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Hippocampal Sharp Wave-Ripples Linked to Slow Oscillations in Rat Slow-Wave Sleep

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Mölle, Matthias, Oxana Yeshenko, Lisa Marshall, Susan J. Sara, and Jan Born. Hippocampal Sharp Wave-Ripples Linked to Slow Oscillations in Rat Slow-Wave Sleep. *J Neurophysiol* 96: 62–70, 2006. First published April 12, 2006; doi:10.1152/jn.00014.2006. Slow oscillations originating in the prefrontal neocortex during slow-wave sleep (SWS) group neuronal network activity and thereby presumably support the consolidation of memories. Here, we investigated whether the grouping influence of slow oscillations extends to hippocampal sharp wave-ripple (SPW) activity thought to underlie memory replay processes during SWS. The prefrontal surface EEG and multiunit activity (MUA), along with hippocampal local field potentials (LFP) from CA1, were recorded in rats during sleep. Average spindle and ripple activity and event correlation histograms of SPWs were calculated, time-locked to half-waves of slow oscillations. Results confirm decreased prefrontal MUA and spindle activity during EEG slow oscillation negativity and increases in this activity during subsequent positivity. A remarkably close temporal link was revealed between slow oscillations and hippocampal activity, with ripple activity and SPWs being also distinctly decreased during negative half-waves and increased during slow oscillation positivity. Fine-grained analyses of temporal dynamics revealed for the slow oscillation a phase delay of approximately 90 ms with reference to up and down states of prefrontal MUA, and of only approximately 60 ms with reference to changes in SPWs, indicating that up and down states in prefrontal MUA precede corresponding changes in hippocampal SPWs by approximately 30 ms. Results support the notion that the depolarizing surface-positive phase of the slow oscillation and the associated up state of prefrontal excitation promotes hippocampal SPWs via efferent pathways. The preceding disfacilitation of hippocampal events temporally coupled to the negative slow oscillation half-wave appears to serve a synchronizing role in this neocorticohippocampal interplay.

INTRODUCTION

Slow oscillatory activity in neuronal networks allows activated neuronal assemblies in distant brain regions to become synchronized. Thereby, slow oscillatory activity may support the binding and consolidation of representations of acquired information extending over distant regions in the brain (Buzsáki and Draguhn 2004). A hallmark of slow-wave sleep (SWS) are EEG slow oscillations, which originate in frontal neocortical networks with a predominant frequency of approximately 0.8 Hz in humans and cats (Achermann and Borbély 1997; Massimini et al. 2003; Mölle et al. 2002; Steriade et al. 1993c; Steriade and Timofeev 2003). During the slow oscillation, the membrane potential of both inhibitory and excitatory neurons oscillates between hyperpolarized "down" and depolarized "up" phases, with firing globally fading during the down state. In contrast, the depolarizing phase is associated with increased firing, which drives the generation of spindle oscillations in thalamoneocortical feedback loops (Destexhe et al. 1999; Steriade et al. 1993b). In humans, the surface-EEG negative half-wave, corresponding to the down state of the slow oscillation, was found to be associated with decreased spindle and faster activity. The following positive-going phase, corresponding to the up state of the slow oscillation, was associated with a strong increase in spindles and faster activity (Mölle et al. 2002). Recent studies in humans have linked slow oscillations with neocortical reprocessing of memories during SWS (Huber et al. 2004; Mölle et al. 2004). An increase in EEG coherence induced during learning of a hippocampus-dependent task (i.e., in humans, a declarative memory task) was observed selectively during the up state, but not during the down state, of slow oscillations in subsequent sleep. These observations suggest that widespread slow oscillatory activity during sleep binds local activation occurring in neocortical and hippocampal networks into a synchronized memory reactivation that eventually subserves the consolidation process (Buzsáki and Draguhn 2004; Mölle et al. 2004; Sirota et al. 2003; Steriade et al. 1993a).

Memory consolidation during sleep is considered to rely basically on a reactivation of previously encoded neural representations, which in the hippocampus-dependent memory system have been preferentially observed during SWS (Buzsáki 1998; Kudrimoti et al. 1999; Lee and Wilson 2002; Mölle et al. 2004; Pavlides and Winson 1989; Peigneux et al. 2004; Qin et al. 1997; Ribeiro et al. 2004; Wilson and McNaughton 1994). The replay co-occurring in neocortical and hippocampal regions has been thought to support a transfer of memory representations between hippocampal and neocortical networks, which leads eventually to a preferential storage in neocortical regions. Importantly, hippocampal reactivation is suggested to occur mainly during bursts of activity known as sharp-wave ripple events (SPWs) (Buzsáki 1989; Buzsáki 1998; Kudrimoti et al. 1999; Wilson and McNaughton 1994). The SPWs are fast depolarizing events generated in CA3 and become superimposed on ripple activity which refers to high-frequency LFP oscillations (100–300 Hz) originating in CA1 (Buzsáki 1986; Chrobak and Buzsáki 1994; Chrobak and Buzsáki 1996; Csicsvari et al. 1999). SPWs have been shown to occur at an increased probability in close temporal association with spindles in thalamo neocortical circuitry (Siapas and

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Wilson 1998). Interestingly, two previous studies have indicated, in addition, that hippocampal SPW events occur in temporal association with up states of cortical excitation, which are thought to be linked to the depolarizing phase of the slow oscillation (Battaglia et al. 2004; Sirota et al. 2003).

To clarify a possible grouping influence of neocortical slow oscillations on hippocampal SPW events, we characterize here the temporal relationships between these phenomena. To extend previous investigations of the relationship between neocortical up and down states in terms of unit activity and SPW events, we first analyzed the temporal association of slow oscillations with multiunit activity (MUA) in the prefrontal cortex, and then with SPW events in the hippocampal CA1 region. The focus being on slow oscillations here should facilitate a comparative interpretation of relevant findings in humans relying on recordings of slow oscillations rather than on MUA.

METHODS

Animals

Recordings were performed in male Sprague-Dawley rats (Charles River Laboratories, Le Genest-St-Isle, France, $n = 10$, weight 350–400 g). The rats were housed individually, handled daily, and kept on a 12-h light/dark cycle with lights off at 8 P.M. Water and food was available ad libitum. All procedures were performed in accordance with the 1986 European Communities Council Directive and Ministère de l'Agriculture et de la Foret, Commission Nationale de l'Experimentation Animal decree 87848.

Surgery, recordings, and treatments

Animals were anesthetized with sodium pentobarbital (initial dose 40 mg/kg, ip, with 0.1-ml supplements given as necessary) and fixed in a stereotaxic frame. Atropine sulfate (0.2 mg/kg) was administered to minimize respiratory distress. For the placement of electrodes and skull screws for surface EEG recordings, the skull was exposed and burr holes were made. Two stainless steel screw electrodes were placed over the left prefrontal cortex (AP = +4.0, L = 0.5; reference: AP = +0.5, L = 0.5). Another skull screw served as ground. Positions of the depth electrodes were confirmed by histological analysis. Hippocampal activity was recorded as local field potentials (LFP, $n = 6$) from CA1. For these recordings, a microelectrode (FHC, Bowdoinham, MA; resistance >1 MΩ) was placed in CA1 of the dorsal hippocampus (AP = −3.5, L = 2.0) at a depth of 2.0–2.5 mm. To compare prefrontal slow oscillations recorded either in the surface EEG or in the LFP from a certain cortical depth with multiunit activity (MUA) in underlying neocortical tissue, two supplementary microelectrodes glued together and mounted on a movable drive were implanted into the medial prefrontal cortex (AP = +3.5, L = 0.5) at a depth of 1.5–2.0 mm ($n = 5$). Also LFPs were recorded from these microelectrodes. The movable drive was used to adapt and optimize unit recording before the experiment proper. Electromyogram (EMG) activity was recorded from the dorsal neck muscles with insulated multistranded wires. Rats had 1 week for recovery after surgery before recordings began.

EEG, LFPs, MUA, and EMG activity were recorded continuously and digitized with 16-bit resolution using a CED Power1401 converter and Spike2 software (Cambridge Electronic Design, Cambridge, UK). EEG and LFP signals were amplified (x 1K) and filtered between 0.01 and 300 Hz (Grass P5 Series PreAmplifier, Quincy, MA). EMG was amplified (x 1K) and filtered at 30–300 Hz. The signals were sampled at 1 kHz. MUA was amplified (x 10K), filtered at 0.3–3 kHz (A-M Systems model 3500, Carlsborg, WA) and sampled at 30 kHz. Unit activity was monitored on-line, and spike templates were built before the recording using Spike2 software. Data were stored on a PC for off-line analysis.

A day before recording, each rat was exposed for 1–3 h to the recording chamber (25 x 25 x 50 cm in size) for habituation. Rats were connected to the amplifier by a cable that allowed free movement within the chamber. Behavior was tracked by a video camera (Quickcam, Logitech, Moulin du Choc, Switzerland) mounted on the top of the recording chamber. The video image was synchronized with electrophysiological recordings. Recordings lasted for 3–5 h between 10 A.M. and 6 P.M. during the light period (i.e., when rats spend most of their time sleeping). After the last recording session, rats were deeply anesthetized with pentobarbital (100 mg/kg) and perfused intracardially; brains were then extracted for histological verification of recording sites.

Data processing and statistical analysis

In four rats, three sessions from each rat were analyzed to assess relationships between slow oscillations in prefrontal EEG and neocortical MUA. In three rats, two sessions from each rat were analyzed to assess relationships between slow oscillations in prefrontal LFP and neocortical MUA. Prefrontal EEG and LFPs from CA1 obtained in six rats with three sessions each were analyzed to characterize relationships between slow oscillations, spindle, and SPW/ripple activity. Data processing was generally performed using Spike2 software and the built-in script language (Cambridge Electronic Design, Cambridge, UK).

Power spectra of delta (1–4 Hz), theta (5–10 Hz), and spindle (12–15 Hz) frequency bands were calculated continuously, and sleep-wake episodes were scored by visual assessment for 10-sec epochs according to the standard criteria (Bjorvatn et al. 1998). Briefly, the "awake" state was marked by the presence of low-amplitude fast activity associated with increased EMG tonus; SWS was identified by continuous high-amplitude slow activity and regular appearance of spindles; and transitions from SWS into REM sleep were identified by a decrease in high-amplitude slow activity, increase of theta activity, and the presence of spindles. REM sleep was characterized by dominant theta activity, low-voltage fast activity, and absence of EMG tonus. Behavioral states were additionally verified by video.

To identify slow oscillations in the prefrontal EEG data, first, we applied a low-pass Finite Impulse Response (FIR) filter of 30 Hz and down-sampled the resulting signal to 200 Hz. Subsequently, this signal was low-pass filtered with an FIR filter of 3.5 Hz to produce the slow oscillation signal. To identify the spindle activity signal in the prefrontal recordings a FIR band-pass filter of 12 to 15 Hz was used. After band-pass filtering, the root mean square (RMS) was calculated at every time point using a time window of 0.1 s. Finally, the RMS signal was smoothed with a moving average of 0.1 s. To identify the ripple activity signal in the hippocampal LFP recordings, we applied a band-pass FIR filter of 150 to 250 Hz. After band-pass filtering the RMS was calculated with a time resolution of 0.005 s (200 Hz) using a time window of 0.02 s.

From the slow oscillation signal of the prefrontal surface EEG, the largest negative half-waves during SWS episodes were selected using an automatic thresholding algorithm (Massimini et al. 2004; Mölle et al. 2002). The peak time of a negative half-wave was selected if the following criteria were fulfilled: *1*) two succeeding zero-crossings of the slow oscillation band signal separated from each other by 0.2 to 1.0 s, *2*) a peak amplitude between both zero-crossings exceeding a threshold of at least −80 μV, and *3*) a negative-to-positive peak-to-peak amplitude >120 μV. Only negative half-waves from artifact-free epochs of SWS were used. For all detected negative half-waves, the original, wideband-recorded prefrontal EEG or LFP, the spindle-RMS signal, and the hippocampal ripple-RMS signal were averaged for intervals of ±1.0 s around the time of the peak amplitude. Grand mean averages across all recording sessions of all animals were

calculated. For an additional analysis, the largest positive half-waves during SWS episodes were selected from the slow oscillation signal using the same thresholding algorithm as described above.

The MUA signal was additionally processed off-line for artifact removal (Spike2 software). Event correlation histograms were calculated for the rate of multiunit spikes (in hertz) with reference to the peak times of negative or positive slow oscillation half-waves identified in the prefrontal EEG or prefrontal LFP. For the histograms, 2-s windows were used with an offset of 1 s (with reference to the peak of the half-wave) and a bin size of 20 ms. The histograms represent a measure of spike probability at a given time before and after the peak of a negative or positive half-wave.

Sharp wave-ripple events (SPW events) were detected by means of an automatic thresholding algorithm on the hippocampal ripple activity signal (Csicsvari et al. 1999; Klausberger et al. 2003; Kudrimoti et al. 1999; Siapas and Wilson 1998). The hippocampal ripple activity signal was derived from the filtered (150–250 Hz) CA1 LFP (see above and Fig. 1). The threshold for ripple detection was set to >3 SDs above the mean RMS signal. The beginning and end of a ripple were marked at points at which the RMS signal dropped below 1 SD, provided that these two points were separated by 25–75 ms. For every marked ripple the troughs of the ripples were detected as the minima of the filtered LFP signal, and the deepest trough was marked as the time point representing the respective SPW event. Event correlation histograms were calculated for SPW events with reference to the peak times of negative slow oscillation half-waves in the prefrontal EEG using 2-s windows (1-s offset with reference to the peak of the half-wave) and a bin size of 20 ms. Grand mean averages of the histograms across all recording sessions of all animals were calculated. Again, event correlation histograms were calculated also with reference to the peak times of the positive slow oscillation half-waves. In four rats (with three sessions each) grand mean averages of the CA1 LFPs and the prefrontal EEG (original, wideband-recorded signals) around the peak of all detected SPWs were calculated. Additionaly, event correlation histograms were calculated for the rate of multiunit spikes (in hertz) with reference to the peak of all detected SPWs.

In a last analysis, three individual cross-correlation functions were calculated: *1*) between prefrontal spindle activity and the prefrontal slow oscillation signal (reference signal), *2*) between hippocampal ripple activity and the prefrontal slow oscillation signal (reference signal), and *3*) between hippocampal ripple and prefrontal spindle activity (reference signal). For these analyses, for spindle and ripple activity the respective RMS signals were used. These calculations were done as gated analyses, i.e., only those sections of SWS data were used that lay within a ±1-s window around the peak of a negative slow oscillation half-wave. Grand mean averages of the cross-correlations were calculated across all recording sessions of all animals. For statistical comparisons in general, two-sided paired *t*-tests were used.

RESULTS

Relationship between slow oscillation and prefrontal multiunit activity

To assess the link of slow oscillations to cortical up and down states of neuronal excitation, we compared MUA in prefrontal recordings with negative and positive slow oscillation half-waves identified in the prefrontal LFP, as well as with simultaneously measured prefrontal EEG activity (at the surface). Analysis of the EEG slow oscillation, as expected, revealed a pronounced decrease of MUA during negative half-waves, averaging 3.3 ± 0.9 Hz ($P < 0.01$, with reference to the first 0.2 s of the 2-s epoch of analysis used as baseline, Fig. 2*A*). Importantly, the peak decrease in MUA occurred approximately 70 ms before the negative peak of the EEG slow oscillation, and this phase advance is likewise apparent in the comparison between MUA and slow oscillations identified in the LFP, recorded from the same electrode as the MUA (Fig. 2*B*).

As expected, in comparison with the slow oscillation picked up from the surface-EEG, the slow oscillation in the prefrontal LFP (layer IV–V) shows a polarity reversal. Accordingly, MUA displays a distinct decrease, averaging 4.1 ± 1.1 Hz ($P < 0.05$, referenced to the first 0.2-s baseline) during the positive phase of the LFP slow oscillation and an enhancement of 4.1 ± 1.7 Hz ($P < 0.05$) during the negative phase. Importantly, the peak decrease again occurred approximately 70 ms before the positive

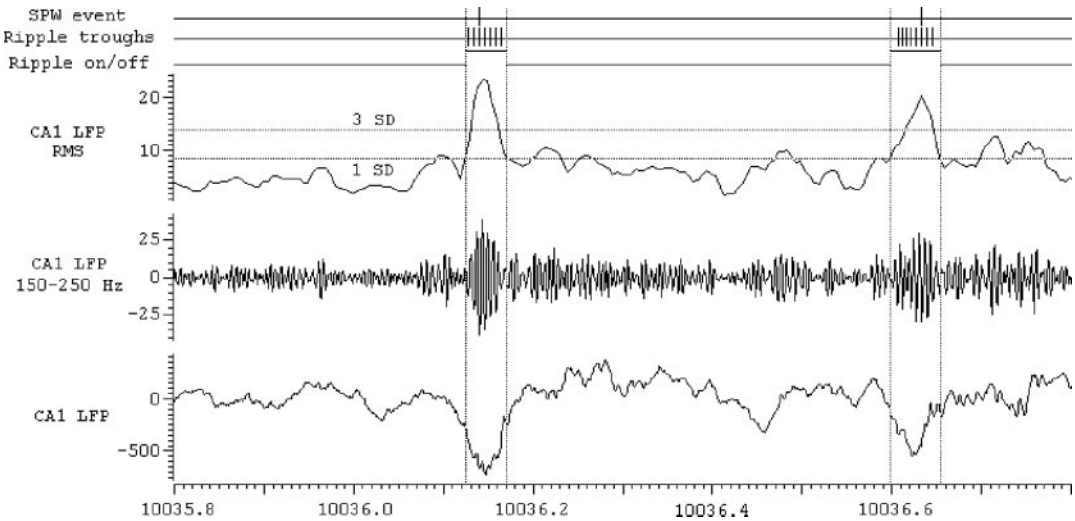

FIG. 1. Detection of SPW events. A 1-s excerpt of a hippocampal LFP recording during SWS in a single rat is shown together with corresponding signal traces and event channels calculated during sharp wave-ripple (SPW) detection. From *bottom* to *top*, CA1 LFP, ripple activity (band-pass, 150–250 Hz), ripple RMS signal, level channel indicating beginnings and ends of detected ripples, event channel containing marked ripple troughs, and event channel containing marked SPW events (largest troughs). Parallel horizontal lines indicate thresholds of 1 and 3 SD of the ripple RMS signal, which were used for SPW detection (see METHODS), and parallel vertical lines indicate beginnings and ends of two detected ripples in this epoch. Numbers at y-axes indicate microvolts and at *x*-axis seconds.

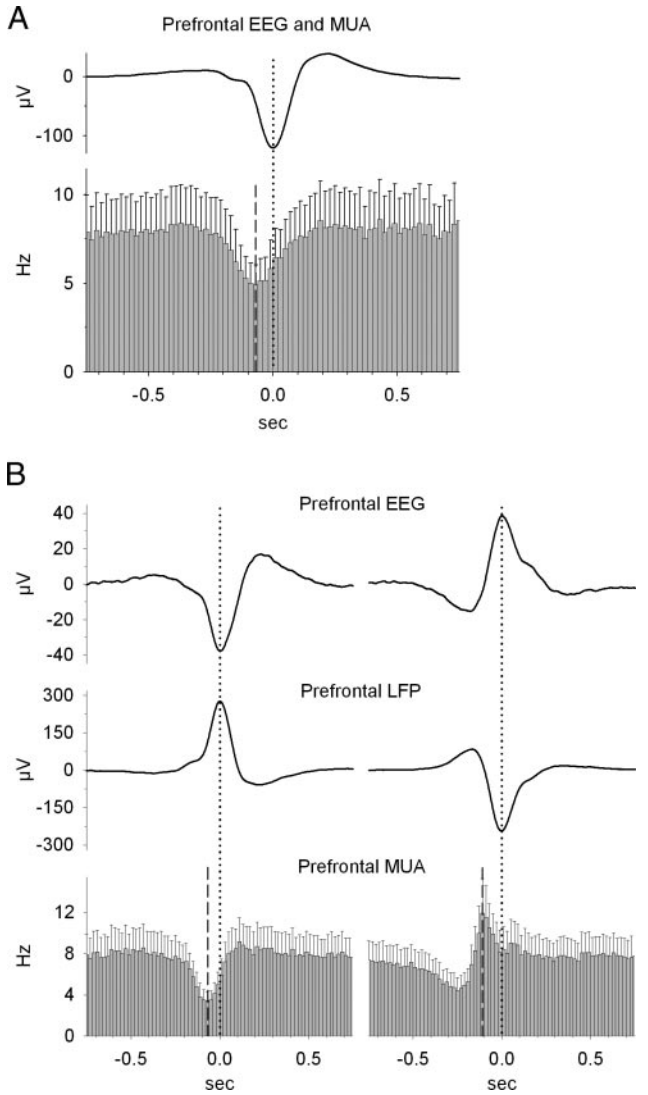

FIG. 2. Prefrontal multiunit activity during slow oscillations. *A*: event correlation histogram shows mean multiunit spike rate (gray bars) +SE during negative slow oscillation half-waves in the EEG. Corresponding mean prefrontal EEG (original, wideband-recorded signal) is indicated above. The event correlation histogram is referenced to the negative peak of the EEG slow oscillation ('0.0 s', dotted line). Note, the minimum in multiunit activity (dashed line) is reached approximately 70 ms before the peak of the negative half-wave. (Recordings from 4 rats, each with three sessions, 1514 half-waves per session.) *B*: event correlation histograms of mean multiunit spike rate (gray bars) referenced ('0.0 s', dotted line) to the peak positive (*left*) and peak negative (*right*) slow oscillation half-waves identified here in prefrontal LFPs recorded from the same electrode as the MUA. The corresponding mean prefrontal LFP (original, wideband-recorded signal) is indicated in the middle trace. Top trace shows corresponding mean prefrontal EEG (original, wideband-recorded signal). In comparison with EEG, LFP recordings show a polarity reversal. Note, the minimum in multiunit activity (*left*, dashed line) is reached ~70 ms before the peak of the positive half-wave in the LFP, and the maximum in multiunit activity (*right*, dashed line) is reached about approximately 110 ms before the peak of the negative half-waves in the LFP. (Averages represent, respectively, 1351 and 1352 positive and negative LFP half-waves per session.)

peak of the LFP slow oscillation and the corresponding negative peak in the EEG slow oscillation. With respect to the enhancement in MUA during the negative-going phase of the LFP slow oscillation, this phase advance amounted to approximately 110 ms (Fig. 2*B*).

Spindle and ripple RMS activity during slow oscillations

Averages time-locked to the negative half-wave of the slow oscillation show a clear relationship between slow oscillations and spindle activity in the prefrontal EEG (Fig. 3). The spindle RMS signal is generally at a lower level in the 1-sec interval before, as compared with the 1-s interval after, the negative half-wave peak (-0.63 ± 0.16 vs. 2.06 ± 0.73 μV, $P < 0.01$). Shortly before the peak negativity of the slow oscillation, spindle RMS activity decreases to an absolute minimum followed by a strong spindle rebound covering the entire subsequent positive phase of the slow oscillation.

An even closer relationship exists between the EEG slow oscillation and hippocampal activity in the ripple frequency range (Fig. 3). Ripple RMS activity in the CA1 LFPs is strongly decreased in a 200-msec interval around the peak of slow oscillation negative half-waves. The peak decrease in ripple RMS activity during negative half-waves amounts to 1.39 ± 0.34 μV ($P < 0.001$, with reference to the first 0.2-s baseline) and is reached on average 37 ms before the peak of the negative slow oscillation half-wave. Maxima in the ripple RMS signal observed around 340 ms before and 230 ms after this decrease also differ significantly from baseline.

Event correlation of SPWs and slow oscillations

Analyses of the LFP recordings from CA1 during SWS revealed 2,598 ± 343 SPW events per session, a number large enough to enable sensitive detection of modulations in SPWs by the slow oscillation. The event correlation histograms of all SPWs detected during the slow oscillation in the prefrontal EEG confirm that SPWs are distinctly decreased during nega-

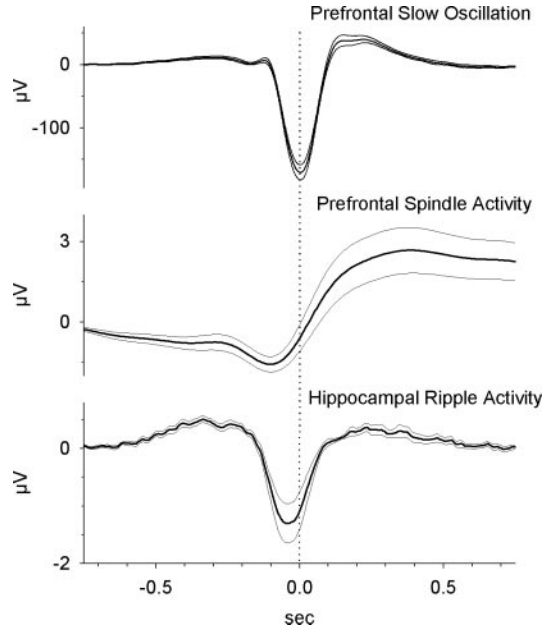

FIG. 3. Spindle and ripple activity during slow oscillations. From *top* to *bottom*, Negative half-wave triggered averages of prefrontal EEG (original, wideband-recorded signal), corresponding prefrontal spindle RMS activity (12–15 Hz), and ripple RMS activity (150–250 Hz) in hippocampal CA1 LFPs. The means (thick lines) ± SEs (*thin lines*) are shown. Spindle and ripple RMS activity are corrected to baseline of −1.0 to −0.8 s. Dotted line indicates reference point of averaging, i.e., the peak negativity of the slow oscillation (averages represent 1732 half-waves per session).

tive half-waves and increased in the beginning of positive half-waves. Specifically, SPWs averaged time-locked to the *negative* slow oscillation peak (Fig. 4*B*, *left*) revealed a maximum decrease in SPWs of 13.5 ± 2.0 events ($P < 0.001$, with reference to the first 0.2-sec baseline) which occurred 45.6 ± 11.3 ms before the negative peak of the slow oscillation. The subsequent increase in SPW events 60–280 ms after the negative peak of the slow oscillation averaged +4.9 ± 1.0 events ($P < 0.001$). In addition, the analysis referenced to the negative half-wave peak indicated a broad increase in SPW counts of +4.7 ± 0.6 preceding the negative slow oscillation peak by 480–140 ms ($P < 0.001$). When time-locked to the *positive* peak of the slow oscillation (Fig. 4*B*, *right*), the decrease in SPWs was greatest 300–260 ms before this positive peak reaching an amplitude of −8.3 ± 1.6 events ($P < 0.001$). The consecutive strong increase in SPWs averaged +19.0 ± 4.5 events and peaked 73.3 ± 7.6 ms before the slow oscillation positive peak ($P < 0.001$).

Prefrontal activity during SPWs

To further clarify the temporal relationships between SPWs and the neocortical slow oscillation, we conversely averaged the prefrontal EEG with reference to SPW events (i.e., the deepest ripple trough, Fig. 5). This analysis revealed a clear-cut positive potential shift in the prefrontal EEG during SPWs. The shift begins about 100 ms before the SPW event and reaches a maximum of 17.5 ± 2.1 μV 43 ms after the SPW ($P < 0.001$, in comparison to baseline). Following this increase, the EEG potential shifts toward negativity to a minimum of −8.1 ± 1.0 μV ($P < 0.001$) reached 314 ms after the SPW. In combination, this pattern indicates that SPWs occur mostly during the positive-going phase of the slow oscillation. MUA also shows a distinct increase during SPWs (Fig. 5), amounting to 0.86 ± 0.19 Hz from −40–160 ms after the SPW event ($P < 0.001$, compared with baseline). Differing from the prefrontal EEG, MUA remains elevated after the SPW event, still averaging 0.52 ± 0.2 Hz 300–500 ms after the SPW event ($P < 0.05$, compared with baseline).

Cross correlation analyses of spindle and ripple activity during slow oscillations

The mean cross-correlation function between the prefrontal slow oscillatory EEG potential (serving as reference signal) and, respectively, spindle RMS activity and ripple RMS activ-

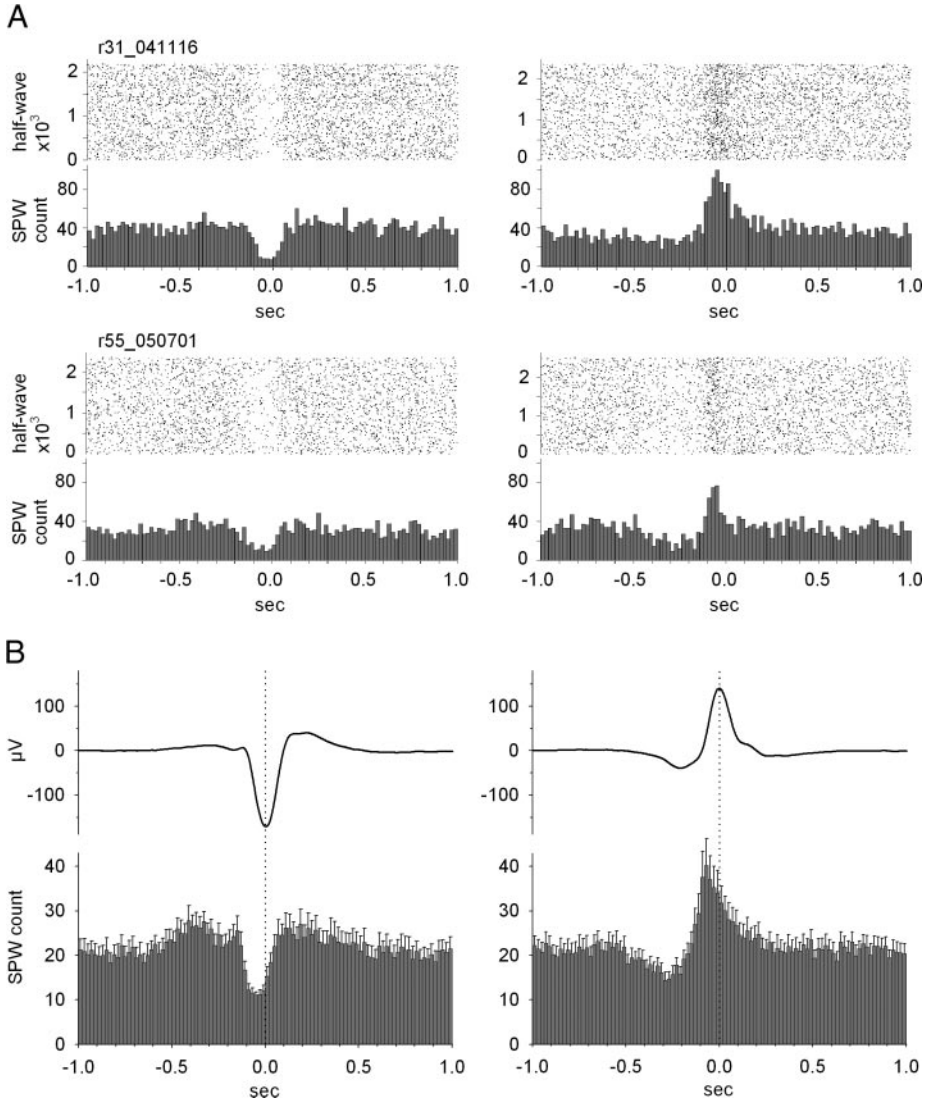

FIG. 4. Event correlation histograms of SPWs during slow oscillations. Event correlation histograms of all SPWs identified during negative (*left*) and positive (*right*) half-waves in prefrontal EEG. *A*: histograms for two individual rats from one session together with corresponding raster plots of SPW events across all negative and positive half-waves, respectively. *B*: mean + SEs of all event correlation histograms across all rats and sessions together with corresponding mean negative and positive half-waves (original, wideband-recorded signal), respectively (black lines, above; per session, respectively, 1732 and 1877 negative and positive half-waves were averaged). Dotted lines indicate reference point of peaks in positive and negative half-waves.

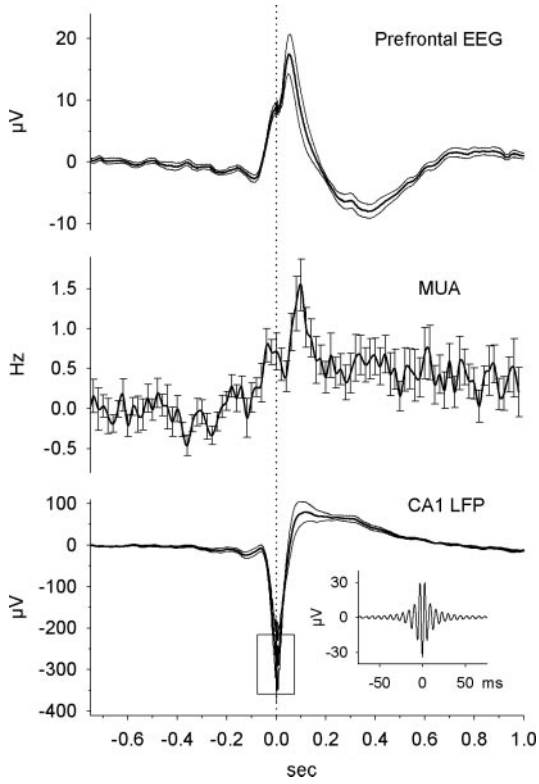

FIG. 5. Sharp wave-ripple complex. Averages of original, wideband-recorded EEG activity (*top*), of event correlation histograms of mean multiunit spike rate (*middle*) and of original, wideband-recorded hippocampal activity in CA1 LFPs (*bottom*) across all sharp wave-ripple (SPW) events detected during SWS. Averaging was performed with reference to the deepest ripple trough of SPW events (*dotted line*, 2598 SPWs per session). Indicated are means (thick lines) ± SEs (thin lines for EEG and CA1 LFP, error bars for MUA). MUA in event correlation histogram is corrected to baseline (–1.0 to −0.8 s interval). Insert in bottom diagram enlarges the −75 ms to +75 ms interval of the mean CA1 LFP filtered between 150–250 Hz.

ity during SWS are depicted in Fig. 6. The cross-correlation function for spindle RMS activity shows a distinct positive peak at a time lag of -57.5 ± 8.1 ms ($P < 0.001$), indicating that decreases in the spindle RMS signal precede shifts toward negativity in the slow oscillatory signal and, correspondingly, that increases in spindle activity precede positive going shifts in the slow oscillation.

The cross-correlation function between slow oscillatory activity and hippocampal ripple activity again indicates a significant ($P < 0.001$) positive peak at a time lag of -26.4 ± 4.8 ms. This peak is even more pronounced than that for spindle activity, with the corresponding time lag indicating that the decreases and increases in the ripple RMS signal slightly precede negative-going and positive-going potential shifts in the slow oscillatory signal, respectively. A smaller but significant ($P < 0.001$) negative peak in this cross-correlation function is found between −300 and −200 ms, which reflects mainly the slight increase in ripple activity seen before the peak of the negative half-wave (see Fig. 3).

Cross-correlations between hippocampal ripple and prefrontal spindle RMS signals during identified slow oscillations were generally more variable, but showed a positive peak at a 0-sec time lag ($P < 0.001$), indicating that spindle and ripple activity tend to vary in parallel (Fig. 6). On the negative (left) side of the cross-correlation function, a small but significant ($P < 0.001$) positive plateau reflects that increased ripple activity precedes increased spindle activity by about half a second, i.e., ripples are more likely in the 1-s epoch before than in the 1-s epoch after increased spindle activity (see Fig. 3).

DISCUSSION

The main results of our experiments indicate that hippocampal sharp wave-ripple (SPW) events during SWS in rats are temporally coupled to slow oscillations of prefrontal cortical origin. Hippocampal SPW events are distinctly decreased during the hyperpolarizing negative phase of the slow oscillations, as measured in the prefrontal EEG, whereas their occurrence distinctly increases during the depolarizing positive phase of the slow oscillation. A fine-grained analysis of the temporal dynamics indicates a phase delay of the slow oscillation with reference to the down and up states of excitation in the underlying neocortex, as defined by MUA, of approximately 90 ms, and with reference to the number of hippocampal SPWs

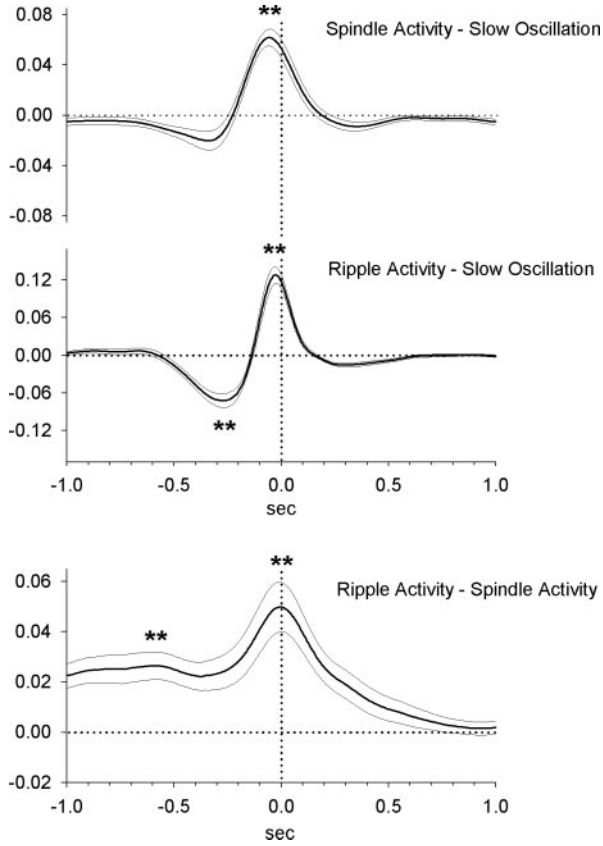

FIG. 6. Cross-correlation analyses of spindle and ripple activity during slow oscillations. *Top*, cross-correlation function between the spindle RMS signal and the slow oscillatory EEG potential in the prefrontal cortex during SWS. The positive peak at about −60 ms indicates that decreases (increases) in spindle activity precede shifts toward negativity (positivity) in the slow oscillation, by this time period. *Middle*, cross-correlation function between the ripple RMS signal of CA1 LFPs and the prefrontal slow oscillatory EEG potential. The positive peak at about −25 ms indicates that decreases (increases) in ripple activity precede shifts toward negativity (positivity) in the slow oscillation, by this time period. *Bottom*, cross-correlation function between the ripple RMS signal of the CA1 LFP and the prefrontal spindle RMS. Positive plateau on the negative side indicates that increased ripple activity precedes increased spindle activity by about half a second, The means (*thick lines*) ± SEs (thin lines) are shown. ** $P < 0.001$.

of approximately 60 ms (Fig. 2 and Fig. 4). This temporal pattern supports the view that excitatory up states in neocortical assemblies preceding hippocampal activity promote, via efferent pathways, the generation of hippocampal SPWs (Sirota et al. 2003).

In combination with previous reports (Battaglia et al. 2004; Sirota et al. 2003), the present finding provides further support for the notion that the grouping influence of neocortical slow oscillations and of underlying neocortical down and up states of excitation is not limited to thalamocortical spindle activity or fast and ripple-like activity generated in the neocortex, but pertains to local hippocampal events as well (Contreras and Steriade 1995; Grenier et al. 2001; Steriade and Amzica 1998). In previous studies in humans, spindle activity was found to be decreased during the hyperpolarizing negative phase of the slow oscillation in the frontal EEG, and to show a strong rebound increase in temporal association with the positive-going depolarizing phase of the slow oscillation (Mölle et al. 2002). An identical temporal dynamic was replicated here by applying the same analyses to prefrontal EEG data in rats, which also exhibited a most prominent rebound of spindle activity during the positive-going, up phase of the slow oscillation (Fig. 3). The picture is well in line with the view derived from studies in cats that the depolarizing component (i.e., surface-positive EEG component) of the slow oscillation is the factor driving the thalamic generation of spindle activity via corticothalamic volleys (Steriade 1999; Steriade 2004; Steriade and Amzica 1998). The distinct rebound-like enhancement in spindle activity after negative half-waves probably reflects a postinhibitory rebound spike-burst in thalamocortical neurons (Contreras and Steriade 1995).

There was an even stronger association of the neocortical slow oscillations with hippocampal ripple activity. Like the spindle signal, CA1 ripple activity is strongly decreased for an interval of about 150–200 ms in temporal proximity to the peak of the negative half-wave (Fig. 3). Moreover, the transition from the down state to the positive up state, indicated by a shift of 200 μV in the wave-triggered average of the prefrontal EEG, is consistently associated with a pronounced shift from low to high ripple activity. This temporal pattern is confirmed by the correlation histograms of detected SPW events showing that the frequency of SPWs was distinctly decreased during negative-going half-waves and at the highest level during positive-going half-waves.

Unexpectedly, the fine-grained analysis of the temporal relationship between slow oscillations and MUA in the prefrontal cortex revealed that downs and ups of MUA preceded the negative and positive going phases of the slow oscillation by approximately 90 ms on average. This finding contrasts with recordings from the somatosensory cortex in cats and mice where MUA and slow oscillations were observed to be almost entirely in synchrony (Destexhe et al. 1999; Mukovski et al. 2006; Sirota et al. 2003). However, in a recent study, recordings from the barrel cortex of rats indicated a similar phase delay of slow oscillations in the local field potential with reference to activity of single units (Zou et al. 2005), and a temporal disparity of population firing rates and slow oscillatory activity in neocortical LFPs in rats has been also reported by others (Battaglia et al. 2004). Our observation cannot be explained by amplifier-induced phase shifts that were <1 ms for the frequency bins of interest and in effect negative (Pallás-Areny and Webster 1999). Also capacitive effects that occur mainly at the skull and preferentially delay the phase of slow frequency oscillations (Nunez 1981) can be ruled out, since the phase advance of MUA was observed similarly for slow oscillations identified in the depth-recorded LFP and in the EEG recorded from skull electrodes. One possible source contributing to our observation that changes in MUA preceded the respective negative and positive-going phases of the slow oscillation could be related to our choice of reference for the EEG and LFP recordings. In supplemental experiments, we recorded in several rats the prefrontal EEG, not only against our more anterior reference, but simultaneously against a more neutral, posterior reference placed over the cerebellum, which has been similarly used by others (e.g., Sirota et al. 2003). Slow oscillations in the prefrontal EEG for these different reference placements showed phase shifts of 25 ms. This analysis demonstrates a clear influence of the placement of the reference on the exact timing of the slow oscillation phase, although it does not fully explain the shift between changes in MUA and slow oscillation. Also, the delay in the prefrontal slow oscillation could be linked to neuron-glial interactions accompanying the generation of slow oscillation, and a greater proportion of glial potential activity picked up in our LFP and EEG recordings. In vivo simultaneously recorded neuron-glia pairs revealed that the depolarizing phase of the slow oscillation in nearby glia cells follows that recorded from the neuron with a lag of 88 ms (Amzica and Massimini 2002), i.e., a time lag very similar to that observed here between MUA and EEG slow oscillations.

Slow oscillations were also found to be delayed in reference to the waxing and waning of hippocampal ripple activity. Yet, this delay was distinctly shorter than that with reference to prefrontal MUA. Specifically, SPW event histograms show a minimum of SPWs 45.6 ms before the peak of negative half-waves, and a maximum of SPWs 73.3 ms before the peak of positive half waves, amounting to an average advance of approximately 60 ms that changes in SPWs precede the slow oscillation. Consequently, since changes in prefrontal MUA precede the slow oscillation by approximately 90 ms, prefrontal MUA precede hippocampal ripple activity by approximately 30 ms. This interval could well cover the time needed for efferent volley originating during neocortical up states of excitation to reach hippocampal CA1 networks and drive SPW events. In this regard our data corroborate previous findings by Sirota et al. (2003) in mice and rats indicating that a slow oscillatory modulation of unit activity in deep layers (V, VI) of the somatosensory cortex toward increased firing precedes SPW events by approximately 50 ms.

A substantial increase of SPW firing at the transitions from the neocortical down states to up states has been also reported by Battaglia et al. (2004). However, those authors reported also that SPWs are more probable during down states than during up states of neocortical activity. While this finding contradicts with ours, it may be related to the determination of up and down states in those experiments. Unlike the present experiments, in which the EEG-defined slow oscillation is taken as point of reference, Battaglia et al. defined up and down states as periods with high and low activity in multiple unit recordings. In fact, those authors emphasize that population firing rates during slow oscillatory activity do not show the bimodality typical for the slow oscillations, but very abrupt upward

transitions and a distinctly more gradual decline during the down phase (Battaglia et al. 2004).

Cross-correlating ripple and spindle activity revealed that the increased occurrence of SPWs during slow oscillation positivity coincided also with an increased spindle activity. A similar synchronization between ripple and spindle activity has been observed previously, whereby hippocampal neuronal firing tends to precede the onset of spindles and thus could feed excitation to subsequent spindle cycles (Siapas and Wilson 1998). Those authors suggested that hippocampal ripple oscillations may serve to bias or select which set of neocortical neurons preferentially participates in a spindle episode based on the information placed into the hippocampal network by past experience. However, the relatively moderate size of the association between ripple and spindle activity in our data as compared with the relationship between slow oscillations and SPWs and spindles does not speak for an immediate influence of hippocampal ripples on the initiation of spindle activity but rather for a joint (synchronizing) influence of slow oscillations on these phenomena. Our results in fact support the view that, in the first place, it is the neocortical activity that biases the probability of hippocampal events. While spindle and ripple activity are grouped by slow oscillations, the finding that during a slow oscillation, ripple activity tends to precede spindle activity, leaves the possibility that hippocampal SPWs additionally exert a weak influence on spindle activity.

The distinct temporal patterns identified here between slow oscillations and associated excitatory down and up states in the neocortical networks, on the one hand, and hippocampal SPWs, on the other hand, could serve a role in the consolidation of hippocampus-dependent memory. Previous studies in humans have pointed to a grouping effect of slow oscillations on a replay of neocortical memory representations during sleep after learning (Huber et al. 2004; Mölle et al. 2004). Moreover, the replay of memory representations encoded in hippocampal networks has been shown to take place preferentially during SPW events (Buzsáki 1998; Kudrimoti et al. 1999; Siapas and Wilson 1998). Assuming that the slow oscillations originate preferentially in those neocortical networks that have been previously involved in the acquisition of information (Huber et al. 2004; Massimini et al. 2004; Mölle et al. 2004), the driving influence of slow oscillation up states on hippocampal SPWs generation indicated here could provide a mechanism that enables, via entorhinal pathways, a selective stimulation of hippocampal neuron populations participating in the SPW event (Sirota et al. 2003). SPWs, in turn, enable reactivated hippocampal memories to be fed back into corticothalamic circuitry at a time when these networks are most excitable, i.e., in an up state, which would ease long-lasting plastic changes in these neocortical networks. Indeed, there are some hints at synchronized memory replay occurring during SWS concurrently in neocortical and hippocampal (and other) brain regions (Qin et al. 1997; Ribeiro et al. 2004). However, a truly binding role of slow oscillations in the coupling of specific replay activity remains to be demonstrated.

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