patterns**

Although the hippocampal formation is generally thought of as a feedforward excitatory path through which coordinated patterns are propelled from the dentate gyrus to the CA1-subicular output regions (Amaral and Witter, 1989), our findings indicate that hippocampal subregions cooperate differently under various conditions. Nearly all dentate gyrus and the majority of CA1 neurons fired preferentially in the UP state concurrent with increased gamma frequency oscillations in the dentate molecular layer and CA1 str. lacunosum-moleculare. This slow oscillation-related “resetting” of hippocampal activity is similar to the periodic decrease of hippocampal unit discharges at the onset of neocortical delta waves in naturally sleeping rodents (Sirotta et al., 2003; Moelle et al., 2006). CA1 neurons active in the UP state might have been driven by either the direct layer 3 entorhinal input (Charpak et al., 1995) or the trisynaptic intrahippocampal path. In the DOWN state, two types of self-organized patterns were present: gamma oscillations and ripples. Previous work in vitro has shown that, under the right pharmacological conditions, gamma oscillations and ripples depend on the CA3 recurrent collateral system (Fisahn et al., 1998; Kubota et al., 2003; Maier et al., 2003). Furthermore, surgical removal of the entorhinal cortex enhances the power of gamma oscillations in the CA3-CA1 regions and increases the incidence of ripple events (Bradein et al., 1995). However, the conditions that determine the switch between gamma oscillations and sharp wave-ripples remain to be explored.

In the naturally sleeping animal, gamma and ripple oscillations in the DOWN state were less dominant perhaps because the short duration of DOWN states associated with delta waves may have limited the emergence of these self-organized patterns. Ripples that emerge in the DOWN state may “replay” stored information within hippocampal circuits and selectively modify intrahippocampal, subicular, and entorhinal cortical connectivity without affecting neocortical targets. Alternatively, ripples associated with the DOWN state may reflect a “correcting” mechanism by eliminating functional pathways no longer needed for hippocampal neocortical information transfer. Independent of these speculations, our findings suggest that the dentate gyrus output can affect the CA3 targets in two different ways. In the UP state, granule cells firing at gamma frequency transmit neocortical information by their potentiating excitatory mossy terminals onto a few selected pyramidal neurons (Henze et al., 2002) while at the same time suppress the...
remaining CA3 population by widespread activation of inhibitory neurons (Acsady et al., 1998). In the DOWN state, the absence of the dentate-mediated suppression allows for the occurrence of transient self-organized gamma and sharp wave/ripple oscillations in the CA3-CA1 circuits. Thus, the hippocampus proper can operate in both neo/paleocortex-dependent and independent manner. These observations indicate that although hippocampal ripples and gamma oscillations can emerge as self-organized patterns, their probability of occurrence and the constitution of the participating neurons in these events may be biased by the state of the neocortex.

Temporal Coordination of Neocortical-Hippocampal Transfer of Neuronal Patterns

The periodic “rebooting” of activity in the neocortical, entorhinal, and hippocampal axis provides a framework for a temporally coordinated communication in these circuits during sleep. It has been hypothesized that experience-related activity modifies synaptic strengths of functional connectivity among the neurons involved and that episodic memory traces embodied in the modified connectivity undergo further modification during sleep (Wilson and McNaughton, 1994; Hasselmo et al., 1995; Buzsaki, 1996; Csicsvari et al., 2000). An important physiological role of slow oscillations is the temporal coordination of locally emerging patterns, such as sleep spindles, gamma oscillations (Steriade et al., 1995c), and sharp wave-ripple events, and their ability to affect the direction of activity spread by phase offset over large cortical areas. After each DOWN state, the neocortex self-organizes its global activity from locally generated patterns (Luczak et al., 2006). Because the spatiotemporal sequences of neocortical neurons during the UP state recur reliably in successive episodes (Luczak et al., 2006), we hypothesize that the output of neocortical patterns can select unique subpopulations of hippocampal neurons. In turn, the targeted hippocampal neurons give rise to ripple-related synchronous outputs back to the still active neocortical assemblies (Chrobak and Buzsaki, 1996). The temporal directedness of these events facilitates conditions in which unique neocortical inputs to the hippocampus and hippocampal outputs to the neocortex may be selectively modified (Sirota et al., 2003). Direct support of this hypothetical mechanism will require demonstration that specific assemblies in the neocortex-entorhinal cortex-hippocampus axis during sleep are brought about by waking experience.

Experimental Procedures

Animal Surgery

For acute experiments, 218 male Sprague-Dawley rats (140–250 g; Hilltop Laboratories) were anesthetized with urethane only (1.5 g/kg, i.p.) or urethane (1.25 g/kg, i.p.) plus additional ketamine/xylazine injections (20 and 2 mg/kg i.m. for maintenance). Data from several of these animals were used in previous studies (Harris et al., 2000; Henze et al., 2000). The body temperature was monitored and kept constant with a heating pad. The head was placed in a stereotaxic frame, the skull was exposed, and a small hole (1.2 mm in diameter) in the skull was drilled above the hippocampus (A, −4.5 mm from the bregma; L, 5.0 mm from the midline; D, 2.0–2.2 mm from brain surface) to insert an extracellular electrode at a 15° angle toward the midline. Another hole in the skull was drilled above the ipsilateral prefrontal cortex (A, 3.0; L, 0.5–1.0), the somatosensory cortex/hippo-
Cortico-Hippocampal Interactions

and Pesaran, 1999) or continuous wavelet transformation (Torrence analyses were carried out using direct multi-taper estimate (Mitra periods containing slow oscillations were used (Figure S1). Spectral tom-written, MATLAB-based programs. For all the analysis, only pe-
tials, extracellular units, and intracellular data were analyzed by cus-
midal layer was filtered for slow oscillations (adjusted for each cell; 
experiments. For phase analysis, the LFP recorded in the CA1 pyra-
eigenvectors corresponding to two largest eigenvalues identified 
was computed in sliding 200 ms windows and was used as a signal for 
spectral analysis in the slow oscillation frequency range (e.g., 
Figure 7). Coherence between the intracellular membrane potential and 
was computed at each recording site was computed and depth profile of high coherence consistent across 
experiments was extracted by principal component analysis. The 
eigenvalues corresponding to two largest eigenvalues identified 
depth profiles of largest covariance of the synaptic currents across 
experiments. For phase analysis, the LFP recorded in the CA1 pyra-
was filtered for slow oscillations (adjusted for each cell; typically ~ 1 Hz), and the instantaneous phase was computed as the 
angle of the Hilbert transformation of the filtered signal. Phase mod-
ulation of spikes and ripples was determined by Rayleigh circular statistics (Fisher, 1993); p < 0.05 was considered significant. For 
all circular statistical tests the nonuniformity of the phase distribu-
tion, due to skewness of the slow oscillation wave shape, was taken 
into account using the cumulative density function-based transforma-
(Siapas et al., 2005). Group comparison tests of circular vari-
ables were performed using circular ANOVA.

Current-source density (CSD) analysis of the simultaneously 
recorded field potentials was used to eliminate volume conduction and 
localize synchronous currents. CSD was computed for each recording 
site according to differential scheme for second derivative and 
smoothed with a triangular kernel (Freeman and Nicholson, 1975). 
Location of the recording electrode sites in the hippocampus was 
determined by computing CSD of sharp waves and evoked poten-
tials in response to perforant path or commissural path stimulation (see Figure 4) (Bragin et al., 1995). In all analyses of the membrane potential signals, an average of the intracellular action potential was computed for each cell, and the membrane potential was inter-
polated for the duration of the spike (typically ~ 3 ms). Spectral power of the membrane potential was computed, and the relative power coefficient (ratio of the peak power and the standard devia-
tion across all frequencies) was used to characterize the strength of the dominant frequency of slow oscillation. For further details, see Supplemental Data.

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/52/5/871/DC1/

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Supplementary Table 1

<table>
<thead>
<tr>
<th>Brain area</th>
<th>Neocortex†</th>
<th>Entorhinal Cortex</th>
<th>Subiculum</th>
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<tbody>
<tr>
<td>Location layer</td>
<td>layer 5</td>
<td>layer 2</td>
<td>layer 3</td>
</tr>
<tr>
<td>Total number</td>
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<td>6</td>
<td>13</td>
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<tr>
<td>Resting membrane potential (mV)</td>
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<td>–67.5 ± 5.0</td>
<td>–66.2 ± 7.3</td>
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<tr>
<td>Spontaneous firing rate (Hz)</td>
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<td></td>
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<td>hippocampal theta period***</td>
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<td>2.56 ± 2.28</td>
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<tr>
<td>hippocampal non-theta period***</td>
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<td>2.67 ± 3.91</td>
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<td>Evoked firing rate‡ (Hz/0.5nA)**</td>
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<td>33.2 ± 12.6</td>
<td>36.0 ± 14.8**</td>
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<tr>
<td>Action potential‡ (mV)</td>
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<tr>
<td>Afterhyperpolarization‡ (mV)</td>
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<td>5.50 ± 2.17</td>
<td>8.15 ± 2.59</td>
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<tr>
<td>Input resistance‡ (Mohm)***</td>
<td>39.5 ± 12.1</td>
<td>31.6 ± 8.58</td>
<td>59.6 ± 17.4**</td>
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</tbody>
</table>

†Medial prefrontal cortex. ‡Calculated from membrane potential responses to depolarizing or hyperpolarizing current injections (±0.1-0.5 nA, 500 ms). Mean ± S.D. *, p < 0.05; **, p < 0.01; ***, p < 0.001; difference among cell groups by one-way ANOVA and Duncan's multiple range test. Note: The data included here was collected by a single experimenter (YI), using the same recording equipment (Axoclamp 2A). Intracellular parameters for hippocampal neurons have been published previously (Henze et al. 2000, 2002).


Supplementary Figures

Figure S1. Characterization of the slow oscillation state. (a) Power spectrogram of local field potential (LFP) in CA1 pyramidal layer (inset shows electrode track) during >2.5 h of recording. Note a clear isolated band of spontaneously occurring theta epoch at 3-4 Hz. (b) Power spectrogram of membrane potential fluctuation in a layer 3 entorhinal cortical neuron (inset). (c) Coherence between LFP and the intracellular potential. Note high coherence values (yellow to red) at ~0.7 Hz from 0 to 4000 sec changing gradually to coherence at theta frequency. (d) Distribution of membrane potential values as a function of time. Note bimodal fluctuation of membrane potential during slow oscillation and the persistent depolarization during UP state, characterized by hippocampal theta activity. Only epochs with strong power of slow oscillations were used in subsequent analyses.

The recording sessions were separated into brain states: theta and slow oscillation periods during anesthesia and slow wave sleep, REM and waking periods in freely-moving animals using spectral features of LFP recorded in the CA1 pyramidal layer and/or neocortex. Because of the variability of electrode positions in different animals, the threshold for the detection of state transitions was visually determined in each file.
Figure S2. Spectral analysis of the relationship between membrane potential and slow oscillation. Each symbol represents the level of coherence and phase shift between membrane potential of one neuron and LFP derived from recording site in CA1 pyramidal layer (reflecting volume-conducted slow oscillations from the neocortex). The values were taken at the frequency of peak power of slow oscillation, individually estimated for each recording session. Data from neurons in neocortex (NC), entorhinal cortex (EC), subiculum (SUB), hippocampal CA1 and CA3 pyramidal layers are represented by respective colors and symbols (see legend in the top right corner). Since phase shift is a circular variable all points are plotted twice with a 0 and 360-degree shift for clarity. Note gradual increase in phase shift from NC to CA3.
Figure S3. Delayed recruitment of superficial entorhinal layer neurons during slow oscillations. Each row represents a normalized slow oscillation phase histogram (color-coded for magnitude) of DOWN-UP transitions of membrane potential in neocortical (NC), entorhinal layer 5 (EC5) and superficial (EC2 and EC3) cells, and subiculum (SUB). The reference LFP electrode was in CA1 pyramidal layer in each experiment. White trace, average LFP. Note similar preferred phase of DOWN-UP transitions in neocortical and deep entorhinal neurons, and consistent phase shift (~60 degrees) in superficial cells and subiculum.
Figure S4. (a) Joint probability density function (JPDF) of the membrane potential in a CA1 pyramidal neuron and the phase of slow oscillation (red indicates high probability). Phase of slow oscillation is derived from LFP recorded in CA1 pyramidal layer (white trace). Two cycles are shown for illustration purposes. Histogram above, marginal phase distribution. Histogram left, marginal distribution of the membrane potential. Note somewhat skewed but unimodal distribution of the membrane potential. To reveal the content structure of JPDF, we decomposed it in two terms: ‘independent’ and ‘dependent’. The independent term can be estimated as a direct product of marginal distributions of the membrane potential and phase, and can be calculated by shuffling the instantaneous membrane potentials and the corresponding phase of the slow oscillation. In essence, the shuffling ‘removes’ the effect of the slow oscillation-
mediated effect. The dependent term can be computed as a difference between the raw JPDF and the independent term, and reveals the relationship between the membrane potential and the phase of slow oscillation even when the membrane potential fluctuation is very small and is not obvious by visual inspection even in the averaged trace. (b) Independent term (JPDF of the shuffled membrane potential and phase). (c) Dependent term. Color in all plots displays values of probability density (red – high, blue – low). Black line, most likely values of the membrane potential extracted from dependent component at each phase. These values were used for the group display in Fig. 5a. Trace above, phase histogram of spike discharge for the same neuron. This neuron fired mostly in the UP state and the effect of slow oscillation was visible in the raw JPDF (a). Examples of the dependent terms of JPDF for 4 different neurons: entorhinal neuron with bimodally distributed membrane potential (d), entorhinal neuron with unimodal, skewed distribution of the membrane potential (e), CA1 neuron with symmetrical distribution of the membrane potential (f) and CA1 neuron with skewed distribution of the membrane potential (g). Note different voltage axes in the four plots. See Supplementary Methods section for more details.
Figure. S5. Firing probabilities of all recorded neurons during slow oscillation. In contrast to Figure 5b in which only significantly modulated cells are tabulated, here all neurons are displayed. Each line is the normalized (color coded) cross-correlogram between a single neuron and the trough of SO (a) or a phase-histogram (b) (n=946; intracellular and extracellular recordings combined). White trace, averaged trace of slow oscillation from CA1 pyramidal layer, aligned to phase axis. NC, neocortex; EC, entorhinal cortex; Sub, subiculum. DG, dentate gyrus neurons. Note the anti-phase activity of most CA3 pyramidal cells and wide phase preference of CA1 neurons as in Fig. 5. Data are from anesthetized animals.
Figure. S6. Phase preference of neurons to slow oscillations is similar in sleep and anesthesia. Histograms are average cross-correlograms of 3 dentate gyrus (DG, blue) and 5 CA3 (green) neurons referenced to the onset of delta waves in slow wave sleep (SWS; upper panels) or to the trough of slow oscillation (onset of UP state) under anesthesia (bottom panels). After recording from a sleep session, the rat was anesthetized with urethane (1.25 g/kg, i.p.) and ketamine/xylazine injections (20 and 2 mg/kg).
Differentiation of gamma oscillation in different hippocampal subfields in relation to entorhinal slow oscillations. (a-c) Spectrograms of LFP recorded in CA1 str. radiatum (rad), str. lacunosum-moleculare (lm) and dentate molecular layer (ml), triggered by the onset of the UP state in the membrane potential of an entorhinal layer 3 neuron (0 sec). Note that gamma spectral power reaches its maximum prior to the UP state in str. radiatum but after in str. lacunosum-moleculare and dentate molecular layer. Note also the different peak frequencies in the different layers. (d) Time distribution of the power maxima for the 16 recording sites, relative to UP state onset. Color of each dot is proportional with gamma power and its radius is proportional to its frequency at the power peak. Note time shift of power dominance in CA1 str. radiatum relative to other layers. (e) Normalized spectra of each channel at times of local power peak. Note distinct frequency ranges in the dentate gyrus (40-55 Hz) and CA1 layers (25-40 Hz). These region-specific frequency differences are reminiscent of those in the drug-free rat (Bragin et al., 1995). (f) Depth profile of coherence between membrane potential of the layer 3 neuron and LFP at each recording site. Note maximum coherence after the onset of UP state in str. lacunosum-
moleculare, the main target of the layer 3 entorhinal projection. Data are from anesthetized animals.
Figure. S8. Comparison between ripple-related and CA1 str. radiatum gamma oscillation-related depolarization of entorhinal neuron (layer 3). The peak of the squared and smoothed envelope of ripple or gamma burst served as a reference event (time 0). Event-related average of the membrane potential (green) triggered by ripples (a) and radiatum (c). This CA1 output-entorhinal cortex relationship is confounded by fact that slow oscillation is modulating the membrane potential (UP-DOWN states) of the entorhinal neurons and phase of both ripples and gamma bursts. Thus event-triggered average is dominated by the slow oscillation modulation of the membrane potential. To remove this confound, we computed the event-triggered membrane potential for events shuffled between DOWN-UP cycles. This procedure keeps time lag of each event relative to onset of the UP state fixed but changes the identity of the DOWN-UP cycle at random for each event. An average of such shuffled event-triggered membrane potential (2500 shuffled events) gives an estimate of the membrane potential that is independent of the hippocampal output on the membrane potential (blue). The difference between the two curves reveals the effect of hippocampal output during ripples (a) and gamma episodes (c). Set of values of the membrane potential at each time lag for each event produced by shuffling allows one to compute the exact p-value of the actual value of the membrane potential at a given time lag. By computing every event and all time lags, the time-dependent statistic of excessive depolarization or hyperpolarization of the membrane potential caused by the event can be estimated. (b) and (d) Color-coded distributions of the p-values (ordinate axis) across all events for each time lag. Time lags that have high probability (red color) of low p-values indicate significantly high values of the membrane potential (~50 msec after the ripple in (b). Conversely, high probability of the high p-values correspond to excessive hyperpolarization.
Figure S9. Neo-/paleocortical input-dependent activity of hippocampus during slow wave sleep. (a) Current-source density (CSD) and superimposed LFP traces of simultaneously recorded events in the neocortex (top) and the hippocampus (bottom). Delta wave (red source in layer 5, LV, marked by black arrow) is followed by a sleep spindle (black star). DOWN-UP state transition is indicated by orange dashed line. In the hippocampus, the entorhinal cortex-mediated DOWN-UP transition is reflected by a phase-reversal of LFP and a sink (blue) in the molecular layer (white arrow). Horizontal arrow, neocortico-dentate time lag (~100 msec). Gray star, sharp wave in CA1 str. radiatum. (b) Average CSD and LFP traces simultaneously recorded in the neocortex (top) and hippocampus (bottom), triggered by peak of delta wave in the neocortex (130 events). Same animal and channel layout as in (a). Note similar time lag between the sink maxima in neocortex (UP state onset) and hippocampal molecular layer (orange and purple lines, respectively). (c) Sink of average CSD of sharp waves (blue) indicates str. radiatum. (d) Comparison of silent periods in neocortex and hippocampal CA1 region. The silence-activity (DOWN-UP) transitions of population firing in neocortex were related to similar silence-activity transitions in the hippocampus. (i.e., events with spiking of CA1 neurons during the DOWN state were excluded). Each color-coded line is a cross-correlogram between the onset of the UP state and the silence-activity periods in the CA1 region (ten sleep sessions).
in 8 rats). Histogram below, mean and standard deviation across experiments. Note approximately 100 msec delay between neocortical UP state and the onset of activity in the CA1 region using either method.
Supplementary Methods

DOWN-UP and UP-DOWN transitions in neurons with bimodally distributed membrane potentials ($V_m$) were detected as follows. First, a filter was designed to detect sharp step-like transitions of membrane potential. Second, wave shapes of UP-DOWN-UP transition segments were passed through semi-automatic sorting procedure, in which the thresholds for DOWN and UP duration, the mean DOWN-UP difference and DOWN variance were selected adaptively in each experiment.

Distribution of the membrane potential was analyzed by several methods. First, a bootstrap-based test for bimodality (Silverman, 1981) on multiple short (20 seconds) segments was used to test for overall bimodality of the membrane potential. Neurons that failed the test for bimodality but had a skewness and kurtosis above a predetermined preset threshold were classified as potentially skewed. In addition, a classical test of skewness (Gupta, 1967) based on a combination of high-order statistical moments was used to classify cells as having skewed membrane potential distribution. The remaining cells were classified as unimodal and symmetrically distributed.

Several methods were used to analyze the relationship between the membrane potential and the slow oscillation (SO) measured from the field potential. First, in order to characterize the relationship between SO power fluctuations in the LFP and the membrane potential, we computed a comodugram, a correlation coefficient between normalized spectral power values of respective signals at all pairs of frequencies. Second, for cells with bimodal distribution of the $V_m$, the SO phase distribution of the onsets of the UP state was tested for nonuniformity (see Supplementary Fig. S3). Third, spectral coherence analysis was performed between the $V_m$ and LFP signal, and coherence values and the phase shift between the two signals at SO frequency were used as a measure of linear phase relationship (Supplementary Fig. S2). These analyses are either limited to neurons with clearly detected DOWN-UP transitions or capture only presence of linear phase relationships. In order to characterize in greater detail the structure (potentially nonlinear) of dependency between the phase of the SO ($\nu$) and $V_m$, we designed an analysis of their joint probability density function (JPDF). The raw JPDF of $V_m$ and $\nu$ was decomposed into
independent term (direct product of the marginal distributions of $V_m$ and $\nu$) and remaining dependent term (Supplementary Fig. S4). The independent term can alternatively be obtained as a JPDF between shuffled $V_m$ and $\nu$. In essence, for each value of $\nu=\nu^0$ the probability of observing a certain value of the membrane $V_m=V_m^0$ decomposed into the additive part, which is derived from the marginal distribution of $V_m$ (independent of $\nu$), and a conditional probability that depends on $\nu$. From this perspective, the actual values of the dependent and independent term are only relevant for significance tests but do not change the structure of relationship between the signals. The most likely values of $V_m$ obtained from the dependent term at each value of $\nu$ served to characterize the non-linear relationship between the phase and the membrane potential and were used in the group data plot in Fig. 5A. The significance of the structure of dependent term was confirmed by comparing this measure to the preferred phase of firing of the same neurons, which in most cases matched the phase of relative depolarization (black dots in Fig. 5A). To test the significance of the structure (phase of relative change in the membrane potential) we performed a 10-fold cross-validation test in a subset of neurons that had sufficiently long recordings. The difference between the most likely $V_m$ values at the opposite phases of slow oscillation was used as a measure of membrane potential fluctuation in each cell.

DOWN-UP transitions in slow wave sleep were detected either by using sufficiently large populations of neocortical or entorhinal units or features of LFP. In the former method, silences that lasted $>150$ msec in population firing were detected, and the putative onset of the UP state was determined as the end of the silent period (Luczak A et al., 2006). For extracting slow oscillations from LFP, a combination of CSD signal from deep and/or superficial layers and gamma power changes were used. A large source in the deep layers was associated with decreased gamma power for the duration of the DOWN state (100-300 msec), followed by a sink and increased gamma power. These changes strongly correlated with the membrane potential shifts of neurons (Mukovski et al., 2006). The time onset of the sink was designated as the beginning of the UP state.

To test for significance of the peaks in cross-correlograms between point processes (e.g., UP state onset and units) the referred point-process was either shuffled across UP state cycles or
jittered to produce 500 surrogate cross-correlograms from which the exact p-value for each time bin was computed. The time bins with p-value of 0.01 or less were considered significant.


