Dentate EEG Spikes and Associated Interneuronal Population Bursts in the Hippocampal Hilar Region of the Rat

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SUMMARY AND CONCLUSIONS

1. This paper describes two novel population patterns in the dentate gyrus of the awake rat, termed type 1 and type 2 dentate spikes (DS1, DS2). Their cellular generation and spatial distribution were examined by simultaneous recording of field potentials and unit activity using multiple-site silicon probes and wire electrode arrays.

2. Dentate spikes were large amplitude (2–4 mV), short duration (<30 ms) field potentials that occurred sparsely during behavioral immobility and slow-wave sleep. Current-source density analysis revealed large sinks in the outer (DS1) and middle (DS2) thirds of the dentate molecular layer, respectively. DS1 and DS2 had similar longitudinal, lateral, and interhemispheric synchrony.

3. Dentate spikes invariably were coupled to synchronous population bursts of putative hilar interneurons. CA3 pyramidal cells, on the other hand, were suppressed during dentate spikes.

4. After bilateral removal of the entorhinal cortex, dentate spikes disappeared, whereas sharp wave associated bursts, reflecting synchronous discharge of the CA3-CA1 network, increased several fold.

5. These physiological characteristics of the dentate spikes suggest that they are triggered by a population burst of layer II stellate cells of the lateral (DS1) and medial (DS2) entorhinal cortex.

6. We suggest that dentate spike-associated synchronized bursts of hilar-region interneurons provide a suppressive effect on the excitability of the CA3-CA1 network in the intact brain.

INTRODUCTION

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 (SW), and the SW-associated high-frequency (200 Hz) oscillation (ripples), sleep spindles, and delta waves of sleep (Bland 1990; Bragin et al. 1993; Buzsáki 1986; Buzsáki et al. 1983, 1992, 1994; Lopes da Silva et al. 1990; O’Keefe and Nadel 1978, Traub and Miles 1991; Ylinen et al. 1995a,b). Hippocampal rhythmic slow activity (theta) is the most studied hippocampal pattern and has been implicated in several functions, ranging from sensory processing to the voluntary control of movement (Grastyán et al. 1959; Vanderwolf 1969).

In the rat, it is associated with exploratory patterns, such as walking, turning rearing and sniffing and theta is the hallmark of the paradoxical phase of sleep. During consummatory behaviors, behavioral immobility, and slow-wave sleep theta is replaced by a mixture of intermittent waves, usually referred to as large-amplitude irregular activity (Vanderwolf 1969). One of the physiologically characterized patterns of the large amplitude irregular activity is a short-duration (40–120 ms) sharp wave (SPW) present in the CA3-CA1-subiculum-entorhinal cortex circuitry (Buzsáki 1986; Buzsáki et al. 1983; Chrobak and Buzsáki 1993; Chrobak and Buzsáki 1995; Suzuki and Smith 1987). 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 conduction with the stratum radiatum SPWs, there are high-frequency field oscillations (ripples) (O’Keefe and Nadel 1978) present in the CA1 pyramidal layer and deep layers of the entorhinal cortex (Buzsáki et al. 1992, Chrobak and Buzsáki 1994; Ylinen et al. 1995).

In the experiments presented here, we describe two new population patterns in the dentate gyrus that emerge in conjunction with a synchronous discharge of inhibitory interneurons of the hilar region. We hypothesize that these population patterns, termed dentate spikes, serve to decrease the network excitability of the CA3 recurrent collateral system during nontheta behaviors in the intact animal.

METHODS

Animals and surgery

Forty-eight male and female rats (250–450 g) 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 𝜇m 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; V, 3.0 mm). Another electrode pair was placed into the ventral hippocampal commissure (AP, −0.8; L, 0.3; 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 (2–4 60-µm tungsten wires) were implanted in the strata pyramidale and radiatum of CA1 and the molecular layer and hilus of the dentate gyrus unilaterally or bilaterally (AP, 3.0; L, 2.6; V, 2.4, 3.0). Microelectrode arrays consisted of four to eight tungsten wires (20 or 60 𝜇m in diameter). Two or three 20-µm wires or a single 60-µm wire was 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. The 20-µm wires within a single silica tube were glued together with varnish. A 3.5 × 1 mm slot was drilled into the skull above the
dorsal hippocampus along the longitudinal or the traverse (dentate CA3) axis of the structure. For simultaneous recording of field potentials and unit activity in different layers, silicon probes micro-machined with thin-film technology (Wise and Najafi 1991) were used in 10 rats. The recording sites (5 × 15 μm², sputtered iridium) were spaced 100 μm. The thickness of the silicon shank was 15 μm throughout. Five or 16 recording sites were available (80 μm wide at the base, narrowing to 15 μm at the tip). In three rats, an epidural screw electrode, driven into the skull above the frontal cortex (AP, 2.5; L, 2.5), was used to record neocortical electroencephalogram (EEG).

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

## Recording and data processing

Four 4-channel MOSFET input operational amplifiers, mounted in the female connector, served to eliminate cable movement artifacts (Buzsáki et al. 1989a). 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 and sampled with 12-bit precision. The data were stored on optical disks. Field potentials were recorded either continuously (1 kHz per channel sampling) or collected as dentate spike-triggered epochs (400 ms) together with unit discharges (10 kHz per channel sampling) in the absence of overt movements. Still alert behavior was defined as immobility with eyes open. Drowsiness was defined by an immobile sleeping posture with eyes open and the head resting on one or both forepaws. Finally, slow-wave sleep was characterized by sleeping posture with eyes closed and the dominance of large amplitude delta waves in the hippocampal EEG.

All analysis was carried out off-line on 486/33 and an IBM RS 6000 computers. The recorded data were digitally filtered at 120 dB/octave to select the frequency of interest: unit activity (500 Hz–5 kHz), dentate spikes (1–1,000 Hz) and SPW-associated high-frequency field ripples (100–300 Hz). The dentate spikes were detected by a window discrimination program and their peaks were used as the zero point for the construction of field potentials. These derived pulses were used as the zero point for the construction of field averages and cross-correlograms. The power of EEG was calculated from 25-s segment EEG during different behaviors.

## Current source density (CSD) analysis

Complete accounts of the theoretical basis of CSD analysis have been presented earlier (Freeman and Nicholson 1975; Mitzdorf 1985). Dentate spikes were first separated into groups (see results) and averaged using their identified positive peaks (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 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 thus are presented as the second derivative of potential as a function of depth, and will be referred to as CSD. The second differences of the voltage profile were divided by the square of the step size in centimeters and by the estimated tissue impedance (300 Ω·cm) to convert them into CSD estimates (Brankack et al. 1993). CSD measurements were plotted as a function of depth at selected time points or at all time points. In the latter case, color plots were constructed with color intensities reflecting sinks and sources in a time (abscissa) versus depth (ordinate) coordinate system. The exact anatomic layers, corresponding to the vertical scale of the CSD maps, were reconstructed with the aid of the histologically identified recording tracks and evoked potentials. The depth profiles of the perforant path and commissurally evoked responses have been well studied in the rat (Brankack et al. 1993; Buzsáki and Czéh 1981; Deadwyler et al. 1975; Leung 1979). Currents sinks and sources associated with the activation of these known anatomic 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–5 kHz) traces to locate the occurrence of spike events (Yang 1988). The spike events were identified by the factorial description of their shape (“feature”) characteristics. 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). For visual display, the spike occurrences within the same target cluster were superimposed and projected relative to the reference event (Ylinen et al. 1995a). The cross-correlograms were calculated from the cumulative number of spikes. Isolation of single units within and across clusters was verified by clear refractory periods (2–3 ms) in their interspike interval histograms.

Several isolated units were identified by physiological criteria. Units that discharged at a shorter latency than the population spike and responded with two or more action potentials in response to perforant path or commissural stimulation were classified as interneurons (Buzsáki and Edelberg 1982; Fox and Ranck 1981). These cells typically fired at high rates (>15 Hz) and discharged in rhythmic groups at the field theta frequency. Units that displayed spontaneous complex burst patterns were classified as pyramidal cells or mossy cells (Ranck 1973; Soltész et al. 1993). Complex-spike cells typically fired at <1 Hz. Physiological criteria for the identification of granule cells are not universally accepted (Buzsáki and Czéh 1992; Mizumori et al. 1989) and criteria for the separation of the various subgroups of interneurons in the hilar region (Annual 1978, Han et al. 1993) are not yet available.

Unit activity was cross-correlated with the peaks of the dentate spikes or with high-frequency field oscillation of the CA1 region (ripples), with the field events serving as reference. Firing rates in a given time epoch (e.g., −10–10 ms of the peak of the dentate spikes) were compared with shuffled spikes, obtained outside of the dentate spike events, and statistical significance was assessed by nonpaired t tests.

## Entorhinal cortex lesion

In a group of seven rats, the entorhinal cortex was removed bilaterally. The electrodes were placed first, and the lesion was made after the completion of the physiological tests. The lesion was made under halothane (2.5%) gas anesthesia. The bone above the entorhinal and perirhinal cortex was removed and the dura was cut. The gray matter and the underlying white matter was aspirated under microscopic vision. The resulting cavity was filled by gelfoam and the wound was closed.

## Colchicine lesion

In seven rats, colchicine toxin (2 μg in 0.5 μl) was injected in the dentate gyrus of the dorsal hippocampus at the following
coordinates: AP, −2.0 and 3.0; L, 1.5 and 2.2; and V, 3.0 and 3.0. These rats were equipped with fixed electrodes (n = 4) or movable electrode arrays (n = 3) 1 mo after the toxin injection. The goal of these experiments was to examine the survival of dentate spikes following toxin damage of the granule cell population.

**Histological procedures**

After completion of the experiments, the rats were anesthetized deeply 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 h, removed, and then postfixed in the same solution for 1 wk. The brains were sectioned with the probes left in the brain on a vibratome at 100 μm in the coronal plane. The sections were stained with the Gallyas silver method (Gallyas et al. 1993). Briefly, the sections were dehydrated with propanol and placed in an esterifying solution (98% propanol, 1.2% sulfuric acid) at 56°C for 16 h. After rehydration and sectioning, they were processed according to the following procedure: pretreatment in 8% acetic acid for 10 min, wash in water for 1 min, physical development with tungstosilicic acid for 10 min, and wash in 1% acetic acid. Finally, the sections were dehydrated, mounted on slides, and coverslipped. Selected sections were stained with cresyl violet.

**RESULTS**

Irregular, sharp transients occurred during immobility, grooming, drinking and slow wave sleep in the hippocampal formation (Fig. 1). Sharp waves (SPW) in the CA1 region have been described in detail earlier (Buzsáki 1986; Buzsáki et al. 1983, 1992; de Curtis et al. 1991; Suzuki and Smith 1987). SPWs represent synchronous discharges of the neurons in the CA3 and hilar regions with resulting current sinks at the termination zones of the associational paths, i.e., the stratum radiatum of CA1 and the inner molecular layer of the dentate gyrus (Buzsáki 1986; Ylinen et al. 1995a). In addition to the wider SPW, large-amplitude (1–4 mV), short-duration (10–40 ms) transients of positive polarity were observed in the hilus of the dentate gyrus, and we termed them dentate spikes. The behavioral correlates of dentate spikes were identical to SPW. They occurred irregularly and at about the same incidence as SPW at 0.01–0.5/ s frequencies during immobility, drowsiness, and slow-wave sleep. Although the incidence of dentate spikes varied as a function of behavior and associated EEG power in the delta band (Fig. 2), the morphological features of dentate spikes, described below, did not depend on behavior. Most dentate spikes were collected in the still alert rat and during slow-wave sleep.

**Two types of dentate spikes**

On the basis of their distinct depth profile and wave duration, dentate spikes could be clearly distinguished from the wider SPWs, which are associated with population bursts of the CA3-CA1 network. SPWs and dentate spikes rarely occurred together in time and SPW virtually never followed dentate spikes within 200 ms.
Quantitative evaluation of the laminar distribution of dentate spikes was carried out on simultaneously recorded potentials from 16-site silicon probes or from successive recordings with wire electrodes at different depths. In the latter case, stationary electrodes were placed in the hilus and molecular layer of the dentate gyrus and averages of dentate spikes were simultaneously obtained from the stationary wires and the movable electrodes at 100 μm steps. The averages obtained from the stationary electrodes were used to normalize the amplitude of the dentate spikes recorded by the movable electrodes. Based on wave morphology and voltage-versus-depth profiles, two types of dentate spikes could be distinguished. The first type (DS1) showed a gradual decrease in amplitude in the molecular layer-hilar axis with a reversal in the outer third of the molecular layer (Figs. 1 and 3). The voltage gradient across the granule cell layer was 2–6 mV/mm. In addition, DS1 occasionally consisted of three to five repetitive spikes of increasing-decreasing amplitude at 70–100 Hz with a large spike in the middle (Fig. 1). In contrast, the second type of sharp transient (DS2) had a fast rise time (0.3–0.5 mV/ms) and the short-duration spike (15–25 ms at the base and 8–15 ms at half-amplitude) was often followed by a longer, small amplitude negative wave (100–300 ms; Fig. 3). DS2 were positive in the hilus and negative in the molecular layer with a sharp reversal in the inner molecular layer of the dentate gyrus. The voltage gradient across the granule cell layer was 12–18 mV/mm. The depth profile of DS2 closely matched the laminar distribution of the evoked responses to medial perforant path stimulation. Based on these distinctive features DS1 and DS2 could be distinguished in records with multiple-site electrodes, or with at least two electrodes straddling the granule cell layer.

The differences in depth profiles of DS1 and DS2 also were reflected by the CSD profiles and maps. Current-source density analysis of DS1 revealed an inward current (sink) in the outer third of the molecular layer (Fig. 4). Both the broader base and the superimposed spike (see Fig. 3) showed similar CSD distribution with depth in color-coded CSD maps (not shown). DS2 had a fast sink-source pair, with the sink located in the middle third of the molecular layer. The spatial position of the sink associated with DS2 and perforant path-induced EPSCs of the granule cells were virtually identical. The sinks of DS1 and especially of DS2 were coupled with sources in the granule cell layer (Fig. 4). These distinct depth profiles were observed in eight out of eight rats equipped with multisite recording probes.

Both types of dentate spikes were present in every rat.
tested. However, DS1 occurred from 1.5 to 3 times more frequently than DS2. Although not investigated systematically, it appeared that this ratio increased with the age of the rat. Quantification of this relationship is subject of a separate paper.

Spatial synchrony of dentate spikes

Synchrony between simultaneously recorded events was evaluated in three different ways. First, dentate spikes recorded simultaneously from different sites were averaged using the peak of the dentate spike from a selected site as the trigger. These derived pulses also were used to construct cross-correlograms between dentate spikes and unit activity recorded from different sites. Second, dentate spikes recorded from different sites were averaged by isolated single units (spike-triggered averages). Third, pulses derived from discriminated dentate spikes of different locations were cross-correlated. DS1 and DS2 showed similar spatial coherence characteristics and therefore they are discussed together.

Longitudinal coherence of dentate spikes was assessed by placing four to eight electrodes in the dorsal hilus along the longitudinal axis of the hippocampus (n = 6 rats) and by simultaneous recordings from the hilus of the dorsal and ventral hippocampus (n = 3 rats). Dentate spikes recorded as far as 1.5 mm intervals in the dorsal hilus were essentially synchronous (Fig. 5). Cross-correlograms between field and unit activity were similar when dentate spikes were recorded from the same or different electrode as the units. Comparison of histological location of the electrode tips with electrical activity suggested that lateral or vertical mismatch of the electrodes relative to the center of the hilus contributed significantly more to the variability than distance. Peaks of field versus unit cross-correlograms were essentially flat, however, when the events were shuffled.

Dentate spikes in the ventral hippocampus were similar

FIG. 4. Current source density (CSD) depth profiles of 2 types of dentate spikes (DS1 and DS2) and evoked responses to stimulation of the perforant path (PP) inputs. Voltage measurements for CSD analyses were taken at the peak of the dentate spikes and 1 ms after the onset of the evoked responses, respectively. Superimposed curves reflect the stability of current distributions during 5 (DS1 and DS2) or 3 (PP) successive averages of 10 events each. Note different sink and source pairs for DS1 and DS2 and the similarity between DS2 and PP-evoked responses. Corresponding hippocampal layers are shown on the left. Intracellularly labeled and reconstructed pyramidal cell and granule cell provide further landmarks for the recording positions. o, stratum oriens of CA1; p, pyramidal layer; r, stratum radiatum; hf, hippocampal fissure; m, molecular layer; g, granule cell layer; h, hilar region.

FIG. 5. Longitudinal synchrony of dentate spikes. A: averages of simultaneously recorded dentate spikes from 5 recording sites (400 ms traces). Distance between electrodes 1 and 5 (shown in D) was 1.5 mm. B: averaged dentate spikes from the ventral hippocampus. Recording sites a, b, and c are shown in C. D: cross-correlogram of dentate spikes recorded simultaneously from the septal and temporal portions of the hilus in a different rat. The recording positions were similar to sites 2 and b in C. cc, corpus callosum; rf, rhinal fissure.
in shape and amplitude. Because of the curvature of the hippocampal axis in the temporal region, it was more difficult to position the electrode tips at similar positions. Nevertheless, dentate spikes in the ventral hilus were also synchronous (Fig. 5) and were coupled tightly to the discharges of the hilar neurons (not shown). When dentate spikes with twice the amplitude of the background activity were discriminated from the dorsal and ventral portions of the hippocampus, their cross-correlograms showed significant peaks at time 0. These findings indicate that neurons located as far as 8 mm in the septal and temporal parts of the hippocampus can discharge within 10 ms during the dentate spike.

The synchrony of dentate spikes in the mediolateral direction was assessed by moving an array of eight electrodes in the coronal plane (n = 7 rats). A pair of stationary electrodes was placed in the hilus and the molecular layer. Locally recorded field potentials from each electrode were averaged by the peak of dentate spikes recorded from the hilus. The averages obtained from the stationary electrode in the hilus were used to normalize the amplitude variations at different depths. The electrode array was moved at 100-μm steps and data were collected from a total of 160–200 locations. Evoked responses and neuronal discharges were used to define the position of the recording electrode array. The resulting averaged dentate spikes were then used to construct two-dimensional voltage maps. As Fig. 6 illustrates, there was little activity outside the dentate region during DS1. The largest amplitude positivity (hot colors) occurred in the granule cell layer surrounded by smaller amplitude negativity (cool colors). The spatial extent of DS2 was similar to DS1, although voltage maps were not constructed.

When electrodes were placed in a symmetrical fashion in both hilar regions, dentate spikes in the two hemispheres occurred virtually synchronously (Fig. 7). Although occasionally lower amplitude (<2 mV) dentate spikes were observed in isolation in one hemisphere, in most cases dentate spikes in the two hemispheres occurred together. Closer inspection of the original records revealed that small delays (0–10 ms) were often present in the individual records, but there was a 0 time lag between the peaks of the averaged dentate spike recorded from the opposite hemispheres. Unit activity simultaneously recorded from both hilar regions (n = 11 rats) had similar peaks independent of whether the reference event for the cross-correlation was the ipsilateral or the contralateral dentate spike (Fig. 7).

Correlations between dentate spikes and unit activity

To reveal the neuronal populations contributing to the dentate spikes, action potentials of physiologically identified single neurons and multiple-unit discharges were cross-correlated with the peak of the dentate spike. Units that were recorded ≥200 μm below the reversal of the perforant path evoked response were regarded as “hilar cells”. A subgroup of these units with complex-spike bursts were categorized as mossy cells (Soltész et al. 1993) or CA3c pyramidal cells (Fox and Ranck 1981; Ranck 1973). Four of these complex spike cells were putative mossy cells because they were localized to the hilus proper. Nine neurons were identified positively as interneurons, based on their multiple action potential response to perforant path and/or commissural stimulation (e.g., Fig. 7C, inset). Identification of granule cells was not attempted in this study, because of the lack of reliable criteria (Buzsáki and Czéh 1992; Mizumori et al. 1989). Putative granule cells recorded within 100 μm of the reversal of the perforant path response fired very rarely during immobility, thus preventing the construction of interpretable cross-correlograms.

The relationship of complex spike cells and the remaining hilar cell group to the dentate spike events was different. All hilar cells (n = 75), including the nine positively identified interneurons and hilar multiple unit groups (n = 50) increased their activity during both DS1 and DS2. In the experiment shown in Fig. 8, four fast-firing hilar cells were recorded simultaneously along with pyramidal neurons of the CA3b and CA1 regions. In association with DS2, hilar cells showed peaks in their cross-correlograms. In contrast, multiple-unit activity in the CA3b site decreased, whereas unit activity in the CA1 region was not altered in this animal. In two out of nine further cases, however, decreased firing of CA1 pyramidal cells was also evident. DS1 or DS2-associated decrease of unit activity was found in 3 out of 14 single or multiple CA3 neurons and one out of the four putative mossy cells. It must be emphasized, that lack of a clear depression in the other cases might be because of the low firing rates of the complex spike neurons. Importantly, complex spike cells never increased their discharges in either CA3 or CA1 regions during the dentate spikes.

To date, we have not identified any cell that discharged only during the peak of DS1 but not DS2 or vice versa. However, the shape of the dentate spkc-versus-unit cross-correlograms were characteristically different for the two events. In association with DS2 the discharge peak was preceded by a depression of firing for 30–100 ms (Figs. 7–9). In contrast, histograms associated with DS1 did not show such a depression (Fig. 9).

Spike-triggered averaging of the local unfiltered potentials by the physiologically identified interneurons also revealed a reliable relationship between unit discharges and local field potentials in the hilus. Putative interneurons discharged significantly more frequently during the dentate spikes as evidenced by the spike-triggered field averages. The shape of these averages was often similar to the algebraic sum of averaged DS1 and DS2, suggesting that the interneurons fired during both events. Shuffled spikes resulted in flat averages.

Relationship between dentate spikes and sharp waves

The two types of dentate spikes appeared mutually exclusive because they virtually never occurred together within a
A right C

400-ms time window. In addition, SPWs and associated high-frequency (200 Hz) field ripples in the CA1 pyramidal layer were never observed after the dentate spikes (<200 ms) nor were they followed by DS2 (<200 ms). These observations are based on 12 rats in which SPWs and dentate spike were simultaneously recorded. In contrast, 5–15% of DS1 were preceded by SPW events. This relationship was revealed by cross-correlograms of DS1 and CA1 ripples as well as DS1 and neuronal discharges of CA1 pyramidal neurons, respectively (Fig. 9, B and C). Increased activity of CA1 neurons and associated 200-Hz field ripples occurred 60–120 ms before the peak of DS1. Conversely, when CA1 ripples were used as reference events, unit discharges of hilar neurons both preceded and followed them (Fig. 9F). Increased hilar neuronal activity before SPW reflected recruitment of these neurons by the CA3 population burst events (Buzsáki 1986; Buzsáki et al. 1983; Scharfman 1995; Strowbridge et al. 1993). The slightly increased neuronal firing after the SPWs likely corresponded to the triggered DS1 events. Despite the occasional coupling between SPW and DS1, it must be emphasized that the occurrence of SPW was not a prerequisite for the occurrence of DS1. The above findings also indicate that the hilar network can be synchronized by two distinct mechanisms: from the entorhinal cortex during dentate spikes (feed-forward drive) and from the CA3 region during SPW (feed-back drive).

Effect of colchicine lesion on dentate spikes

Colchicine injection into the dentate gyrus of the dorsal hippocampus resulted in nearly complete elimination of the granule cells in one rat and partial damage in the remaining three animals. However, both the cresyl violet staining and the Gallyas silver impregnation methods (Gallyas et al. 1993) revealed that not only granule cells but a large portion of the hilar neurons also were damaged by the toxin. The amplitude of dentate spikes, in general, was considerably lower in these rats than in the intact animals. The toxin effect especially was convincing in two cases where the longitudinally placed electrode arrays surveyed areas with and without granule cells. In areas with complete elimination of granule cells, the amplitude of dentate spikes was two- to threefold lower than the simultaneously recorded spikes from areas with intact granule cells. These observations suggested that the currents underlying the dentate spikes are generated mostly by the granule cell population. However, the immediate course of neuronal synchrony could not be determined because of simultaneous damage of the hilar cell population.

Effect of entorhinal lesions on dentate spikes and sharp waves

Histological analysis of the brains revealed that in five of the seven rats the entorhinal cortex was removed completely (Fig. 10). In all cases, the extent of the lesion was larger than the boundaries of the entorhinal cortex and involved damage to the perirhinal and/or occipital cortical areas, as well. In two cases, a small portion of the medial entorhinal cortex remained in connection with the angular bundle. Evoked responses from both perforant path and the commissural inputs were decreased on the day of the lesion but both recovered to normal values 24 h later. The threshold of the
perforant path response increased progressively day by day, but small amplitude evoked field patterns in response to high intensity stimulation were observed for at least 7 days. In

FIG. 8. Simultaneous recording of field and unit activity from the hilus (H), CA3 and CA1 regions during DS2, From the hilar recording electrode 4 units could be separated, Independence of the units were verified by the refractory periods in the autocorrelograms, Single traces of multiple units are shown for the CA3 and CA1 recordings sites, Right column, averaged field potentials (n = 50) and cross-correlograms (300 ms traces), Time 0: peak of DS2, Note increased activity of the hilar neurons (H1-H4) and decreased activity of CA3 pyramidal cells (arrow) during DS2.

FIG. 9. Relationship between dentate spikes, hilar neuronal activity and SPW-associated bursts in the CA1 region, A and B: cross-correlograms between the 2 types of dentate spikes (DS1 and DS2) and SPW-wave associated high-frequency oscillation (ripple) in the CA1 pyramidal layer, Time 0: peak of dentate spikes, C: DS1 vs. multiple unit discharge of CA1 pyramidal neurons, Note that SPW-associated activity in CA1 preceded DS1 by ~100 ms, D and E: cross-correlograms between the 2 types of dentate spikes (DS1 and DS2) and multiple unit activity in the hilus, Note that unit activity is suppressed before the occurrence of DS2 and increased during both DS1 and DS2, F: cross-correlogram between SPW-associated ripples recorded from the CA1 pyramidal layer and multiple unit activity in the hilus, The early increase (↑) is due to the SPW-associated population burst of CA3 and hilar region neurons, The late increase of neuronal activity (↑) is likely due to DS1-concurrent unit activity that often followed the SPW bursts, Number of sweep are indicated above the histograms.

the two rats with partial lesions, the perforant path evoked responses survived to the end of the observation period, The commissurally evoked responses also predicted the extent of the lesion, In the intact rat, commissural stimulation evoked a late (20–25 ms latency) response in the dentate gyrus (not shown). This late reverberatory potential reflects sequential
activation of the CA1-entorhinal cortex-dentate gyrus circuitry (Deadwyler et al. 1975). This late potential permanently disappeared in rats with complete lesions but survived in the two partially damaged rats.

Changes in theta activity and associated gamma waves after entorhinal cortex damage have been described earlier (Bragin et al. 1995). In contrast to the intact rat, large amplitude fast EEG activity was no longer a characteristic feature of the hilar region. Large interictal spikes (>4 mV) occurred for 1–4 days after the lesion in every rat. These interictal spikes were largest on the second postoperative day and their depth profiles and form were virtually identical with the perforant path-evoked responses. Tentatively, we assume that these large transients were brought about by spontaneous synchronized discharges of the perforant path fibers due to the demyelination process. Apart from these large-amplitude sharp spikes during the early postoperative days, dentate spikes were observed only rarely during the postlesion observation period (21 days; Fig. 11). The surviving dentate spikes were small amplitude sharp events (<1 mV) and emerged after the first postoperative week (Fig. 11, A and B). However, based on the depth of polarity reversal and shape these were similar to DS1 (Fig. 11B). In the two rats with partial lesion, dentate spikes survived and, despite the large extent of the entorhinal cortex damage, their incidence and amplitude returned to the preoperative levels after 2 wk. These findings suggest that the entorhinal cortex plays a primary role in triggering dentate spike events.

In contrast to the drastic reduction of the dentate spikes, SPWs occurred more frequently after entorhinal cortex damage (Fig. 11, A and C). Not only did the total number of SPWs increase but the incidence of double SPWs (2 events within a 400-ms time window) also increased significantly. The amplitude of the SPWs, however, was not significantly different from the preoperative level. Occasionally, SPWs occurred rhythmically at 3–6 Hz for several seconds. In addition, postlesion SPWs often were followed by a "tail" of 50–100 Hz oscillation (Fig. 11D).

**DISCUSSION**

This study revealed the occurrence of short-duration and large-amplitude field transients in the hilar region of the intact rat brain. These field transients, termed dentate spikes, invariably were associated with the synchronous bursting of the hilar-cell population. The dentate spikes and associated population bursts of the putative hilar interneurons occurred virtually simultaneously along the long axis of the hippocampus and in the two hemispheres and emerged independently from the SPW-associated bursts of the CA3 region.

**Cellular-synaptic generation of dentate spikes**

Two types of dentate spikes (DS1 and DS2) could be distinguished. They had different voltage-versus-depth profiles and different spatial distribution of their current sinks. After bilateral removal of the entorhinal cortex, dentate spikes were virtually eliminated. Both types of dentate spikes had similar spatial synchrony and were associated with a synchronous population burst of putative dentate interneurons. However, the hilar-region population burst was consistently preceded by a decreased discharge probability of hilar interneurons before the occurrence of DS2 but not before DS1. Frequently, DS1 appeared as a brief oscillatory event, whereas DS2 was an isolated event followed by a longer negative field event. DS1 and DS2 appeared to be mutually
hippocampal dentate spikes

most of the observed characteristics of DS2 support the hypothesis that they are triggered from a synchronous burst of layer II stellate cells of the medial entorhinal cortex. Based on the voltage-versus-depth profile and a large sink of DS2 in the middle molecular layer, we assume that extracellular negativity at this level reflects synchronous depolarization of granule cells, basket and chandelier cells and hilar interneurons by the medial entorhinal input (Gemroth et al. 1989; Han et al. 1993; McNaughton and Barnes 1977; Steward 1976). Overall, these findings suggest that a dominant source of the extracellular currents underlying DS2 derives from synchronous excitatory postsynaptic currents impinging upon the dendrites of granule cells in the middle third of the dentate molecular layer.

other observations, on the other hand, suggest that the current source observed in the granule cell layer during DS2 is not simply a passive return current. In agreement with previous observations (Buzsáki and Eidelberg 1982), several putative interneurons responded earlier and at a lower current threshold than the granule cell population spike evoked by stimulation of the perforant path. The anatomic basis of this observation is that basket cells and several hilar cell types extend their dendrites into the molecular layer of the dentate gyrus (Amaral 1978; Han et al. 1993; Scharfman 1991). These neurons therefore could be driven monosynaptically by layer II stellate cells of the medial entorhinal cortex during DS2. Independent of whether basket cells and hilar interneurons were activated by the perforant path, granule cells, or by other means, their terminals on the somatic/perisomatic region of granule cells likely produced a concerted inhibition of the latter neurons. We may hypothesize, therefore, that at least part of the extracellular current flow, associated with the source in the granule cell layer, arises from synchronously active inhibitory postsynaptic currents (IPSCs) on the granule cells. This conclusion is also supported by the survival of lower amplitude dentate spikes after bilateral removal of the dentate gyrus. Further discussion about the role of somatic/perisomatic inhibition in the generation of field events is hampered by the lack of consensus regarding the direction of inhibitory current flow in granule cells. Patch-clamp experiments suggest that γ-aminobutyric acid-A (GABA_A)-mediated
IPSCs in granule cells are depolarizing because the resting membrane potential in these cells is considerably more negative than the chloride equilibrium potential (Soltész and Mody 1994; Staley et al. 1997). In experiments with sharp electrodes, on the other hand, presynaptic activation of identified single interneurons resulted in short-latency hyperpolarization of the granule cells from the resting membrane potential (Buhl et al. 1994). Because similar measurements are not yet available in vivo, these in vitro experiments do not directly support or refute the interpretation of the currents underlying DS2. We conclude, therefore, that the major electromotive forces underlying DS2 correspond to active inward and outward synaptic currents at the dendrites and somata of granule cells, respectively. Because these active currents are segregated spatially, their summation in the extracellular space should give rise to a large extracellular field.

One apparent aspect of DS2 that the above reasoning fails to explain is the prominent decrease of the firing rate of hilar cells 30–100 ms before the peak of the field DS2. This transient decrease of discharge rate in hilar interneurons may be because of a subcortically mediated inhibitory process that precedes the synchronous population burst in layer II neurons of the entorhinal cortex. A candidate mechanism for such subcortical inhibition of hilar and possibly entorhinal cortex interneurons is the septal GABAergic projection on hilar and possibly entorhinal cortex interneurons (Freund and Antal 1988; T. F. Freund, personal communication). An experimental verification of this hypothesis will require simultaneous recordings of dentate spikes and medial septal neurons. Alternatively, population bursts in the entorhinal cortex may emerge from a rebound of synchronous hyperpolarization of layer II stellate cells. In the latter case, the decreased firing of hilar cells preceding DS2 is a disfacilitation event due to a transient decrease of the entorhinal drive. Although the cellular basis of such hyperpolarization-rebound bursts in the entorhinal cortex has yet to be demonstrated, such intrinsic mechanisms are known to form the basis of network neuronal bursts in other parts of the brain (Llinás 1988; Steriade et al. 1993).

The cellular-synaptic generation of DS1 appears different from that of DS2. Typically, DS1 was a brief oscillatory event with one large and two to four small surrounding waves at ~100 Hz. The voltage-versus-depth profiles and CSD findings as well as the entorhinal cortex lesion experiment are compatible with the hypothesis that a major part of the currents underlying DS1 are generated by the lateral entorhinal cortical synapses in the outer molecular layer (McNaughton and Barnes 1977; Steward 1976). Acceptance of the suggestion that the field events in the dentate gyrus underlying DS1 reflect depolarization of the distal dendrites of granule cells, brought about by the population burst events of the entorhinal cortex, carries the explicit assumption that the intrinsic and/or circuit properties of layer II neurons are different in the medial and lateral entorhinal cortex, because the wave forms and unit correlates of DS1 and DS2 were different.

The importance of extrahippocampal drive in the triggering of dentate spikes is supported further by observations that sharp transients in the dentate hilar region, likely analogous to the dentate spikes, were reduced by damaging the amygdala in the cat (Paré et al. 1994). The role of the entorhinal input in the genesis of dentate spikes also may be interpreted from a different viewpoint. It may be assumed that population bursts in the entorhinal cortex served merely as a trigger for initiating the synchronous discharge of the hilar network. In hippocampal slices, bath application of 4-aminopyridine induces burst discharges in hilar neurons even when N-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepro-pionic acid-mediated excitatory neurotransmission is blocked pharmacologically (Michelson and Wong 1991; Muller and Misgeld 1990; Soltész and Mody 1994). Parallel to bursting of hilar cells, synchronously occurring giant IPSPs are seen in granule cells and CA3 pyramidal cells accompanied by positive field potentials. These in vitro observations are compatible with the dentate spike-concurrent large positive field potentials in the granule cell layer, synchronous discharges of hilar-region interneurons and inhibition of CA3c pyramidal cells (Michelson and Wong 1991). Both sets of findings may be explained by the hypothesis that population synchrony of inhibitory cells in the hilar region is due to depolarizing responses mediated by GABA receptors on inhibitory interneurons (Michelson and Wong 1991). An alternative mechanism for the fast recruitment of hilar cells during dentate spikes is a temporary increase of the efficacy of gap junctions. Katsumaru et al. (1988) have demonstrated that the majority of hilar-region interneurons, but not other cell types, are connected through gap junctions. Because the efficacy of gap junctions is modulated by pH, temperature (Church and Baimbridge 1991) and possibly neurotransmitters, such a mechanism could provide an efficient and rapid means for the synchronization of interneurons during dentate spikes.

Individual dentate spikes could emerge from virtually any segment of the dentate region as evidenced by the latency and amplitude differences of simultaneously recorded single events. However, averages of field events and units histograms recorded from different locations had 0 time lags. When recordings were carried out from the septal and temporal ends of the hilar region many dentate spikes occurred synchronously. Spatial synchrony of the dentate spikes may be explained by the widespread projection of the perforant path to the dentate area (Amaral and Witter 1989) and/or by our recent observation that certain types of hilar interneurons have extremely large axonal arbors covering longer than one-third of the longitudinal extent of the dentate gyrus (Sik et al. 1994).

**Dentate spikes and sharp waves**

In the absence of theta activity, two kinds of irregular burst events occur in the intact hippocampus: SPWps and dentate spikes. SPWps are initiated in the CA3 region and invade the CA1 region and retrogradely the dentate networks (Buzsáki 1986), whereas dentate spikes are initiated in the hilar regions and tend to suppress the emergence of SPW bursts.

A small percentage (~2%) of DS1 were preceded by SPW-associated population bursts but such relationship was
not observed with DS2. Because layer V neurons of the medial entorhinal cells discharge synchronously with hippocampal SPW but layer II cells do not alter their firing patterns (Chrobak and Buzsáki 1994), we have to assume either that the physiological connectivity between layers V and II connections are different in the medial and lateral parts of the entorhinal cortex or that the entorhinal cortex in not involved in SPW-triggered DS1 events. An alternative explanation for the SPW-triggered DS1 events is based on the population dynamics of the CA3-hilar region network. After the SPW burst, CA3 pyramidal cells become silent due to the burst-induced long-lasting hyperpolarization in the pyramidal cells and to recurrent inhibition. Thus transiently reduced excitations of hilar interneurons by the CA3 pyramidal cells and hilar mossy cells (Li et al. 1994; Scharfman 1994) may create conditions favorable for hilar network synchrony (disfacilitation induced rebound).

Synchronous discharge of a large number of hilar neurons within a narrow time window is likely to be an important event for the operation of the hippocampus. Full synchrony of these neurons is dependent on the driving force of the entorhinal cortex. On the other hand, dentate spikes were never followed by SPWs or by an increased discharge of CA3 and CA1 pyramidal cells. Instead, dentate spikes tended to suppress pyramidal cell activity in the CA3 region. Such a scenario does not support the generally held view of the hippocampal circuitry as a series of unidirectionally excited groups of cells from the entorhinal cortex to dentate granule cells $\rightarrow$ CA3 $\rightarrow$ CA1 pyramidal cells and subicular neurons (Amaral and Witter 1989; Andersen et al. 1971). Instead, it suggests that during the dentate spikes the net output of the dentate gyrus to the CA3 region in the intact brain is mainly inhibitory. This view is further supported by the increased excitability of the CA3-CA1 network, as reflected by the higher incidence of SPWs, after entorhinal cortex lesion. Suppression of the recurrent CA3 network can be accomplished by feed-forward excitation of interneurons in the CA3 region (Frotscher 1989) or by a direct inhibitory action of hilar interneurons on the CA3 pyramidal cells. The anatomic substrate for the latter possibility has yet to be demonstrated. However, in line with such a suggestion, we have recently recorded intracellularly from a hilar chandelier cell, which fired bursts of action potentials during dentate spikes. Reconstruction of the in vivo labeled cell revealed extensive axonal arborization in the fascia dentata, hilus and the CA3c region (Sik et al. 1994).

Based on the relationship between dentate spikes and SPWs, it may be suggested that a possible physiological function of dentate spikes and associated population bursts of hilar interneurons is to delay the occurrence of SPW-concurrent network bursts in the CA3-CA1-subiculum-layer V entorhinal cortex circuitry. Thus dentate spikes may be conceived as a "disable" signal which can prevent the occurrence of the powerful feedback from the hippocampus to the neocortex by the entorhinal cortex. The latter mechanism has been postulated to play a critical role in transferring information from the hippocampus to neocortical areas (Chrobak and Buzsáki 1994).

**Dentate spikes and interictal spikes**

Increased synchrony of the dentate cell population may lead to epileptic interictal spikes (Michelson and Wong 1991; Müller and Misgeld 1990, 1991; Scharfman and Schwartzkroin 1990). Indeed, the polarity and form of the dentate spikes are similar to type 2 interictal spikes observed in several epilepsy models (Buzsáki et al. 1989b, 1991; Fujita et al. 1983; Wadman et al. 1983), whereas type 1 interictal spikes represent excessive recruitment of CA3 and CA1 pyramidal neurons (Buzsáki et al. 1983, 1989b; Wong and Traub 1983). The involvement of the different neuronal types (hilar neurons and pyramidal cells, respectively) and their opposite target effects (inhibition and excitation, respectively) in these population events may explain why different categories of interictal spikes suppress or promote epileptic seizures (Engel 1989; Stevens et al. 1972). Experimental support for this hypothesis will require identification of interictal events as SPW-like (type 1) or dentate spike-like (type 2) and correlation of their incidence with the occurrence of afterdischarges.

**Conclusions**

In the intact rat large amplitude, positive field potentials are present in the dentate gyrus in the absence of theta waves. The entorhinal cortex is involved critically in triggering dentate spikes and synchronous discharges of layer II stellate cells in the lateral and medial entorhinal cortex are hypothesized to be the immediate course of two types of dentate spikes. At least part of the extracellular current flow underlying these intermittent field events is due to synchronous inward currents to the granule cell dendrites. Dentate spikes are invariably associated with a burst discharge of hilar interneurons. In the immobile rat and in slow-wave sleep, dentate spikes provide a physiological means to suppress the excitability of the CA3-CA1 network.

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**REFERENCES**


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**REFERENCES**


Soltesz, I., Bourassa, J., and Deschenes, M. The behavior of mossy cells


