Sharp Wave–Associated High-Frequency Oscillation (200 Hz) in the Intact Hippocampus: Network and Intracellular Mechanisms

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Sharp wave bursts, induced by a cooperative discharge of CA3 pyramidal cells, are the most synchronous physiological pattern in the hippocampus. In conjunction with sharp wave bursts, CA1 pyramidal cells display a high-frequency (200 Hz) network oscillation (ripple). In the present study extracellular field and unit activity was recorded simultaneously from 16 closely spaced sites in the awake rat and the intracellular activity of CA1 pyramidal cells during the network oscillation was studied under anesthesia. Current source density analysis of the high-frequency oscillation revealed circumscribed sinks and sources in the vicinity of the pyramidal layer. Single pyramidal cells discharged at a low frequency but were phase locked to the negative peak of the locally derived field oscillation. Approximately 10% of the simultaneously recorded pyramidal cells fired during a given oscillatory event. Putative interneurons increased their discharge rates during the field ripples severalfold and often maintained a 200 Hz frequency during the oscillatory event. Under urethane and ketamine anesthesia the frequency of ripples was slower (100–120 Hz) than in the awake rat (180–200 Hz). Halothane anesthesia prevented the occurrence of high-frequency field oscillations in the CA1 region. Both the amplitude (1–4 mV) and phase of the intracellular ripple, but not its frequency, were voltage dependent. The amplitude of intracellular ripple was smallest between –70 and –80 mV. The phase of intracellular oscillation relative to the extracellular ripple reversed when the membrane was hyperpolarized more than –80 mV. A histologically verified CA1 basket cell increased its firing rate during the network oscillation and discharged at the frequency of the extracellular ripple. These findings indicate that the intracellularly re-

corded fast oscillatory rhythm is not solely dependent on membrane currents intrinsic to the CA1 pyramidal cells but it is a network driven phenomenon dependent upon the participation of inhibitory interneurons. We hypothesize that fast field oscillation (200 Hz) in the CA1 region reflects summed IPSPs in pyramidal cells as a result of high-frequency barrage of interneurons. The sharp wave associated synchronous discharge of pyramidal cells in the millisecond range can exert a powerful influence on retrohippocampal targets and may facilitate the transfer of transiently stored memory traces from the hippocampus to the entorhinal cortex.

[Key words: hippocampus, oscillation, unit activity, field potential, plasticity, memory, network, behavior, rat]

The principal cell types of the hippocampus are the granule cells and the CA3 and CA1 pyramidal neurons. The information transmitted from the entorhinal cortex can reach the pyramidal cells directly and indirectly by way of the granule cells (cf. Amaral and Witter, 1989; Lopes da Silva et al., 1990). An apparent difference between pyramidal cells of the CA3 and CA1 regions is the extensive recurrent collateral system of CA3 neurons (Lorente de Nó, 1934; Ishizuka et al., 1989; Li et al., 1993) in contrast to the extremely limited interconnectivity of CA1 pyramidal cells (Christian and Dudek, 1988; Thomson and Radpour, 1991). The question to ask, then, is what new processing is taking place in the CA1 region that is lacking in CA3? A previous suggestion is that the CA1 pyramidal cells are a type of “and” neurons that discharge only if several CA3 pyramidal neurons converge on them (Lynch, 1986; Mizumori et al., 1989). In this article we present evidence that a highly organized pattern emerges from the interaction between CA1 pyramidal cells and their inhibitory interneurons as a result of a synchronous population burst in the CA3 region.

Various population patterns, as reflected by spontaneous field potentials and rhythms, are present in the hippocampal formation, including theta activity and associated gamma pattern (40–100 Hz), hippocampal sharp waves (SPW) and the SPW-associated high-frequency (200 Hz) oscillation (“ripple”), two types of dentate spikes, sleep spindles and delta waves of sleep (O’Keefe and Nadel 1978; Buzsáki et al., 1983, 1992; Buzsáki, 1986; Bland, 1990; Lopes da Silva et al., 1990; Traub and Miles, 1991; Bragin et al., 1992, 1993). Hippocampal rhythmic slow activity (RSA or theta) is the most studied pattern and has been implicated in several functions, ranging from sensory processing to the voluntary control of movement (Grastyan et al., 1959; Vanderwolf, 1969). In the rat, hippocampal theta activity occurs during exploratory behaviors, such as sniffing, rearing, walking,

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and the paradoxical phase of sleep (Vanderwolf, 1969). During theta, interneurons show a marked increase in firing rate phase locked to the theta oscillation (Ranck, 1973; Fox and Ranck, 1981; Buzsáki et al., 1983). In contrast, principal neurons are generally silent and only a limited number of pyramidal and granule cells, representing, for example, the spatial position of the animal, show a transient increase in firing as the animal moves into unique spatial locations (Ranck, 1973; O'Keefe and Nadel, 1978; Jung and McNaughton, 1993).

In the absence of theta, intermittent sharp waves (SPW) of 40–120 msec duration are observed during consummatory behaviors, behavioral immobility, and slow wave sleep (Buzsáki et al., 1983; Buzsáki, 1986; Suzuki and Smith, 1987). The frequency of the intermittent SPWs ranges from 0.02 to 3/sec. The immediate cause of SPW in the CA1 region is the synchronous discharge of a large number of CA3 pyramidal neurons and the consequent near-simultaneous depolarization of CA1 pyramidal cells. In conjunction with the stratum radiatum SPWs high-frequency field oscillations ("ripples"; O'Keefe and Nadel, 1978) are present in the CA1 pyramidal layer (Buzsáki et al., 1992). The goal of the experiments presented here was to reveal the emergence and control of this high-frequency oscillatory pattern. We combined current source density analysis and multisite single unit recording in the awake rat with intracellular recording from identified cells in the anesthetized animal to unveil the cellular-synaptic origin of electromotive forces underlying hippocampal SPW and the associated high-frequency field oscillation in the CA1 region.

Materials and Methods

Chronic studies. Twenty-eight male and female rats (300–450 gm) of the Sprague-Dawley strain were used in this study. The rats were anesthetized with a mixture (4 ml/kg) of ketamine (25 mg/ml), xylazine (1.3 mg/ml), and acepromazine (0.25 mg/ml). Pairs of stainless steel wires (100 μ m in diameter) with 0.5 mm vertical tip separation were placed in the angular bundle on the right or both sides to stimulate the medial perforant path afferents to the hippocampus (AP = -7.0 mm from bregma, L = 3.5 mm from midline, and V = 3.0 mm). Another electrode pair was placed into the ventral hippocampal commissure (AP = -0.8, L = 0.5, V = -4.2) to stimulate the commissural afferents to the CA1–3 regions and the dentate gyrus.

Three different recording electrodes were used: stationary wire electrodes, microelectrode arrays and multisite recording silicon probes. Stationary electrodes (two to four 60 μ m tungsten wires) were implanted in the strata pyramidale and radiatum of CA1, the molecular layer, and hilus of the dentate gyrus (AP = -3.0, L = 2.6, V = -2.1 to -3.1). Microelectrode arrays consisted of eight tungsten wires (60 μ m in diameter). Single 60 μ m wires were inserted into a parallel array of fused silica tubes with 0.3 mm horizontal separations. The wires protruded 3–4 mm from the guiding tubes. For simultaneous recording of field potentials and unit activity in different locations and layers, silicon probes micromachined with thin-film technology (Wise and Najafi, 1991) were used. In the 24 site probe each of the six shanks had four recording sites (5 $\times$ 15 μ m² platinum-plated pads) that were spaced 50 μ m. The shanks were 300 μ m apart. The thickness of the silicon shank was 15 μ m throughout. In the 5 and 16-site single shank probes the recording sites were 100 μ m from each other in the vertical plane (80 μ m wide at the base, narrowing to 15 μ m at the tip; Fig. 1A). A 3 $\times$ 1 mm slot was drilled into the skull above the dorsal hippocampus along the long axis of the structure through which the recording electrodes were inserted after cutting the dura mater under a dissecting microscope.

The silicon probes and parallel arrays of tungsten electrodes, which were attached to a movable headstage, were inserted into the neocortex or corpus callosum during surgery. Following recovery the tips were gradually lowered into the hippocampus. During the experiment the evoked field potentials helped guide the microelectrodes (Fig. 1). Two stainless steel watch screws driven into the bone above the cerebellum served as indifferent and ground electrodes.

Unit activity was identified by physiological criteria. Units that discharged at a shorter latency than the evoked population spike and responded with two or more action potentials in response to perforant path or commissural stimulation were classified as interneurons (Fox and Ranck, 1981; Buzsáki and Eidelberg, 1982). These cells typically fired at high rates (> 15 Hz) and discharged in rhythmic groups at the field theta frequency. Units that displayed spontaneous complex bursts patterns were classified as pyramidal cells (Ranck, 1973). Complex-spike cells typically fired less than 1 Hz.

Recording and data processing. Four to six four-channel MOSFET input operational amplifiers, mounted in the female connector, served to eliminate cable movement artifacts (Buzsáki et al., 1989). The movement of the rat was recorded by a sensitive magnet-coil velocity detector attached to the transparent home cage. Physiological data were recorded wide-band (1 Hz to 1 kHz for field or 1 Hz to 5 kHz for units and field) and sampled at 10 kHz/channel (16 channels) or at 20 kHz/channel (8 channels) with 12 bit precision. The data were stored on optical disks.

All analysis was carried off line on a 486/33 and/or IBM RS 6000 computer. The recorded data were digitally filtered at 120 dB/octave in order to select the frequency of interest: unit activity (500 Hz to 5 kHz), various EEG bands (1 Hz to 150 Hz), high-frequency oscillations (50–250 Hz). The peaks of the fast oscillatory events were determined by a peak detection algorithm. These derived pulses were used as the zero reference point for the construction of auto- and cross-correlograms.

Current source density (CSD) analysis. Complete accounts of the theoretical basis of CSD analysis have been presented earlier (Freeman and Nicholson, 1975; Mitzdorf, 1985). Field patterns (theta, SPW, fast oscillatory waves) were first averaged using the identified positive or negative peaks of the filtered events ($n = 50$ –200). Smoothing of the averaged potential profiles was accomplished by convoluting the potential as a function of depth with a three-point rolling average of the voltage in depth. The second spatial derivative was calculated from a polynomial interpolated through the smoothed data points. Although some resistivity differences are present in the different hippocampal layers (Holsheimer, 1987), in practice these are not large enough to significantly modify the calculated distribution of current generators. Therefore, isotropy of the extracellular space is assumed in the CSD analysis. The results are therefore presented as the unscaled second derivative of potential as a function of depth, and will be referred to as CSD. The spatial filtering procedure disproportionately attenuated fast events, such as population spikes, but the CSD profiles in this paper are not intended to faithfully represent these events. The exact anatomical layers corresponding to the vertical scale of the CSD maps were reconstructed with the aid of the histologically identified recording tracks (Fig. 1) and evoked potentials. Currents sinks and sources associated with the activation of the perforant path and commissural afferents provided precise landmarks for the identification of the recording sites. In addition, unitary activity in the CA1 pyramidal layer provided further help for the depth calibration of the electrodes.

Spike separation and analysis. A Haar transformation was performed on the digitally filtered (500 Hz to 5 kHz) traces to locate the occurrence of spike events (Yang, 1988). The digitized spikes were reconstructed by the combination of three sinus waves. The spike events were identified by the factorial description of their shape ("feature") characteristics (peak amplitude, pre- and post-peak valley amplitudes, "pre-peak valley to peak" and "peak to post-peak valley" amplitudes, spike width, time to peak and time to valley). Event sorting was carried out by an IBM RS6000 using a perceptron version of the incremental conceptual clustering procedure (SpikePerceptron, MUA Technology BT, Pécs, Hungary) that combined algorithms of Cheng and Fu (1985), Fisher and Langley (1985), Lebowitz (1987), and DeCaestecker (1989). Artifacts and clustered noise were rejected manually. Further analysis was carried out by a PC-386. For the construction of cross-correlation between different clusters (units vs units or field vs units), a reference cluster and a set of target clusters were selected. The target clusters were scanned by the reference cluster within a certain time window. For visual display, the spike occurrences within the same target cluster were superimposed and projected relative to the reference event. The cross-correlograms were calculated from the cumulative number of target-events. For the field events, the average and standard deviation were calculated. Isolation of single units within and across clusters was verified by clear refractory periods (2–3 msec) in their interspike interval histograms. Pyramidal cells in the CA1 region occasionally emit complex-spike bursts with spikes of decreasing amplitude (Ranck, 1973). Our spike classification method inevitably clustered these event in separate groups.

Figure 2. Depth profile of a single sharp wave event (SPW) and associated high-frequency oscillation. *Left*, Simultaneous recordings of a SPW event from 16 sites in the CA1–dentate gyrus axis (wide band; 1

However, since previous observations with sharp electrodes revealed that these complex events occur at approximately 10% of all action potentials during SPW states (Buzsáki et al., 1983; Suzuki and Smith, 1987), probably less than 10% of the spikes were falsely clustered.

Histological procedures. Following completion of the experiments the rats were deeply anesthetized and perfused through the heart first with cacodylate-buffered saline (pH 7.5) followed by a cacodylate-buffered fixative containing 4% paraformaldehyde and 5.9% calcium chloride (pH 7.5). Brains were left *in situ* for 24 hr, removed and then postfixed in the same solution for 1 week. The brains were sectioned with the probes left in the brain on a vibratome at 100 μ m in the coronal plane (Fig. 1*B*). The sections were stained with the Gallyas silver method (Gallyas et al., 1990). Briefly, the sections were dehydrated with propanol and placed in an esterifying solution (98% propanol, 1.2% sulfuric acid) at 56°C for 16 hr. After rehydration and sectioning they were processed according to the following procedure: (1) pretreatment in 8% acetic acid for 10 min, (2) wash in water for 1 min, (3) physical development with tungstosilicic acid for about 10 min, and (4) wash in 1% acetic acid. Finally, the sections were dehydrated, mounted on slides, and coverslipped.

Intracellular methods. Seventy-three female Sprague–Dawley (250–350 gm) rats were anesthetized with urethane (1.3–1.5 gm/kg) and placed in a stereotaxic apparatus. The body temperature of the rat was kept constant by a small animal thermoregulation device. The scalp was removed and a small (2.0 mm) bone window was drilled above the hippocampus (anteromedial edge at AP = 3.3 and L = 2.2 mm from bregma) for extra- and intracellular recordings. A pair of stimulating electrodes (100 μ m each, with 0.5 mm tip separation) was inserted into the left fimbria–fornix (AP = 1.3, L = 1.0, V = 4.1) to stimulate the commissural inputs to the CA1 region. The cisterna magna was opened and the cerebrospinal fluid was drained to decrease pulsation of the brain. The extracellular recording electrodes (three 20 μ m insulated tungsten wires) were inserted into the brain at the medial edge of the bone window and placed into the upper part of hippocampal CA1 pyramidal cell layer. After the intracellular recording electrode was inserted into the brain, the bone window was covered by a mixture of paraffin and paraffin oil in order to prevent drying of the brain and decrease pulsation. The distance of the intracellular and extracellular electrodes was 0.5–1.5 mm in the anteroposterior and 0.0–0.5 mm in the lateral directions.

Micropipettes for intracellular recordings were pulled from 2.0 mm of capillary glass. They were filled with 1 M potassium acetate (or 1 M potassium chloride in four experiments) in 50 mM Tris buffer, containing also 3% biocytin for intracellular labeling. *In vivo* electrode impedances varied from 60 to 100 M Ω . Once stable intracellular recordings were

Hz to 5 kHz). *Right*, Filtered derivatives (50–250 Hz) of the records on the left. Note high-frequency oscillation (180–200 Hz; “ripple”) associated with the SPW peak with an amplitude maximum at the CA1 pyramidal cell layer (*p*). The ripple reversed in phase below electrode 7 (not visible at this resolution). The layers from which the recordings are derived from are indicated by letters. *o*, Stratum oriens; *p*, stratum radiatum; *hf*, hippocampal fissure; *g*, granule cell layer; *CA3c*, hilar portion of the CA3 pyramidal layer. The position of these layers is inferred from the depth profiles of the evoked responses, unit discharges at different electrodes and their relationship with the histological sections containing the electrode.

Figure 1. *A*, Silicon probe with 16 recording sites. *B*, Silicon probe in the hippocampus at the final recording position. The upper part of the probe was lost during sectioning (double arrowhead). Probe is viewed from side in this coronal section (Gallyas stain) with the tip (large arrowhead) in the thalamus. Arrowheads 1 and 16 indicate the extent and position of recording sites for *C* and *D*. *o*, Stratum oriens; *p*, pyramidal layer; *hf*, hippocampal fissure; *g*, granule cell layer; *h*, hilar region. *C*, Averaged evoked field potentials in response to stimulation of the commissural input (arrow, *com*) and averaged sharp waves (SPW) from the 16 sites. *D*, Current-source density (CSD) maps of evoked field responses to perforant path (PP) and commissural (*com*) stimulation and SPW patterns. Sinks (inward currents) and sources (outward currents) are indicated by cold and warm colors, respectively, in this and all other CSD figures. *iso*, Zero current flow. White arrows, stimulation. Red (PP) and black stars (*com*) mark population spikes in the granule cell and pyramidal layers, respectively. Recorded layers, indicated on the left, are the same as in *B*. On the right are drawings of a CA1 pyramidal cell and granule cell (SPW). Sinks *a* and *aa* reflect current generated by perforant path EPSCs in the middle third of the molecular layer; sinks *b*, *bb*, and *bbb* represent the current generated by excitation of the apical dendrites of CA1 pyramidal cells by the Schaffer collaterals and commissural inputs; sinks *c* and *cc* represent inward currents to activation of the basal dendrites by the commissural inputs and Schaffer collaterals from the CA3a and -b subregions (Li et al., 1994); sink *d* is postulated to be the current due to activation of the inner third of molecular layer by the associational and commissural afferents.

Figure 3. Current-source density maps of evoked responses during theta activity, SPW and SWP-associated high-frequency oscillation (ripple). All maps are based on averages of 50–200 events. Note gradually shifting sinks and sources with depth during theta. *SPW*, Sink (*s*) in the CA1 stratum radiatum (*r*) represents the current generated by excitation of the apical dendrites of CA1 pyramidal cells by the Schaffer collaterals and commissural inputs. The source (*so*) in the pyramidal layer (*p*) is postulated to be a combination of the passive return current and an active outgoing current due to interneuronal activity. *Ripple*, The large source associated with the ripple was mainly confined to the CA1 pyramidal layer. Data are from the same rat as shown in Figure 2.

obtained, evoked and passive physiological properties of the cell were determined. Field activity was recorded through the extracellular electrode and filtered between 100 Hz and 5 kHz. A threshold function was used to capture 400 msec epoch containing a fast oscillatory events. The threshold was adjusted for fast field oscillation burst (ripples) that occurred simultaneously with hippocampal SPWs. After the physiological data had been collected biocytin was injected through a bridge circuit (Axoprobe-1A) using 300 msec depolarizing pulses at 2–10 nA at 1 Hz for 10–60 min. Neuronal activity was followed throughout the procedure.

After 2–12 hr postinjection survival times the animals were given an urethane overdose and then perfused intracardially with 100 ml physiological saline followed by 400 ml of 4% paraformaldehyde and 0.2% glutaraldehyde dissolved in phosphate buffered saline (pH = 7.3). The brains were then removed and stored in the fixative solution overnight. Sixty or 100 μ m thick coronal sections were cut and processed for biocytin labeling as described previously (Li et al., 1992, 1994).

Results

Awake animals

Hippocampal SPW and associated high-frequency oscillations were recorded in the awake immobile and in the drowsy rat. A single SPW event and associated high-frequency oscillation (“ripple”) in the CA1–dentate gyrus axis is shown in Figure 2. The largest-amplitude SPW was observed in the middle of the stratum radiatum. SPWs reversed their polarity above the pyramidal cell layer. The laminar profile of the SPW was identical to the trisynaptic evoked potential in the CA1 region in response to perforant path stimulation (Fig. 1C,D). Power spectra of the epochs containing SPW revealed a peak at approximately 200 Hz with a maximum amplitude at the level of the CA1 pyra-

midal layer. Simultaneous recording from the CA1 pyramidal layer and CA3c/hilar region showed that this peak was present only in the CA1 region although a lower frequency and wider peak (50–120 Hz) was often localized to the CA3c pyramidal layer–hilar area.

The laminar differences in the frequency domain analysis were also reflected by the CSD maps. For the construction of one-dimensional CSD maps, SPWs were first averaged using their peaks as reference events (Fig. 1C). The field ripples were also averaged by using the positive peaks of the filtered (50–250 Hz) waves recorded from the CA1 pyramidal layer. CSD analysis of SPWs showed a large inward current (sink) in the middle third of the stratum radiatum and a smaller sink in the stratum oriens of CA1 with an outward current (source) at the level of the pyramidal layer (Figs. 1D, 3, SPW). The spatial position of these sink-source pairs was virtually identical with similar dipoles representing activation of the CA3 to CA1 associational/commissural system by stimulation of the perforant path or the ventral hippocampal commissure (Fig. 1D). However, they were different from sink-source distribution of theta waves recorded during exploration (Fig. 3, theta). These findings confirm the previous suggestion that SPWs in the CA1 region reflect activation of the apical and basal dendrites due to synchronous activity of the Schaffer collaterals (Buzsáki et al., 1983). CSD analysis of the fast oscillatory waves revealed narrow source-sink pairs confined to the CA1 pyramidal layer (Fig. 3, ripple). The alternating sink-source pairs at the level of pyramidal cell somata may reflect an active inward current associated with action potentials of the discharging cells and/or an active out-

Figure 4. Single "ripple" events recorded simultaneously from the CA1 pyramidal layer along the longitudinal axis of the hippocampus in the awake rat. *A*, Traces recorded by a six-shank silicon probe; 300 μm shank distance, $9 \times 9 \mu\text{m}$ recording area. *B*, Traces recorded by seven flatly cut tungsten wires (60 μm in diameter); 300 μm electrode distance. *Lower traces*, expanded portions of the traces above. *Arrows* indicate near 0 msec phase lag.

ward current due the activity of inhibitory interneurons. The membrane currents discussed above were consistently present in all seven animals implanted with 16 site probes.

Spatial coherence of high-frequency oscillation in CA1

Coherence measurements among patterns recorded in the CA1-dentate gyrus axis with 16-site probes indicated further that the high-frequency activity was confined to the pyramidal layer. Coherence values dropped below insignificant values (<0.3) 200–300 μm from the pyramidal cell layer in both directions. In contrast, when activity was assessed along the longitudinal axis of the hippocampus with the 24-site silicon probes or wire electrode arrays placed in the pyramidal layer, very high coherent values (0.6–0.94) were present in the 150–200 Hz frequency band even when the electrodes were placed as far as 2.1 mm. The high coherence values observed in the frequency domain were also reflected by the minimum time lags between simultaneously recorded traces. When the oscillatory activity was averaged by any of the recording sites in the pyramidal layer the simultaneously recorded averages showed a zero time lag. Visual analysis of individual events revealed, however, that the zero time lag was a result of averaging (Fig. 4). The individual traces had a small but consistent and measurable jitter (0.0–1.5 msec). These findings suggest that the synchrony that builds up during the ripple is not consistently initiated from the same site, but emerges simultaneously from several locations.

Unit correlations

Both pyramidal cells and interneurons increased their discharge probability during SPW-associated fast oscillatory events. The simultaneously recorded and isolated units were cross-correlat-

ed with the filtered (50–250 Hz) local field potentials (Fig. 5). By using the 24-site probes with all the recording tips placed in the pyramidal layer up to 50 single units could be isolated. The number of simultaneously firing units with a given fast oscillatory event was small and varied from 0 to 40% (Fig. 5*f*). On the average, approximately 10% of the recorded neurons fired in the time window of the SPW-associated fast oscillatory event (50 msec). The probability of participation of individual pyramidal cells in the group oscillatory events, however, were not equal. Some neurons discharged up to 40% of the successive oscillatory episodes, whereas other pyramidal cells, discharged very rarely (Fig. 5*e*). When an individual pyramidal cell discharged its action potential tended to be phase locked to the negative peak of the local field oscillation. Since pyramidal cells only rarely fired, such a relationship was not obvious by visual inspection. However, when all simultaneously recorded pyramidal cells were displayed together with the locally derived field potential a tight relationship between the two events became more obvious (Fig. 5*a,b*). Cross-correlation of unit discharges and the field the oscillatory events clearly revealed that pyramidal cells, as a group, were tightly coupled to the negative peaks of the field ripple waves recorded from the pyramidal layer (see Figs. 5, 7).

In contrast to the pyramidal cells, physiologically identified interneurons discharged with every SPW event (Figs. 5, 7). Some of the interneurons fired on virtually every single fast oscillatory wave. This frequency increase was clearly visible on the individual records and was quantified by the autocorrelation function of the interneurons. When interneuronal activity was cross-correlated with the field oscillation the peaks of the cross-correlograms lagged 0.0–2.5 msec (0–180° of the ripple cycle)

Figure 5. Relationship between high-frequency field oscillation and neuronal spike activity. Simultaneous recording from 40 pyramidal cells and two putative interneurons (24 site probe). *a*, Single event. *b*, Averaged field event (bottom, $n = 157$) and superimposed spike events from seven recording sites in the CA1 pyramidal layer ($300\ \mu\text{m}$ horizontal tip separation). Rows, Isolated (clustered) pyramidal cells and interneurons (*i*). Waveforms of pyramidal cell units and putative interneurons are displayed in *c1* and *c2*, respectively. *d*, Cross-correlogram between field oscillation and summed pyramidal cell activity (*PYR*) and interneuronal firing (*INT*). Continuous line, Gabor function curves. *e*, Number of action potentials (*z*-axis) emitted by the 40 pyramidal cells (*units*) during successive 157 ripple episodes. Note that an isolated unit only rarely discharged more than once during a given field oscillatory episode. Note also that many cells were silent during most of the ripple events whereas some cells discharged during almost all of the ripples. *f*, Percentage of pyramidal cells discharging during successive ripple episodes. Continuous line, average; dotted lines, 95% confidence intervals. Note that between 0 and 40% of the recorded units can be active during a single ripple episode.

behind the peaks of the cross-correlograms of the pyramidal cells. Interneurons, recorded in the pyramidal layer ($n = 10$) fired earlier (0.0–0.1 msec; Figs. 5*d*, 6) than interneurons recorded from the stratum oriens ($n = 6$; 0–2.4 msec; Fig. 7). The frequency of the interneurons steeply increased at the occurrence of the field ripple and the discharge rate was often maintained between 150–250 Hz for the duration of the event (Fig. 6*d*). The above findings are compatible with the hypothesis that the fast oscillatory event is brought about by inhibitory interneurons and reflects summated IPSPs in the pyramidal cells.

Cross-correlation of the high-frequency oscillation in the CA1 pyramidal layer and unit activity recorded from the contralateral CA1 region did not result in significant correlations ($n = 7$). Oscillatory patterns were also present in the CA3 region in association with SPWs. However, these were less regular than in the CA1 field and their frequency varied between 80 and 140 Hz. In addition, when the CA1 field ripple was correlated with unit activity of the ipsilateral CA3 region no peaks were observed in the cross-correlograms ($n = 5$). These observations suggest that the 200 Hz oscillatory pattern emerges from the CA1 cell population rather than passive response to a high-frequency drive by the CA3 input.

Intracellular recordings in anesthetized rats

The main characteristics of SPWs and associated unit and fast field oscillatory events were also observed in rats anesthetized by urethane ($n = 63$ rats). SPW periods alternated with theta periods spontaneously by a 20–40 min cycle. Stroking of the back or slight pinching of the tail suppressed SPW activity and induced theta waves. The SPW had the same laminar profile as in the awake rat. Also, the amplitude of the SPW-associated fast field oscillation was greatest at the level of CA1 pyramidal layer. The intraripple frequency under anesthesia, however, was significantly lower (90–130 Hz) than in the awake animal. In addition, ripple episodes tended to be longer under anesthesia. Pyramidal cells discharged phase-locked to the negative portion of the field oscillatory waves. The peaks of the cross-correlograms usually lagged 0.2–1 msec behind the peak negativity (Fig. 8).

Simultaneously recorded fast field oscillations from the CA1 pyramidal layer and intracellular recordings were available from forty two pyramidal cells. All pyramidal cells included in this study had stable resting potentials (–60 to –70 mV) and overshooting action potentials. Depolarizing current steps typically

Figure 6. Interneuron activity during high-frequency oscillation. *a*, Single trace of wide band recording from the CA1 pyramidal layer (100 Hz to 5 kHz). Note rhythmic discharge of the single cell during the field oscillation. *b*, Cross-correlation between field activity and interneuronal firing. *c*, Firing pattern of the interneuron during 106 successive "ripple" events. Time zero was defined by the positive peak of the largest ripple deflection. Vertical ticks, action potentials. *d*, Instantaneous frequency of the interneuron during the events shown in *c*. Note transiently increased firing frequency between 150 and 200 Hz during the field oscillation event.

induced a bursts of action potentials with progressively longer interspike intervals. Evoked and spontaneously occurring bursts were followed by large-amplitude (5–10 mV) and long-lasting hyperpolarizations (>0.5 sec). Commissural stimulation evoked pure IPSPs or an EPSP/IPSP sequence in putative pyramidal cells. Increasing the stimulus intensity often discharged the neuron at a latency of the extracellularly recorded population spike. The above physiological features identified these neurons as pyramidal cells (Wong and Prince, 1978; Nicoll and Alger, 1981; Thompson et al., 1985). Ten of the physiologically identified pyramidal neurons were injected with biocytin and histologically verified. Putative pyramidal neurons with deteriorating membrane potentials and/or fast discharge rates (>2 Hz) were excluded from further analysis.

Intracellular recordings from CA1 pyramidal cells often showed a depolarization of the membrane potential during SPW-concurrent fast oscillations, due most likely to the depolarization of the dendrites by the Schaffer collaterals (Buzsáki et al., 1983). This depolarization force was clearly revealed when the cell was hyperpolarized (Figs. 9*a*, 10*c*). In contrast, hyperpolarizing currents had a different effect on the membrane potential during theta activity. With successive hyperpolarization steps the intracellular theta showed an amplitude minimum between –70 and –80 mV and increased again with further hyperpolarization. However, at hyperpolarized levels (<–80 mV) the phase of intracellular theta was reversed relative to the resting membrane potential (Fig. 9*b*). Similar observations were obtained

from eight pyramidal cells. These findings confirm previous intracellular observations and suggest that a large portion of the membrane fluctuation during theta activity is due a chloride-mediated GABA_A inhibition (Yim and Leung, 1986; Fox, 1989; Soltész and Deschênes, 1993). In contrast, during SPW events the synchronous afferent barrage from the CA3 region resulted in a powerful depolarization of the pyramidal cell dendrites.

The membrane potential of pyramidal cells exhibited a 1–5 mV oscillation during extracellularly recorded SPW-concurrent fast oscillation (Fig. 10). Such fast oscillations of the membrane potential were present only in association with extracellular ripples. The fast membrane oscillation most often coexisted with the SPW-related depolarization. However, in some cases fast membrane oscillations were observed without obvious depolarization (Fig. 10*b*). These observations suggest that the two field events (SPW and fast oscillations) are generated independently despite their frequent coexistence.

In the absence of a field ripple event, depolarization or hyperpolarization of the membrane failed to induce the fast oscillatory pattern. Furthermore, the frequency of the intracellular fast oscillation was independent of the membrane potential but was phase-locked to the extracellular event. In contrast, the amplitude and phase of the intracellularly recorded fast oscillation were voltage dependent. For a quantitative verification of the visual observations, the intracellular oscillation was averaged using the peaks of the extracellularly recorded fast waves as zero reference. For these averages, epochs containing action

Figure 7. Interneuronal activity during high-frequency oscillation. *a*, Averaged field event (thick trace, $n = 256$) and superimposed spike events from four recording sites in the CA1 region (100 μ m vertical tip separation). Site 1 was in the stratum oriens and recorded from a single interneuron (INT). Sites 2–4 recorded pyramidal cells (PYR) from the pyramidal layer. *b*, Cross-correlogram of the interneuron and field oscillation. *c*, Cross-correlogram of all pyramidal neurons ($n = 24$) and field oscillations. Continuous lines in *b* and *c*, Gabor functions of the histograms. Note phase-locked discharges of pyramidal cells to the negative peak of the field oscillatory waves and the phase-shifted oscillatory pattern of the single interneuron in the stratum oriens.

potentials were edited. To correct for a possible error due to volume conduction, the extracellular averages were subtracted from the intracellular averages. In addition, extracellular recording by the micropipette after the intracellular analysis was completed provided further information about phase relationship of the ripple waves recorded by the micropipette and the extracellular tungsten electrode. The amplitude of the intracellular fast oscillation was smallest between -70 and -80 mV. The phase of the intracellular oscillation relative to the extracellular ripple reversed when the membrane was hyperpolarized more than -80 mV (Fig. 11). At resting membrane potential the positive peaks of the intracellular fast oscillation coincided with the rising portion of the extracellularly recorded ripple waves and matched closely the peaks of the cross-correlograms of the extracellular units (compare Fig. 8).

Intracellular diffusion of Cl^- ions from KCl-filled electrodes ($n = 4$) decreased or abolished the voltage-dependent phase relationship between extracellularly and intracellularly recorded fast oscillations. The phase shift at resting membrane potential

Figure 8. Comparison of high-frequency oscillation in the anesthetized (urethane) and awake rat. *a* and *b*, Averages of simultaneously recorded field (*a*) and unit (*b*) activity from the same electrode in the CA1 pyramidal layer. The reference zero for the field and unit histograms was the positive peak of the ripple. *c* and *d*, Simultaneously recorded ripple (*c*) and SPW (*d*) activity from the CA1 stratum pyramidale and radiatum, respectively. Single traces, Note lower frequency (120 Hz) oscillation in the urethane anesthetized rat compared to the awake animal (190 Hz).

with KCl electrodes was similar to the phase shift observed at hyperpolarized levels (-80 mV or less) with K-acetate electrodes (Fig. 12). Such phase-reversals were observed in two of the four cells tested.

A histologically verified CA1 basket cell was also studied during SPW-associated fast oscillations. The interneuron was located in the pyramidal layer. Its axons formed numerous baskets in the pyramidal layer. Physiological analysis of the cell revealed characteristics of an interneuron (Kawaguchi et al., 1987; Lacaille and Schwartzkroin, 1988a,b), including short-duration action potentials, high-frequency spontaneous activity (10–30 Hz), short-lasting and large-amplitude afterhyperpolarization and limited spike frequency accommodation upon membrane depolarization (Fig. 13). Commissural stimulation evoked double or triple action potentials, with the first spike preceding the extracellularly recorded population spike. Although the neuron was recorded from for longer than an hour, only a few SPW-concurrent oscillatory epochs occurred during the observation period. Nevertheless, when large-amplitude extracellular ripples were present the interneuron increased its firing frequency and discharged at ripple frequency. Strong depolarization of the soma during the biocytin injection procedure (2–4 nA) resulted in a long series of action potentials at 150–300 Hz followed by high-frequency fluctuation of the membrane potential (Fig. 13c).

Effects of ketamine and halothane on the fast oscillatory pattern

Rats used in the chronic experiments were first tested from 2 to 24 hr after the electrode implantation done under ketamine anesthesia. SPW-associated field oscillations were often present when the rat was sitting or lying immobile. The frequency of the field oscillation was between 90 and 150 Hz and did not reach the frequency typical for the drug-free state even 24 hr after the initial anesthesia.

Figure 9. Intracellular correlates of field SPW (*a*) and theta activity (*b*, *c*). *a*, A single ripple event (*extra*) showing the trigger pulse used for intracellular averaging (*intra*). Note that intracellular hyperpolarization reveals a strong depolarization force during the ripple. Intracellular traces (*intra*) are averages of 10–20 repetitions. *b*, Extracellular and intracellular averages of theta ($n = 80$ –200 repetitions) at three different polarization levels. Note that hyperpolarization leads to a reversal of intracellular theta (*dotted lines*). *c*, Single epochs of extracellular and intracellular theta activity at different membrane potential levels. *Middle traces*, amplified ($3\times$) and filtered (1–1000 Hz) derivatives of the intracellular traces. Note that intracellular activity is virtually flat at -80 mV and membrane fluctuation is larger at both -65 mV and -90 mV.

Ten rats with extracellular electrodes and intracellular glass pipettes in the pyramidal layer and were monitored for fast field oscillations from 4 to 12 hr under halothane anesthesia. Clear fast field oscillatory patterns were never observed in any of these rats, even though the level of anesthesia was systematically varied from deep (3%) to shallow (1%). When the concentration was decreased transiently below 1% continuous theta activity was present. With deepening anesthesia theta disappeared and irregular sharp wave events with concurrent increase of neuronal activity in the CA1 pyramidal layer were observed. However, high-frequency rhythmicity was absent. In one rat, small-amplitude, irregular oscillations between 50 and 200 Hz were de-

tected on four occasions during a 5 hr observation period. These findings suggest that the volatile anesthetic halothane blocks the mechanism responsible for the emergence of fast oscillation in the CA1 region.

Discussion

The main findings of the present experiments are that (1) extracellular currents underlying the high-frequency field oscillation in the CA1 region are concentrated at the level of the cell body layer, (2) only a small percentage of pyramidal cells discharge during a given oscillatory event, (3) interneurons increase their discharge rates severalfold during the field ripple and can

Figure 10. Intracellular correlates of high-frequency oscillation in a CA1 pyramidal neuron. *a* and *b*, Oscillation of the membrane potential with and without SPW-associated depolarization. Resting potential was -65 mV. *c*, Hyperpolarization to -85 mV revealed a large SPW-associated depolarization. *extra*, Filtered extracellular activity (50–250 Hz) recorded by the extracellular electrode. *intra (f)*, Filtered (50–250 Hz) intracellular activity. *intra*, Unfiltered intracellular traces. Note bursts of action potentials in *c*.

maintain a 200 Hz frequency transiently, (4) urethane and ketamine anesthesia decelerate the oscillation frequency (100–120 Hz), whereas halothane blocks it altogether, and (5) the amplitude and phase, but not the frequency, of the intracellular oscillation is voltage dependent. These findings indicate that the intracellularly recorded fast oscillatory rhythm is not dependent on interactions of membrane currents intrinsic to the CA1 pyramidal cells but is a network driven rhythm with critical participation of inhibitory interneurons.

Origin of high-frequency oscillation in the CA1 region

The CA1 region of the hippocampus is a highly organized laminar structure with a parallel alignment of pyramidal cells (Ramón y Cajal, 1911). The main afferents to the apical dendrites are the laminarily segregated commissural, associational (Schaffer collateral) and perforant path fibers. Part of the commissural and associational afferents terminate on the basal dendrites of pyramidal neurons (Amaral and Witter, 1991; Ishizuka et al., 1991; Li et al., 1994). Based on *in vivo* intracellular staining of the axon arbor of CA3 pyramidal neurons, it was estimated that a single pyramidal cell can influence between 10,000 and 30,000 neurons in the ipsilateral CA1 region (Li et al., 1994). In addition to the pyramidal cells, the associational and other inputs also activate local circuit neurons in a feed-forward manner (Buzsáki and Eidelberg, 1982). A large portion of the local circuit interneurons, including basket cells and chandelier cells, are excited by the recurrent collaterals of the CA1 pyramidal cells as well (Lacaille and Schwartzkroin, 1988a,b; Li et al., 1992).

Based on the anatomical circuitry and the physiological observations presented here we suggest that during the SPW-burst two antagonistic polarizations compete with each other: dendritic excitation and somatic/perisomatic inhibition. Dendritic depolarization is a direct consequence of the excitatory barrage

of pulses from the CA3 pyramidal cells. Interneurons are also driven by the CA3 collaterals and axon terminals of basket and chandelier cells can induce somatic and axon initial segment inhibition of CA1 pyramidal cells concurrently with dendritic depolarization.

Several observations of the present experiments support the importance of somatic/perisomatic inhibition in the emergence of rhythmic group discharge of pyramidal cells. First, interneurons increased their discharge rates and were phase-locked to the network rhythm. Second, the current sink-source pairs were confined to somatic region of the pyramidal cell population. Third, the amplitude of intracellular ripple was smallest between -70 and -80 mV. The phase of intracellular oscillation relative to the extracellular ripple reversed when the membrane was hyperpolarized more than -80 mV. In addition, intracellular diffusion of chloride ions in experiments with KCl electrodes abolished the voltage dependence of the intracellular oscillation. Fourth, the frequency of the intracellular ripple in CA1 pyramidal cells was not voltage dependent. The latter observation indicates that the fast membrane oscillation of the pyramidal cells did not emerge as a result of intrinsic, voltage-dependent ionic mechanisms in these cells. Overall, these findings support the view that during SPW bursts repetitively firing interneurons can produce fast fluctuation of the membrane around the chloride equilibrium potential in CA1 pyramidal cells by activating GABA_A receptors. Rhythmic and coherent firing of interneurons would thus be responsible for the timing of action potentials of CA1 pyramidal cells during SPW-associated population bursts.

The phase relationship between firing of interneurons and population oscillation of pyramidal cells varied from zero to almost 180° . The time delay (0–2 msec) between pyramidal cell firing and discharges of the interneurons appear shorter than what might be expected from recurrent activation of these cells

Figure 11. Phase-reversal of intracellular oscillation by hyperpolarization. *A*, Averages of simultaneously recorded extracellular (*extra*) and intracellular (*intra*) oscillation at different levels of membrane potential. Each average consists of 16–60 repetitions. The three extracellular averages are superimposed. Note approximately 90° phase shift relative to the extracellular field at resting membrane potential (–65 mV) and reversal of the intracellular oscillation upon hyperpolarization. Note also that the intracellular ripple is smallest in amplitude at –80 mV. Calibration bar refers to both extracellular and intracellular traces. *B*, Photograph of the dendritic arbor of the pyramidal cell from which data in *A* and *C* were obtained. *C*, Responses of the neuron to depolarizing and hyperpolarizing current steps.

by the CA1 pyramidal neurons. In the CA3 region, the minimum time between the presynaptic pyramidal cell discharge and the onset of the action potential of unidentified interneurons and anatomically identified basket cells is 2–3 msec (Miles and Wong, 1987; Gulyás et al., 1993; Han et al., 1993). These con-

straints on timing reduce the possibility that high-frequency, rhythmic firing of CA1 interneurons during the fast field oscillation results solely from a rhythmic excitatory drive from the recurrent collaterals of pyramidal cells. Instead, they support an alternative view that intrinsic properties of interneurons are

Figure 12. Phase reversal of the intracellular fast membrane oscillation by KCl infusion. *a*, Averages of extracellular and intracellular potentials during SPW-concurrent high-frequency oscillations. The intracellularly recorded neuron was histologically verified as a CA1 pyramidal cell. K-Acetate electrode. *b*, Intracellular recording with a KCl-filled electrode. Note opposite polarity of intracellular averages in *a* and *b*. *a* and *b* are from different rats.

the primary determinants of the frequency of the high-frequency population oscillation. We hypothesize that the excitatory barrage by the discharging CA3 and CA1 pyramidal neurons results in a relatively steady depolarization of the target interneurons. This transient depolarization, in turn, would activate voltage-dependent channels resulting in rhythmic oscillation of the cell membrane and phase-locked firing (Llinás 1988). In this hypothesized scenario sustained firing of interneurons would be the primary event and the time-locked firing of the pyramidal neurons is a consequence. Basket cells and chandelier cells are the likely candidates for entraining the pyramidal cells into the population oscillation. First, these inhibitory interneurons provide fast somatic/perisomatic inhibition (Buhl et al., 1994), compatible with our current-source analysis findings. Second, basket cells and chandelier cells extend their axons 400–800 μm in both the transverse and the longitudinal directions and contact more than a thousand pyramidal cells (Struble et al., 1978; Li et al., 1992; Gulyás et al., 1993; Han et al., 1993; Buhl et al.,

1994; our unpublished findings). Third, computer simulations suggest that chandelier cells do not necessarily function as all-or-none gates but instead they may simply delay the occurrence of the Na^+ spike, thus may play an important role in the timing of the action potentials of pyramidal cells (Lytton and Sejnowski, 1991; Douglas and Martin, 1992).

Origin of extracellular currents underlying high-frequency network oscillation

We hypothesized above that fast field oscillation in the CA1 region reflects summed IPSPs in pyramidal cells as a result of high-frequency barrage of interneurons. Thus, the spatiotemporal summation of active outward currents (IPSPs) in the somata of regularly arranged CA1 pyramidal cells is a significant source of the extracellularly recorded oscillatory field potential. As a result of coordinated interneuronal firing the discharge of pyramidal cells becomes phase locked. Indeed, action potentials of pyramidal cells occurred most often on the negative portion of the local oscillatory waves. Since the temporal overlap of action potentials on the negative phase of the fast waves (2.5–3 msec) is considerable, the active inward current associated with the action potential generation in neighboring pyramidal cells should also be regarded as a significant source of extracellular currents underlying the extracellular ripples (“mini” population spikes; Buzsáki, 1986). The alternating dipoles in the current source density maps are compatible with either or both of the above mechanisms.

Longitudinal coherence of high-frequency oscillation

A notable aspect of the high-frequency network oscillation in the CA1 region is its high spatial coherence. This observation indicates that a large number of pyramidal cells can be transiently entrained into an ensemble pattern with a high temporal precision. Our maximum recording distance was approximately 2 mm (20% of the longitudinal length of the rat hippocampus), and within this area we did not observe any significant decrease of coherence either in the septal or temporal direction. No particular site was leading the oscillation as evidenced by the zero time lag in the field averages.

It is generally believed that synchronization with zero time lag is indicative of a common input to the sampled neuronal populations (Gerstein and Perkel, 1972). This is not very likely in our case, however. The driving force behind the high-frequency oscillation in the CA1 region is the transiently increased population burst of CA3 pyramidal neurons. Although single CA3 pyramidal neurons project to one-half to two-thirds of the longitudinal extent of the CA1 region (Ishizuka et al., 1991; Tamamaki and Nojyo, 1991; Li et al., 1994), SPW-concurrent population oscillation in CA3 cells was highly variable and its frequency was always below 150 Hz. In addition, phase locking between the CA3 and CA1 field oscillations were not observed. Finally, high-frequency oscillations in the CA1 regions of the right and left hippocampi were not coherent (Buzsáki et al., 1992). We suggest, therefore, that the high spatial coherence is

Figure 13. Intracellular recording from a basket interneuron during high-frequency oscillation. *a*, Single trace of extracellular (*extra*) and intracellular (*intra*) ripples. Note high-frequency discharge and membrane oscillation of the interneuron in association with the field ripple. *b*, Response of the neuron to depolarizing and hyperpolarizing current steps. *c*, Wide band (DC–10 kHz) and filtered traces during a strong (4 nA) depolarizing current step. Note high-frequency (200–300 Hz) discharge of the cell initially and membrane oscillation after action potential failure. *d*, Biocytin labeled basket cell (UR32A). Arrow, Cell body in the pyramidal layer (*p*). Numerous axon boutons were observed in the pyramidal cell layer in this and several neighboring sections (not shown).

intra (50-250) Hz

brought about by a spatially distributed process confined to the CA1 region. These processes may involve dynamically activated gap junctions, pyramidal cell–interneuron interactions, reciprocal interactions between pyramidal cells and field (ephaptic) effects. Significant contribution of the latter two mechanisms is less likely since excitatory collaterals among CA1 pyramidal cells are very scarce (Christian and Dudek, 1988; Thomson and Radpour, 1991), and the voltage gradient of the high-frequency oscillation across the pyramidal cell layer (1–2 mV/mm) is significantly below the experimentally determined minimum values for such effects (8–15 mV/mm; Jefferys and Haas, 1982; Dudek and Traub, 1989).

Synchronization through gap junctions is supported by some previous and the present findings. First, Katsumaru et al. (1988) has demonstrated that the majority of hippocampal interneurons are connected through gap junctions. Second, the efficacy of gap junctions is modulated by pH, temperature, and possibly neurotransmitters (Church and Baimbridge, 1991). Third, we observed that halothane anesthesia completely abolished high-frequency oscillation in the CA1 region. This finding is notable, since this effect was selective (ripples were present under urethane and ketamine anesthesia) and because halothane is the most frequently used drug for blocking gap junctions in cardiac and smooth muscle research (Terrar and Victory, 1988; Burt and Spray, 1989; Moore et al., 1991; Finkbeiner, 1992). Although direct evidence has yet to be obtained, transiently activated gap junctions could provide an efficient and rapid means for the synchronization of interneurons during the SPW burst.

An alternative or complementary mechanism for the fast recruitment of spatially distributed pyramidal cells and interneurons into the network oscillation can occur via the reciprocal connections between these cell populations. As discussed earlier, axon terminals of basket cells and chandelier cells span 400–800 μm in the longitudinal axis. In turn, axon collaterals of CA1 pyramidal neurons course in the stratum oriens and alveus, project both septally and caudally and emit numerous local collaterals on their way to septal and subiculum/entorhinal cortex targets (Finch et al., 1983; Tamamaki and Nojyo, 1987). In addition, interneurons are synaptically connected, as well (Misgeld and Frotscher, 1986; our unpublished findings). Despite the significant conduction delays in these collaterals, synchronization with zero-phase lag can be brought about by reciprocal interactions. When during the SPW burst a critical number of spatially distributed interneurons discharge at approximately 200 Hz, due to their depolarization-dependent firing properties, they will affect the timing of the action potentials in their common pyramidal cell targets. In turn, pyramidal cells with the most synchronously occurring action potentials will phase-reset the intrinsic oscillation of their common target interneurons in the next cycle(s), thereby establishing a highly coherent, spatially distributed rhythmic inhibition in a large number of pyramidal cells. Thus, by means of spatial diversion and converging overlap of connections between CA1 pyramidal cells and interneurons phase-locking of the action potentials of pyramidal cells into the population rhythm will be enhanced. In this scenario no particular site is leading the oscillation, but temporal coherence within a given ripple event is determined by the most synchronously discharging pyramidal neurons in that event. Action potentials of pyramidal cells that are not linked monosynaptically by excitatory collaterals can be synchronized with better than 5 msec precision via their shared interneurons that are equipped with intrinsic properties to oscillate at high frequen-

cies. From this perspective a major role of the inhibitory basket and chandelier neurons is in adjusting the timing of action potentials in principal cells rather than their classically assigned “or” function for preventing the generation of Na^+ spikes. Dampening of the network oscillation is postulated to be a passive process due to the waning of the depolarization barrage by the CA3 region.

Physiological significance of high-frequency oscillation

An important finding of the present and previous (Buzsáki et al., 1992) experiments is the demonstration of a network effect that is not evident in the firing patterns of single pyramidal neurons. Pyramidal cells discharged at a considerable lower frequency than the parallel output of the CA1 region during the SPW-associated field oscillation. The transient, network-bound oscillation of these neurons became evident only by simultaneous sampling of a large number of the participating elements. Highly synchronous, time-locked discharge of slowly discharging pyramidal cells emerged dynamically from a seemingly non-organized activity within a few milliseconds. The number of pyramidal cells in a given ripple event was relatively small and the probability of participation of single pyramidal cells in successive network oscillatory events was not random. Some neurons fired spikes on a large percentage of the successive SPW events, whereas others were engaged very seldom in the population bursts. The probability of participation of a given neuron in the population event was likely determined by the previous history of the network.

Coherent discharge of several thousand neurons within a time window of less than 5 msec is likely to be a significant event for the brain. Temporal coordination of presynaptic neuronal activity is among the most important conditions for the induction of synaptic modification (Hebb, 1949). The high-frequency intraburst oscillation (200 Hz) of the CA1 network is the optimal frequency range for the induction of long-term potentiation (Douglas 1977; Larson and Lynch, 1986; Diamond et al., 1988). Since such population bursts have been shown to induce long-term synaptic modification *in vitro* (Buzsáki et al., 1987; Ben-Ari and Gho, 1988; Azouz et al., 1992), the issue is whether SPW-associated ensemble oscillation of CA1 neurons encodes meaningful information. We have hypothesized earlier that synaptic modification in the hippocampus occurs in two stages. In the first (exploratory, theta) stage extrahippocampal coactivation of a set of CA3 pyramidal cells results in strengthening of their mutually active excitatory collaterals, so as to form a cell assembly. In the second (SPW) stage members of the cell assembly become relatively constant partners and will initiate SPW-concurrent population bursts (Buzsáki, 1989). Thus, the circuitry to be modified by the SPW bursts is same that was selectively active during theta-related exploratory behavior. CA1 pyramidal cells that succeed in synchronizing their discharges in a precise temporal window during high-frequency network oscillation are expected to exert a powerful impact on their retrohippocampal targets. In accordance with this assumption, we have recently found that high-frequency network oscillation of the CA1 network is followed by a synchronized population burst in layer V neurons of the presubiculum and entorhinal cortex (Chrobak et al., 1994). These latter neurons, in turn, are known to have connections with a very large part of the neocortex (Swanson and Köhler, 1986; van Hoesen and Pandya, 1975). Since SPW-concurrent high-frequency network oscillation is an “*au naturelle*” counterpart of the tetanic stimulus for

long-term potentiation (Bliss and Collingridge, 1993), we hypothesize that by way of the cooperative, oscillatory network discharge CA1 pyramidal cells may address and modify entorhinal/neocortical circuits.

Implications for epileptic interictal patterns

The number of cells participating in a given SPW-burst and their speed of synchronization is a homeostatically regulated process. In the intact brain, the duration of SPW and associated burst activity varies between 40 and 150 msec (Buzsáki et al., 1983; Suzuki and Smith, 1987) and its amplitude never exceeds 3 mV. Therefore, the oscillatory interplay between pyramidal cells and interneurons during the SPW burst can be viewed as a dissipative mechanism to decelerate and limit population synchrony of pyramidal cells. Blockade of GABAergic inhibition or surgical removal of subcortical inputs to the hippocampus results in significantly faster recruitment of a larger number of pyramidal cells as reflected by large-amplitude (>5 mV) and short-duration (<20 msec) "augmented" SPWs, termed type 1 interictal spikes (Buzsáki, 1986; Buzsáki et al., 1989; 1991). Such fast synchronized field patterns are not associated with rhythmic oscillation of the CA1 network. Instead, a large proportion of pyramidal cells may discharge simultaneously as evidenced by large population spikes riding on the wider field event, followed by a prolonged discharge of putative basket cells (Buzsáki et al., 1991). Therefore, in contrast to the bifurcation from rest to a rapid limit-cycle oscillation that characterizes the physiological SPW event, network activity moves rapidly from rest into instability during interictal spikes. Based on these observations it may be suggested that an important role of the interneuron-mediated oscillation in the CA1 region is to prevent the "all-or-none" discharge of the activated pyramidal cells by protracting the recruitment process and limiting the number of participating neurons. Failure of the oscillatory process to limit neuronal recruitment is postulated to be the immediate cause of epileptic interictal spikes.

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