Single Neuron Burst Firing in the Human Hippocampus During Sleep

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ABSTRACT: Although there are numerous non-primate studies of the single neuron correlates of sleep-related hippocampal EEG patterns, very limited hippocampal neuronal data are available for correlation with human sleep. We recorded human hippocampal single neuron activity in subjects implanted with depth electrodes required for medical diagnosis and quantitatively evaluated discharge activity from each neuron during episodes of wakefulness (Aw), combined stage 3 and 4 slow-wave sleep (SWS), and rapid eye movement (REM) sleep. The mean firing rate of the population of single neurons was significantly higher during SWS and Aw compared with REM sleep ($p = 0.002$; $p < 0.0001$). In addition, burst firing was significantly greater during SWS compared with Aw ($p = 0.001$) and REM sleep ($p < 0.0001$). The synchronized state of SWS and associated high-frequency burst discharge found in human hippocampus may subserve functions similar to those reported in non-primate hippocampus that require burst firing to induce synaptic modifications in hippocampal circuitry and in hippocampal projections to neocortical targets that participate in memory consolidation. *Hippocampus 2002;12:724–734.*

KEY WORDS: slow-wave sleep; REM sleep; firing rate; high-frequency discharge

INTRODUCTION

The marked changes in central neuronal activities that accompany mammalian sleep have generated numerous theories on the function of sleep (for review, see Rechtschaffen, 1998). For example, "dream" sleep has been proposed as a period during which memory traces are refined by weakening disruptive synaptic connections among neuronal networks (Crick and Mitchison, 1983). The importance of the hippocampal formation for memory is well established on the basis of neuroanatomical and electrophysiological studies (Squire and Zola, 1996; Eichenbaum, 1999). The idea that sleep subserves memory consolidation derives from results showing that hippocampal "place" cells exhibit experience-dependent replay of firing patterns during episodes of slow-wave sleep (SWS) (Wilson and McNaughton, 1994; Kudrimoti et al., 1999) and rapid eye movement sleep (REM) (Poe et al., 2000; Louie and Wilson, 2001).

During SWS, the non-primate hippocampal encephalogram (EEG) is dominated by irregular large-amplitude activity with the intermittent appearance of sharp waves (SPW) and related high-frequency (100–200-Hz) "ripple" oscillations. The spontaneous activation and dynamic interaction between hippocampal pyramidal cells and interneurons underlie the SPW and associated ripple events (Ylinen et al., 1995; Draguhn et al., 2000). During REM sleep, hippocampal SPW-ripple events are replaced by a pronounced rhythmic slow activity (RSA) that reflects a shift from irregular synchronized firing to rhythmic hippocampal burst firing (Buzsaki et al., 1983; Cobb et al., 1995). On the basis of these neurophysiological correlates of sleep, it has been suggested that during SWS, high-frequency bursts of hippocampal neuronal activity serve to consolidate and transfer stored representations to neocortical networks (Buzsaki, 1998). REM sleep has been described as a period during which weak associations among neocortical networks are strengthened and representations relayed back to the hippocampus (Stickgold, 1998).

Given the abundant animal literature on hippocampal neuronal activity across behavioral states, it is important to determine what state-dependent changes occur in hu-

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man hippocampal neuronal activity, in contrast to that recorded in non-primate hippocampus. For example, does human hippocampus show neuronal burst firing associated with the large amplitude irregular activity that characterizes non-primate hippocampal activity during SWS sleep? Also, does human hippocampal activity show the prominent theta frequency bursting observed in non-primates during REM sleep? Perhaps of greater importance is whether there are patterns of discharge during sleep states that are suitable for the transfer of hippocampal input or output to and from neocortical networks. To address these questions, we used the opportunity to record spontaneous human hippocampal single neuron activity in subjects with chronically implanted hippocampal depth electrodes required for medical diagnosis. During a period of overnight recording, single neuron firing properties were correlated with states of wakefulness, SWS, and REM sleep. After these recordings, we quantitatively evaluated hippocampal activity for high-frequency burst discharge, and on the basis of rates, patterns, and variability of firing.

MATERIALS AND METHODS

Subjects

Wide-band, high-frequency recordings were obtained from eight patients with medically intractable complex partial seizures. Before depth electrode implantation to investigate and localize areas of seizure onset, patients gave their informed consent for participation in these studies under the approval of the UCLA Internal Review Board. Each patient was surgically implanted with 8–14 flexible polyurethane depth electrodes stereotactically targeted to clinically relevant brain areas, which were monitored on a 24-h basis to find those in which spontaneous seizure activity began first (Fried et al., 1999). Patients in whom a seizure onset area could be localized became candidates for surgical removal of epileptic sites if resection of the area would not produce an unacceptable neurological deficit. Localization was based on the recording of 3–10 seizures during the average 2 weeks that patients spent in the hospital. For each subject, functional and anatomical data and, when applicable, follow-up reports from resective surgery were used to identify the epileptic area (Engel, 1996).

Electrodes and Localization

High-frequency EEG was recorded from bundles of nine platinum–iridium microwires, which were inserted through the lumen of seven-contact clinical depth electrodes, so that they extended 3–5 μm beyond the tip of the clinical electrode. Microwires were 40 μm in diameter with impedances from 200–400 kΩ. Electrode tips were localized using the combined information from co-registered postimplant computed tomography (CT) scans, pre-implant 1.5-T magnetic resonance imaging (MRI) scans, and skull radiographs. The imaging software used (Brain Navigator, Telefactor, Philadelphia, PA) allowed for visualization and highlighting of electrode tip locations on CT, which were automatically registered to the MRI scan. Anatomical boundaries were based on references of hippocampal anatomy by Duvernoy (1998) and Amaral and Insausti (1990). Only microwires verified to be located in the hippocampus were used in analyses (Fig. 1).

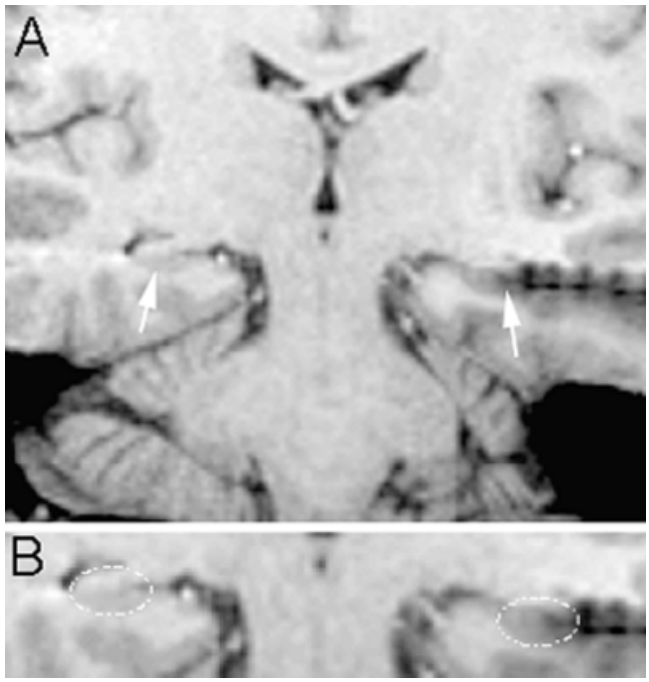

FIGURE 1. Coronal magnetic resonance imaging (MRI) scan illustrating position of depth electrode. A: Electrode entering from the lateral aspect of the temporal lobe and the tip of the electrode lying within the hippocampus (right arrow). The arrow on the left points to the contralateral hippocampus. B: Enlarged view from A. Dotted circles outline the hippocampus. Electrode position is indicated on scan as signal dropout. The area of signal dropout is much larger compared with the actual size of the electrode. Only those electrodes verified to be positioned within the hippocampus were included in the analyses.

Overnight Polysomnographic Sleep Studies

Sleep studies were conducted on the hospital ward within each subject's room. Studies were conducted 48–72 h after surgery and typically began between the hours of 10 PM and midnight and ended at 6 AM the following morning. Patients continued taking their standard doses of anticonvulsant medications during this period. Sleep staging was carried out according to the criteria of Rechtschaffen and Kales (1968). The sleep record consisted of two electro-oculogram leads recording eye movements, two electromyogram (EMG) leads placed on the chin to record submental muscle tone, and two O Flexon scalp leads placed during surgery at "10–20" positions C3 and C4 each referred to the contralateral ear to record cortical EEG activity (Fig. 2). Sleep–wake stages were categorized as waking, drowsy, stage 1–4 sleep, and REM sleep. Single neurons recorded during the stages defined as waking (Aw), stages 3 and 4, hereafter referred to as slow-wave sleep (SWS), and REM sleep were analyzed for firing rate and bursting activity. States of drowsiness, stages 1and 2 were not included for analyses in order to better contrast hippocampal activity during states of wakefulness and non-REM sleep.

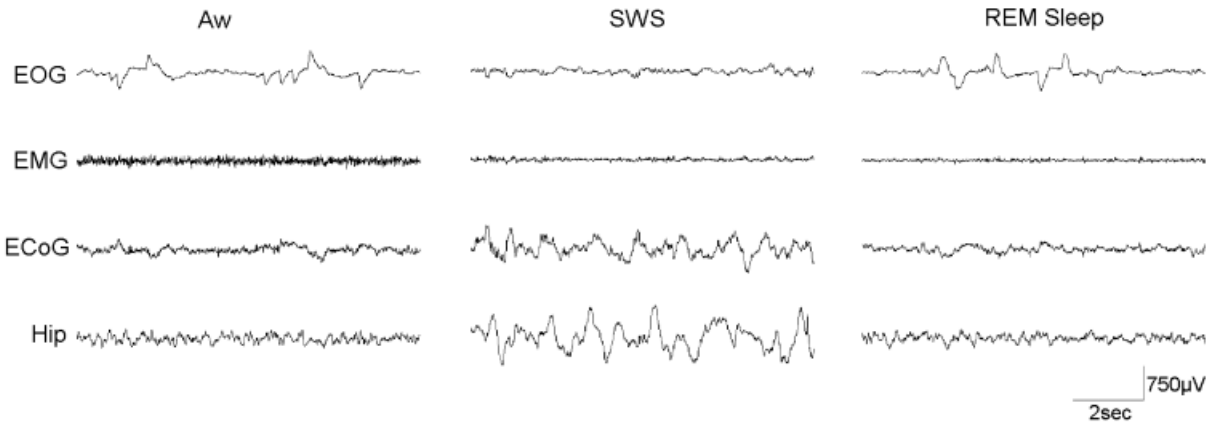

FIGURE 2. Example of hippocampal electroencephalogram (EEG) during Aw, slow-wave sleep (SWS), and rapid eye movement (REM) sleep. These three 10-s samples were taken from a sleep record that was acquired during one patient's overnight polysomnographic sleep study. Sleep staging was carried out using a sleep record consisting of recorded eye movements (EOG), muscle tone (electromyogram [EMG], chin), and scalp EEG (EcoG, C3–A2 derivation). Note the irregular large amplitude, slow-wave activity in hippocampus (Hip) and EcoG during SWS, in contrast to the low-amplitude, mixed frequency activity in Hip and EcoG during Aw and REM sleep.

Electrophysiology

Continuous high-frequency EEG was recorded wide-band (0.1–5 kHz) and sampled at 10 kHz with 12-bit precision using RC Electronics software (Santa Barbara, CA). Data files were copied onto 1-GB Jaz disks for off-line analysis, and later archived on CD. Data channels of interest were high-pass filtered at 300 Hz (roll-off 36 dB) and visually examined for the presence of neuronal activity.

Extracellularly recorded action potentials were triggered and discriminated using DataWave Technologies CP Analysis software (Longmont, CO). High-frequency, bi- or triphasic action potential waveforms ("spikes") with amplitude >3:1 signal-to-noise (S:N) ratio were triggered using window and duration ($0.1 <$ width <2.0 ms) discriminators (Fig. 3); 3 ms surrounding the main negative peak of the triggered spikes were saved in detection files. Detection files were replayed to identify spike waveform characteristics that could be used in spike sorting. Up to eight parameters were extracted from each waveform and used in a spike-sorting method called "cluster cutting." Waveform parameters included amplitude and width of the maximal negative peak (width measured from peak negativity to the following peak positivity) and amplitude and duration of peaks or valleys surrounding the maximal negative peak. Various combinations of the waveform parameters were graphically displayed in x,y-point plots such that spikes with similar waveform parameters would form clusters of points. Boundaries separating point clusters were visually set in order to group points representing similar waveforms belonging to a putative single neuron. Maximum boundary thresholds on the eight extracted waveform parameters were set at ± 2.5 SD of the mean value of the point cluster. After replaying the clustered spike waveforms to visually confirm the accuracy of the cluster boundaries, autocorrelograms with a time base of 1,000 ms and a bin-width of 1 ms were constructed for each single neuron. A spike train with counts less than the mean firing frequency of the neuron in the 0–2-ms bins (refractory period) was considered a single neuron. A spike train with counts greater than mean firing frequency during the refractory period was considered multiple neurons, and re-clustered. Upon re-clustering, a spike train with an absence of a clear refractory period was termed multiple neuron activity and omitted from the analysis. Cross-correlograms with 1-ms bin-widths were constructed for all simultaneously recorded neurons. Pairs of neurons recorded on different microwires in the same bundle that demonstrated 0-ms coincident interactions exceeding 99% confidence (Abeles, 1982) were considered the same neuron, and one neuron was eliminated.

Single Neuron Analysis

To segregate hippocampal neurons on the basis of pathology, two major criteria were used: hippocampal atrophy and electrographic seizure onsets. A single neuroradiologist at UCLA evaluated every subject's MRI scans for the presence or absence of hippocampal atrophy and its location as part of the clinical workup. Electrographic seizure onsets were recorded during the patient's depth electrode telemetry monitoring, and attending neurologists in the UCLA Seizure Disorders Center determined locations of seizure onset. Neurons from atrophic hippocampi or from hippocampi where seizure onsets were recorded were omitted. Neurons that were successfully recorded during Aw, SWS, and REM sleep for $\geq$600 s in each state were included in the analysis.

Discharge activity was characterized for hippocampal neurons by measuring mean firing rate, median interspike interval (ISI), mean interval-to-interval variability, bursts per minute (burst rate), bursts per 500 spikes (burst ratio), percentage of spikes in bursts to total spikes, burst duration, and mean number of spikes in bursts. Interspike interval variability was measured by calculating the coefficient of variation (standard deviation/mean) between adjacent ISI within each spike train (known as C_{V2}) (Holt et al. 1996). Thus, for a spike train of n spikes, there are $n - 2$ C_{V2} values. The C_{V2} measure effectively reduces the variation in the neuron firing that occurs on a time scale longer than the mean ISI. For compar-

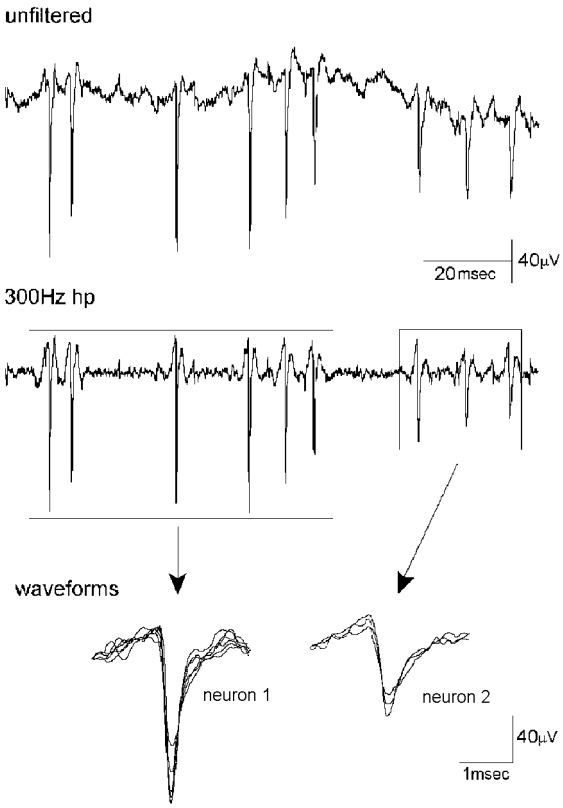

FIGURE 3. Detection and separation of single neuron activity. Continuous wide-band electroencephalogram (EEG), digitized at 10 kHz, showing multi-neuron activity (top trace) was high-pass filtered (middle trace) in order to set window discriminators to trigger neuronal activity. Action potential waveforms ("spikes," bottom) with amplitudes >3:1 signal-to-noise (S:N) were triggered and separated based upon extracted waveform characteristics using a graphical cluster cutting method. In this example, the action potentials within the two "boxed" sections of the filtered trace would be clustered to represent the activity from two different neurons. Spike amplitudes were typically >50 μV. However, for bursting neurons with spike amplitude attenuation, it was occasionally observed that spikes toward the end of the burst (i.e., 3rd spike in burst of neuron 2) fell below the minimum 3:1 SNR threshold and would not be detected. Given that not all bursts were comprised of spikes with attenuating amplitude, and that an average of only 10% of spikes were within bursts during any given state, the loss of these "subthreshold" spikes would be negligible.

ison across states, we calculated the mean C_{V2} for each spike train. Burst detection involved serially scanning each spike train to identify short duration, high-frequency discharge episodes. A burst was defined by a series of three or more spikes with an ISI <20 ms. Rhythmic burst discharge was measured using autocorrelograms constructed with a time base of 2 s and bin-width of 1 ms. Significance for rhythmic discharge was established as the appearance of a peak(s) beyond the initial 20 ms that consisted of ≥5 consecutive bins exceeding the 99% confidence interval of the mean firing rate.

Discharge variables were analyzed using an analysis of variance (ANOVA) repeated measures design. Consistent with the requirement for normality, variables were transformed with a logarithmic function such that $X' = \log(X)$. Significant ($\alpha = 0.05$) "main effects," i.e., state (Aw vs SWS vs REM sleep), were further analyzed using a Bonferroni post hoc analysis. Comparison of median ISI values and the number of spikes per burst were made using a one-way Kruskal–Wallis and post hoc analysis with the Wilcoxon signed-rank test.

RESULTS

The primary goal of this investigation was to characterize the firing patterns of hippocampal neurons during Aw, SWS, and REM sleep. We found significant reductions in mean firing rate among single hippocampal neurons during REM sleep. During periods when cortical EEG recordings consisted of large amplitude low-frequency waves, which characterize SWS, we observed an increase in hippocampal high-frequency burst discharge that was significantly greater than the bursting activity recorded during episodes of Aw and REM sleep.

Properties of the Single Neuron Population

A total of 76 well-isolated single neurons recorded in 17 patients were localized in hippocampus using post-implant CT and MRI (Figs. 1, 3). Nineteen neurons in four patients were excluded on the basis of localization in atrophic and/or epileptic (seizure-generating) hippocampi during each state. An additional 34 neurons recorded in five patients did not meet the minimum sampling criterion of 600 s recorded during each polysomnographically defined state (Fig. 2). For the remaining 23 single neurons, a total of 13.4 h of recorded activity was analyzed from eight subjects during the states of Aw, SWS, and REM sleep. For localization of recording electrode tips using the techniques described in the Materials and Methods section, hippocampi were visualized with 1.0-mm MRI coronal slices. Starting at the anterior most point and proceeding posteriorly along the longitudinal axis of the hippocampus, 11 of the 23 neurons were located within the anterior hippocampal region (also known as uncal hippocampus or pes hippocampi). Three neurons were located in the middle or body of the hippocampus (at anteroposterior levels identified by the presence of the lateral geniculate nuclei on the coronal slices), and the remaining nine neurons were located in the posterior hippocampus or hippocampal tail. Of the 23 hippocampal neurons that were analyzed, six were recorded in hemispheres contralateral to epileptic sites localized to the frontal lobe, 13 neurons were in hemispheres contralateral to epileptic mesial temporal lobe sites, and four neurons were 3–5 cm distant from an ipsilateral epileptic site localized in the lateral temporal lobe. MRI-defined hippocampal atrophy was present in two of the eight patients, and in both patients, all seven neurons were contralateral to both the atrophic hippocampus and the electrophysiologically defined epileptic site. Behavioral attributes common to all Aw episodes were that subjects were lying supine in bed, eyes open, and participating in quiet conversation with one of the investigators. The average epoch analyzed for each neuron during each state was 699 ± 27 s (mean ± SE). The mean number of spikes comprising each spike train was 1,301 ± 170 spikes. The minimum number of spikes analyzed

within one spike train was 34, while the maximum was 6,674 spikes.

Firing Rate

The mean firing rate during Aw, SWS, and REM sleep showed significant state-related changes in hippocampal single neuron activity ($F_{2,66} = 11.94$, $P < 0.0001$). We observed a significant reduction in hippocampal activity during REM sleep compared with Aw and SWS (Aw vs REMS, $P = 0.002$; SWS vs REMS, $P < 0.0001$) (Fig. 4A). Twenty of the 23 hippocampal neurons decreased discharge during REM sleep, in comparison with Aw and SWS rates, while three showed an increase. No significant difference in mean firing rate was observed between the two electrographically distinct states of Aw and SWS. However, the distribution of firing rates (Fig. 4B) illustrates that during Aw, 11 of the 23 total neurons and, during REM sleep,12 of the 23, had firing rates <1 spike/s, while during SWS only two neurons had firing rates this low.

Because neuronal firing rates may be influenced by the period from when it occurred during the sleep process, we divided each subject's sleep recording into three time periods: beginning (22:00–01:00), middle (1:00–4:00), and end (4:00–7:00), and compared the neuronal activity recorded during Aw, SWS, and REM sleep on the basis of these time periods. Mean firing rate during Aw episodes that occurred during the beginning period of the sleep recording (n = 5) was significantly higher compared with firing rate during Aw episodes that occurred during the middle period of the sleep recording (n = 18; t = 2.92, df = 21, $P = 0.008$; 5.08 ± 1.49 vs 1.17 ± 0.19 spikes/s). None of the Aw episodes occurred during the end period of the sleep recording. Six of the eight subjects had SWS episodes during the middle of the sleep recording (n = 21), negating any meaningful comparison between SWS episodes that occurred at the beginning (n = 1) or end (n = 1). There was no difference in mean firing rate during REM sleep episodes that occurred during the middle period of the sleep recording (n = 11) compared with those that occurred during the end period of the recording (n = 11; $P = 0.3$). One subject had a REM sleep episode occur during the beginning of the sleep recording (n = 1).

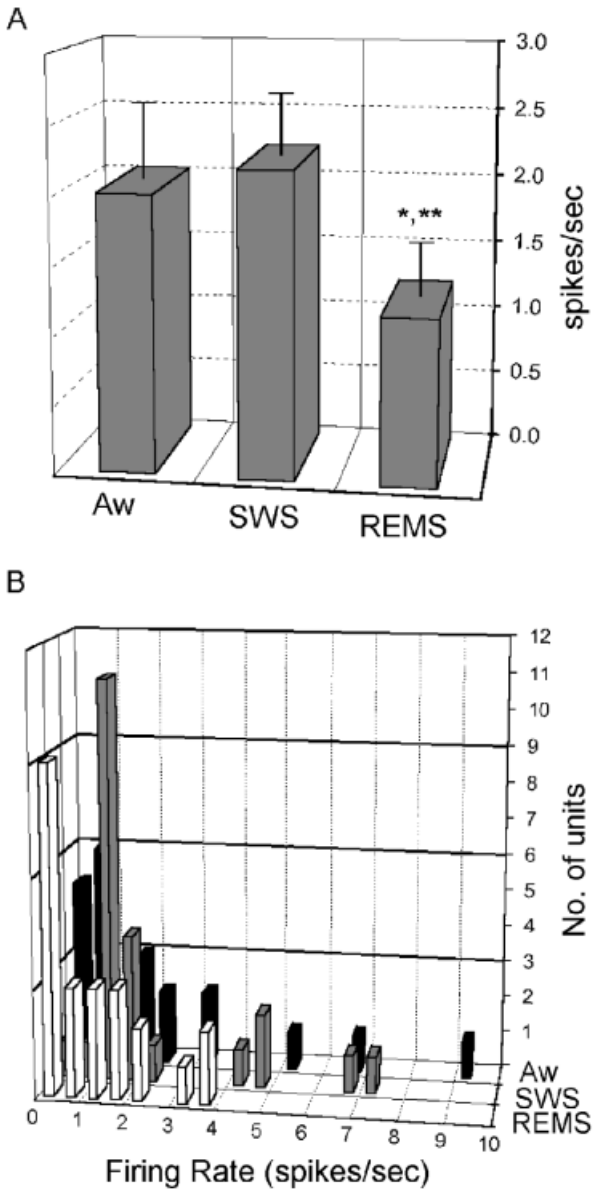

FIGURE 4. Firing rate of hippocampal neurons across states. A: Mean firing rate for all 23 hippocampal neurons during states of Aw, slow-wave sleep (SWS), and REM sleep (REMS). REM sleep was associated with a significant reduction in firing rate compared with Aw and SWS. No difference in firing rate was observed between Aw and SWS. B: Frequency distribution of firing rates during each state. Note the number of neurons firing >1 spike/s during SWS compared with Aw and REMS, and the lack of neurons firing >4 spikes/s during REMS. *Aw vs REMS, $P = 0.002$; **SWS vs REMS, $P < 0.0001$.

Variability of Firing

Having observed differences in firing rate associated with sleep–wake states, we sought to characterize the variability of neuron firing by quantifying the ISI distribution. Visual inspection of ISI histograms, like those in Figure 5A, typically revealed a positively skewed distribution, thus the median was used instead of the mean to estimate central tendency. Fluctuations in hippocampal discharge were reflected in ISI histograms as a broad range of ISI values, from a minimum >2 ms to ISI values exceeding 1,000 ms. The state-dependent differences observed in firing rate (Fig. 4A) inversely correlated with the median ISI values for each state ($H_{2,66} = 10.27$, $P = 0.005$). Significantly lower firing rates during REM sleep were associated with significantly longer median ISI values (389 ms) compared with the median ISI values during Aw (199 ms; $P = 0.005$) and SWS (146 ms; $P < 0.0001$).

Because a variable burst pattern of firing has been implicated in many studies correlating hippocampal activity and behavior, we employed joint ISI plots to obtain further information about differences in the patterns of firing that were observed across states. All the neurons we analyzed demonstrated the capacity to discharge spikes at short intervals, i.e., high-frequency discharge. This is illustrated as a peak near the origin of the ISI histogram (Fig. 5A)

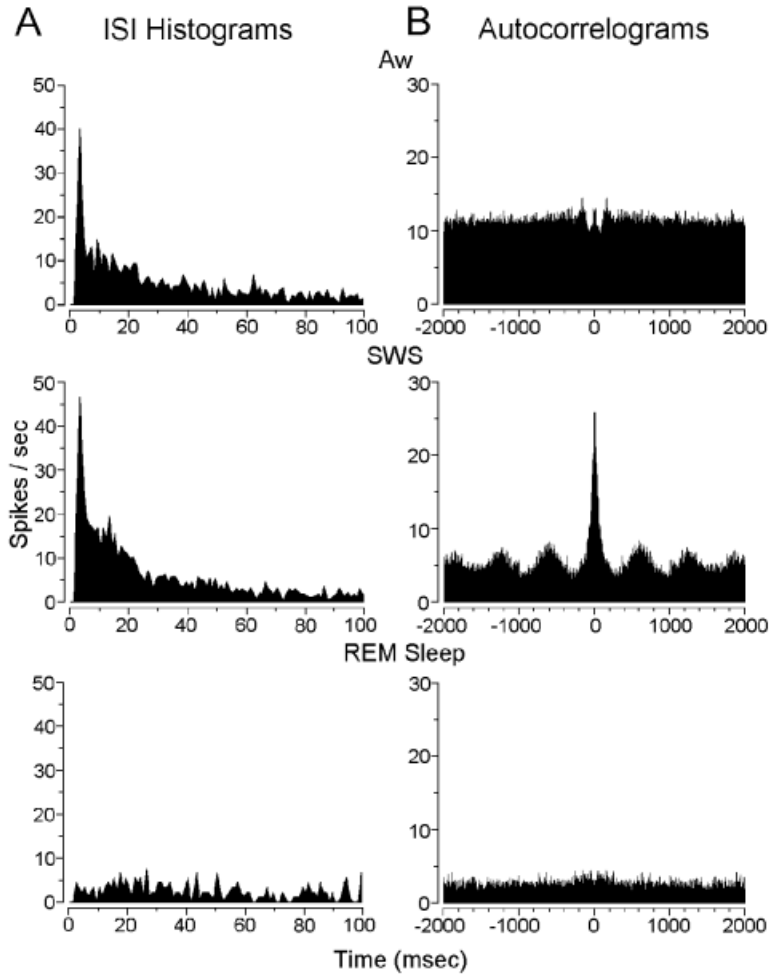

FIGURE 5. Interspike interval (ISI) histogram and autocorrelogram of two different single neurons during awake (Aw), slow-wave sleep (SWS), and rapid eye movement (REM) sleep. A: ISI histograms for this single neuron illustrate it was during the initial 20 ms that the differences in ISI values across state were most pronounced, showing a tendency for burst firing during SWS and Aw, but no bursting during REMS. B: Autocorrelogram for a different single neuron reveals rhythmic discharge with an approximate frequency of 1.5–2 Hz during SWS. Note that rhythmic firing of higher frequencies at 4–7 Hz was detected in two additional neurons during episodes of Aw and REM sleep. ISI histograms and autocorrelograms were constructed with 1-ms bin width.

or on the joint ISI plots (Fig. 6A) as the clustering of points near the origin of the axes. The joint ISI is a plot of successive pairs of ISI values (ISI_i vs ISI_{i+1}) to identify patterns that may exist in neuron firing. Fluctuations in neuron firing were most pronounced during SWS. Such major changes in spike discharge indicate fluctuations in firing that occur on a time scale longer than that predicted by the mean ISI. To effectively reduce the amount of variability associated with such long time-based rate changes, the C_{V2} (S.D./mean between all adjacent ISI values) was calculated and plotted for all 23 neurons combined during each state (Fig. 6B). A uniform distribution of values of 0–2 would be expected if spike discharge followed a Poisson process. As can be seen in the plots, the distributions are relatively uniform over this range. However, during SWS, the dark "cloud" of points at the top of the plot represents the increased variability of neuron firing corresponding to short–long or long–short interval–interval changes in neuron firing. Computing the mean C_{V2} during each state, results revealed state-specific differences in the spiking pattern ($F_{2,66} = 3.52$, $P = 0.03$). Discharge variability was highest during SWS with a C_{V2} of 1.14 ± 0.03, compared with Aw, which demonstrated the lowest C_{V2} value of 1.07 ± 0.02 ($P = 0.01$). REM sleep was intermediate to both Aw and SWS at 1.09 ± 0.03.

Burst Patterns and Spikes Per Burst

The ISI histograms (e.g., Fig. 5A) showed that the largest difference in the frequency of ISI values across states was found during the initial 20 ms of the histogram. To better differentiate state-dependent differences in high-frequency discharge, we set our burst criteria as a series of three or more consecutive spikes separated by ISI values of <20 ms in duration. The hippocampal burst events observed in our recordings were often characterized by decrementing amplitude during the successive discharge of spikes (Fig. 3). The mean duration of a hippocampal burst event was 28.0 ± 0.0003 ms (n = 2321). No statistical difference was noted in burst duration across states. However, the mean number of

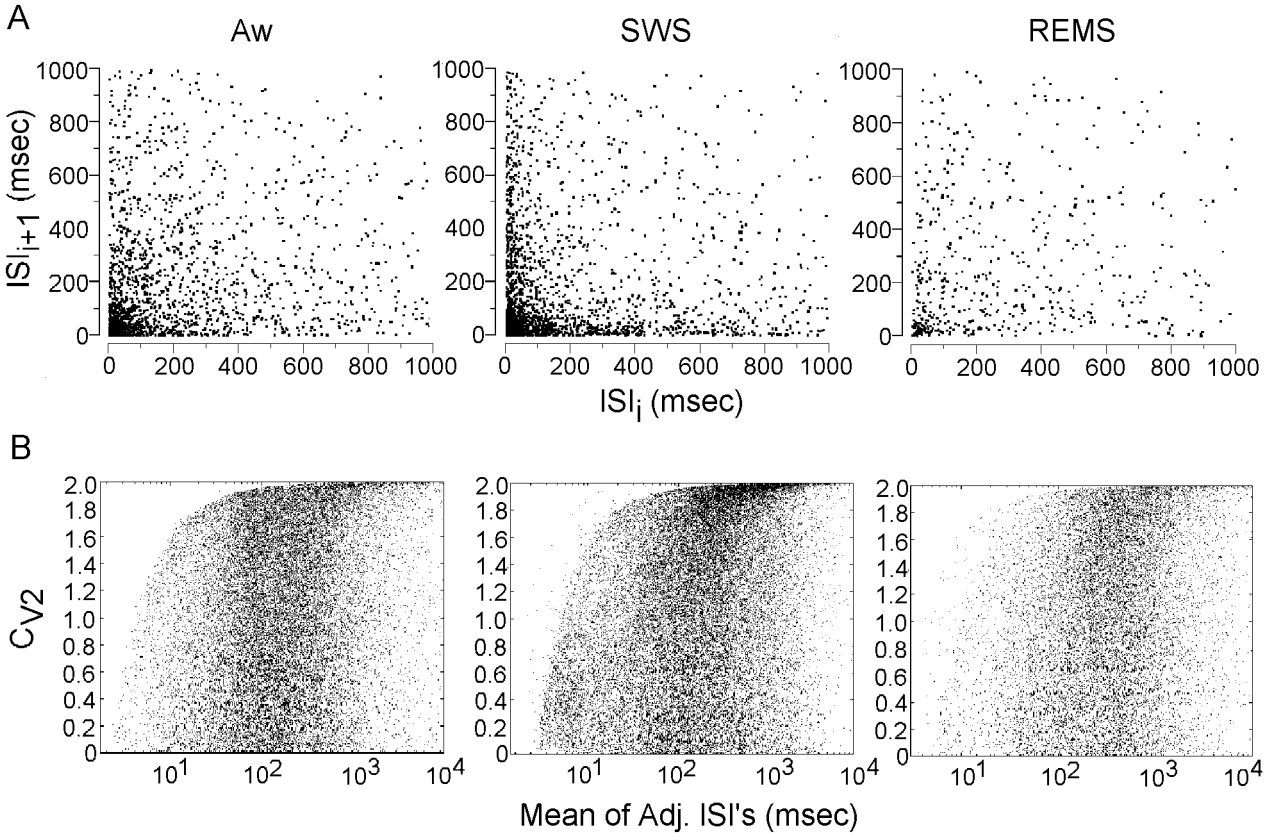

FIGURE 6. Interval-by-interval fluctuations in hippocampal neuron firing during wakefulness, slow-wave sleep (SWS), and rapid eye movement (REM) sleep. A: The spike train from a single hippocampal neuron was represented by plotting each pair of consecutive ISI values as a point along the x- and y-time axes. Pairs of ISI values plotted for awake (Aw), n = 2,354; slow-wave sleep, n = 2,834; REMS, n = 910. Note that some of the ISI values, which exceeded 1,000 ms, are not illustrated in the plot. B: Variability in neuronal firing was plotted as the mean of adjacent ISI values versus C_{V2} (refer to Methods for C_{V2} calculation). Neuron activities from all 23 hippocampal neurons during each state are represented (Aw, n = 32790; SWS, n = 37,498; REMS, n = 19386). The mean of adjacent ISI values was plotted on a logarithmic scale.

spikes discharged during a burst event did vary across state. Overall, there were fewer spikes discharged per burst during REM sleep than there were during Aw and SWS ($H_{2,2321} = 9.18$, $P = 0.01$). During REM sleep, the average number of spikes per burst was 3.6 ± 0.08 spikes, which was significantly fewer in comparison to the 4.1 ± 0.08 spikes/burst during Aw ($P = 0.005$) and the 3.9 ± 0.04 spikes/burst during SWS ($P = 0.003$). Higher-order bursts (>4 spikes/burst) (Suzuki and Smith, 1985b) represented 11.8% and 11.6% of total bursts detected during Aw and SWS, respectively, and 7.1% of the total bursts during REM sleep. One hypothesis explaining the state differences in spikes per burst is that faster firing cells discharge more spikes per burst than do slower firing cells. We addressed this hypothesis by testing whether there was a correlation between neuron firing rate and mean number of spikes in bursts during each state. Using Spearman's rank correlation, we did not detect any significant correlation between a neuron's firing rate and the number of spikes per burst ($r = -0.003$, $P = 0.9$). Despite differences in the number of spikes per burst across states, the mean ISI between spikes discharged during a burst event was similar across states. The mean rate of discharge within bursts was 162 ± 1.5 spikes/s, ranging from 75 spikes/s to 400 spikes/s.

Burst Propensity

Bursts were detected in 22 of the 23 hippocampal neurons. Changes in state were associated with significant changes in burst rate (number of bursts per minute; $F_{2,66} = 18.89$, $P < 0.0001$). During SWS, the mean burst rate of hippocampal neurons was 5.1 ± 1.2 (Table 1). During Aw, the burst rate was significantly lower than SWS ($P = 0.001$), while during REM sleep, it was significantly less during Aw or SWS, respectively ($P = 0.01$ and $P < 0.0001$).

Mean burst rate of neurons recorded during Aw episodes occurring during the beginning period of the sleep recording were significantly higher compared with the burst rate during Aw episodes that occurred during the middle period of the sleep recording (n = 18; t = 2.19, df = 18, $P = 0.04$; 9.98 ± 3.80 vs 1.45 ± 0.55 bursts/min). There was no difference in mean burst rate during REM sleep episodes that occurred during the middle period of the sleep recording, compared with those that occurred during the end period.

Of the 22 bursting hippocampal neurons, four neurons demonstrated rhythmic burst discharge at frequencies of 2–7 Hz. The autocorrelograms from a single neuron in Figure 5B illustrates

TABLE 1.

*Measures of Hippocampal Bursting**

Burst measure	State		
	Aw	SWS	REMS
Burst rate	3.3 ± 1.1**	5.1 ± 1.2†,‡	1.0 ± 0.5
Burst ratio	12.4 ± 2.8	18.2 ± 2.7†,‡	8.4 ± 2.8
Percentage spikes within bursts	9.8 ± 2.2%	14.1 ± 2.3%†,‡	6.3 ± 2.1%

Aw, awake; SWS, slow-wave sleep; REMS, rapid eye movement sleep.
*All three burst measures show significantly higher burst firing during SWS compared with both Aw and REM sleep. Burst rate is the number of burst per minute. Burst ratio is the number of bursts per 500 spikes. Values are mean ± S.E.
**Aw vs. REMS, $P < 0.005$
†SWS vs REMS, $P < 0.0001$.
‡SWS vs Aw, $P < 0.01$.

rhythmic discharge only during SWS. Rhythmic bursting was observed during SWS in two of the four neurons, during Aw in one neuron, and during both Aw and REM in one neuron.

As measured above, burst propensity is based on burst rate, i.e., the number of bursts over time. Although burst rate is the most common measure reported, to a certain extent, increases in firing rate might inflate burst rate (Fig. 4). To remove differences found in burst firing due to changes in firing rate, we calculated a second measure of burst propensity, called the burst ratio, which was based on the number of bursts per 500 spikes. Although similar state-dependent modulation for the burst ratio was observed as seen for burst rate, this measure did unmask bursting during REM sleep that was not detected with the first measure. Seventeen of the 22 bursting hippocampal neurons showed greater burst discharge during SWS than during Aw and REM (Fig. 7A), and five neurons demonstrated variable bursting patterns (Fig. 7B). The burst ratio for all 23 neurons was 18.2 ± 2.7 during SWS (Table 1). This burst ratio was significantly greater than the bursting found during Aw ($P = 0.001$) and REM sleep ($P < 0.0001$). In contrast to burst rate, there was no difference in the burst ratio between Aw and REM sleep ($P = 0.1$).

A third technique by which we quantified bursting of hippocampal neurons was calculation of the percentage of spikes found within bursts out of the total number of spikes in the spike train, i.e., the extent to which the burst firing contributes to the total amount of neuron firing. State significantly affected the proportion of spikes found within bursts ($F_{2,66} = 12.78$, $P < 0.0001$; Table 1). An average of 14.1 ± 2.3% of spikes discharged by hippocampal neurons were found within bursts during SWS. This was a significantly higher proportion than that calculated for Aw ($P = 0.001$) and REM sleep ($P < 0.0001$). Statistically, Aw and REM sleep failed to demonstrate any difference in the ratio of spikes within bursts to total spikes discharged ($P = 0.2$). No differences were observed in the either mean burst ratio or proportion of spikes found within bursts across sleep–wake episodes that occurred during any of the three periods of sleep recording.

DISCUSSION

The results of this study provide evidence for several significant alterations in firing properties of human hippocampal single neurons associated with different states of consciousness. Overall, the mean firing rate was lowest during REM sleep compared with Aw and SWS, but no significant difference was observed between hippocampal neurons during Aw and SWS. More specific analysis of firing patterns within the spike trains revealed significantly higher ISI variability during SWS compared with Aw; it also showed that the state of SWS was associated with a significant increase in high-frequency burst firing compared with Aw and REM sleep.

Physiological Identification of Hippocampal Cell Types

Since the recordings made by Ranck (1973) describing complex-spike cells and theta cells, three criteria have commonly been used to discriminate putative pyramidal cells from interneurons: spike duration, firing rate, and burst propensity (Skaggs and McNaughton, 1996; Csicsvari et al., 1999). Spike durations >0.4 ms, firing rates <10 Hz and high-frequency spike bursts with attenuating amplitude have been associated with pyramidal cells. The hippocampal neurons we recorded discharged an average of 2 spikes/s and 96% demonstrated decrementing amplitude, complex-spike-like bursts. In spite of the 3:1 or greater S:N ratio in our recordings, the sampling rate was insufficient to reduce variable spike waveform alignments, limiting our ability to measure averaged spike duration accurately. The success with which single neurons were reliably isolated single neurons using 40-μM-diameter microwires suggests that the microwire tips were recording from

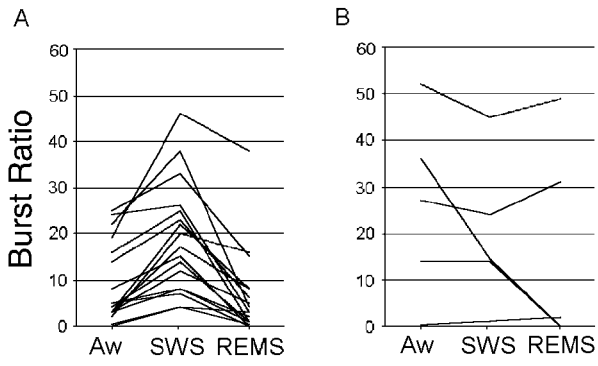

FIGURE 7. State-related burst patterns of individual neurons as measured by burst ratio. A: Seventeen of the 22 bursting neurons demonstrated enhanced burst firing during the period of slow-wave sleep (SWS) compared with awake (Aw) and rapid eye movement (REMS). B: SWS episodes were associated with an activation of burst firing for most hippocampal neurons; five neurons had variable burst patterns across sleep–waking states.

relatively sparsely packed large neurons rather than the densely packed small neurons of the granule layer of the fascia dentata. These electrophysiological data combined with the characteristics of our recording electrodes lead us to believe that a large proportion of our neurons were pyramidal cells.

Firing Rate

Studies in the rodent have found an increased probability of pyramidal cell and interneuronal discharge during SPW-associated episodes of SWS and waking immobility (Buzsaki, 1986). However, pyramidal cell discharges decreased during the periods when rodents show robust RSA associated with REM sleep and ambulatory behavior (Suzuki and Smith, 1987; Chrobak and Buzsaki, 1996). Similar sleep-related changes in firing rate were found in our human hippocampal single neuron recordings, as well as in two previous studies, one in the rodent (Suzuki and Smith, 1985a) and the other in human recordings of multi-unit hippocampal activity (Ravagnati et al., 1979). In contrast, human functional neuroimaging studies during sleep have revealed a deactivation of the hippocampal formation during SWS (Maquet et al., 1997) and activation during REM sleep (Nofzinger et al., 1997). While imaging results provide evidence for a net increase in hippocampal activity during REM sleep, the electrophysiological evidence in non-primate point to specific cell types, and human studies point to hippocampal locations, that preferentially decrease discharge activity during REM sleep.

Burst Propensity

The structure and propensity for single neuron spike bursts are also consistent with some of the same studies cited above regarding firing rate in animals (Ranck, 1973; Suzuki and Smith, 1985b) and human hippocampal single neuron burst activity (Colder et al., 1996). Burst structure analysis revealed similarities in burst duration and mean ISI between spikes in bursts, which suggest that mechanisms involved in burst generation were stable across all behavioral states. However, we did find a significant increase in the number of spikes per burst during SWS and Aw compared with REM sleep. This state-related increase in higher-order bursts might enhance the transfer of hippocampal output (Huerta and Lisman, 1995).

Neuronal burst rate and firing rate were higher during Aw episodes that occurred at the beginning period of the sleep recording compared with those that occurred during the middle period of the recording. The reduction in firing rate and burst rate from Aw episodes that occurred during middle period of the sleep recording may reflect the level of monoaminergic and cholinergic activation from preceding sleep episodes imposed on the waking state. No difference was observed between single neuron discharge parameters during REM sleep episodes that occurred during the middle period compared with the end period of the sleep recording. No comparisons could be made for SWS because six of eight subjects experienced SWS episodes during the middle period of the sleep process.

Propensity for burst firing recorded from our hippocampal single neurons was most pronounced during SWS compared with Aw and REM sleep. While our single neuron analysis methods did not allow us to quantify the hippocampal EEG for the presence of SPWs, the increased propensity for hippocampal neuron burst firing during SWS reflects a synchronized state (Fig. 2) in which the probability of hippocampal SPW occurrence is greatest. Conversely, the reduction in burst firing associated with Aw and REM sleep reflects states in which the hippocampal EEG is low amplitude, mixed frequency and the probability for SPW occurrence is reduced. High-frequency burst discharge has been shown to increase the probability of signal transmission between neurons, as well as induce long-term synaptic modifications (for review, see Lisman 1997). In accordance with Buzsaki's theory (1998), the greater propensity for hippocampal burst firing we observed during SWS would increase the probability of hippocampal output influencing neocortical networks.

Discharge Variability

Variable spike discharge has been observed in cortical neurons in response to repetitive stimuli, as well as in spontaneous activity from hippocampus, thalamus, and midbrain nuclei during sleep (Yamamoto et al, 1986; Kodama et al., 1989; Britten et al. 1993; Softky and Koch, 1993). In the present study, analysis of ISI values revealed a state-dependent increase in the variability of spike discharge during SWS compared with Aw. The primary source of this increased variability is probably an increase in burst firing. In spite of similarities in mean firing rate between Aw and SWS, C_{V2} plots (Fig. 6B) identified high variability at mean ISI values of approximately 1,000 ms in duration. This variance reflects long intervals of neuronal silence after periods of high-frequency burst firing. During REM sleep, lengthy ISI values ($>$1,000 ms) contributed to the significantly longer median ISI values compared with Aw and SWS, contrary to a previous finding of short ISI hippocampal neuron firing during REM sleep containing theta rhythm (Noda et al., 1969). Discharge variability during REM sleep in human hippocampus may be due to a greater influence of sporadic burst firing upon a reduced background of neuronal activity. This pattern of firing is in contrast to the highly rhythmic bursting that accompanies the theta activity dominating REM sleep in non-primate hippocampus. Our analysis did find one rhythmically firing neuron at a frequency of 6 Hz during Aw and REM sleep, while two neurons during SWS (one neuron illustrated in Fig. 5B) and one neuron during Aw rhythmically discharged at 2 and 7 Hz, respectively. We believe that the limited number of neurons we analyzed, combined with the large proportion of putative pyramidal cells that comprise our sample, may not fully represent the rhythmicity of neuronal activity in the human hippocampus. Other recordings from human hippocampus and entorhinal cortex in this laboratory (unpublished data) have shown ample evidence of rhythmic bursting during all three states.

The application of our findings to the current understanding of normal hippocampal activity during sleep was limited to the analysis of 23 hippocampal neurons, so that we could base our conclusions only on single neurons showing clear isolation, and from neurons recorded in sites free from any electrographic or anatomical evidence of epileptic potential; i.e., no seizure onsets were recorded from these electrodes, and no hippocampal atrophy was detected in these recording sites on the basis of neuroradiologic

analysis of MRI. While we cannot discount the presence of interictal epileptiform EEG activity in our recordings, studies have shown interictal epileptiform discharges are widespread during SWS (Sammaritano et al., 1991; Malow et al., 1998), and that areas demonstrating epileptic potential positively correlate with maximal interictal EEG spike rates (Lieb et al., 1978). In addition, human studies have failed to demonstrate a clear relationship between interictal EEG spikes and neuronal burst discharge (Wyler et al., 1981; Telfeian et al., 1999). Although there is evidence that epilepsy can influence sleep architecture (Shouse and Sterman, 1983; Gigli and Gotman, 1992; Bazil et al., 2000), characterization of neuronal activity was based on stable, 10-min episodes of wakefulness, SWS, and REM sleep that were clearly defined by standard polysomnographic criteria. Nevertheless, evaluation of these data must take into consideration the possible influences of epileptic activity and anticonvulsant medications to which these hippocampal neurons were exposed.

Implications

Our results demonstrate state-related changes in the frequency and pattern of hippocampal neuron firing. The timing of such neuronal discharge in relation to the network EEG rhythm may determine whether neuronal synaptic transmission is enhanced or depressed (Otto et al., 1991; Debanne et al., 1996). Several studies have demonstrated that cortical and thalamic neurons discharge rhythmically in the low-frequency range (0.5–4 Hz) during states characterized by high-amplitude, low-frequency EEG, i.e. SWS (Steriade et al., 1996; Contreras and Steriade, 1997; Weyland et al., 2001). The SWS-related interaction between cortical and thalamic neurons is effective in increasing their spatiotemporal coherence over widespread brain areas (Steriade, 1999). As a requisite step in a model of memory consolidation, concerted burst firing during SWS may provide the critical increase in hippocampal output that is required for potentiation of neocortical targets. The greater hippocampal burst discharge during SWS we observed may correspond to SPW-like episodes optimal for hippocampal output to influence neocortical targets, while the reduction in firing during REM sleep may signal a shift in hippocampal state favorable to the reception of neocortical feedback. Further studies of the specific structure of synchronization of human hippocampal neuronal burst firing are needed to distinguish the comparative roles of SWS and REM sleep in memory processing.

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