Spontaneous Waves in the Dentate Gyrus of Slices From the Ventral Hippocampus

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INTRODUCTION

The hippocampal EEG of freely moving rats alternates between rhythmic oscillations and intermittent, monophasic waves. Exploration and other locomotor behaviors are typically accompanied by theta, a large amplitude, regularly timed rhythm with a frequency of 4–7 Hz that is driven by pacemaker cells in the medial septum/diagonal bands complex (Leung 1998; Petsche et al. 1962; Vertes and Kocsis 1997). Higher frequency beta (20 Hz) and gamma (40 Hz) rhythms are also thought to be initiated by ascending cholinergic projections from the basal forebrain (Leung 1992, 1998). Aperiodic sharp waves (SPWs), negative-going waves with a mean frequency of about 3 Hz, replace rhythmic oscillations as the dominant hippocampal EEG pattern when the animal is not moving and presumably not actively sampling the environment (e.g., during awake immobility and slow-wave sleep) (Buzsaki 1986; Buzsaki et al. 1983; Jouvet et al. 1959; Suzuki and Smith 1987). SPWs, which are highly irregular with respect to rate and size, occur in near-synchrony across a substantial portion of the CA3 pyramidal cell population (Buzsaki 1986; Kubota et al. 2003) and are endogenously generated events that do not depend on extrinsic afferents (Buzsaki et al. 1987a, 1988). While a sizeable body of literature supports the idea that cholinergically dependent rhythms are intimately involved in information processing and memory formation (Jensen and Tesche 2002; Mizumori et al. 1990; Winson 1978), there is no widely accepted hypothesis regarding the function of SPWs.

Recent work has described a second form of spontaneous spike-like activity in hippocampus. These potentials occur in the dentate gyrus and have a lower mean frequency than SPWs, but nonetheless appear during the same behavioral states (Bragin et al. 1995; Bramham 1998; Karlsson and Blumberg 2004; Penttonen et al. 1997). Termed dentate spikes, these waves disappear following bilateral lesions of the retrosplenial region and thus may be triggered by population bursts arriving via the perforant path from the entorhinal cortex (Bragin et al. 1995). If dentate spikes involve discharges of large numbers of granule cells, then, through the potent mossy fiber system, they should trigger substantial excitation in field CA3. However, this has not been described. Similarly, the origins and distribution of the spikes across the inner and outer blades of the dentate gyrus are poorly understood. The authors recently described spontaneous waves in slices from the temporal portion of rat hippocampus that closely resemble in vivo SPWs (Kubota et al. 2003). The experiments reported here tested for spontaneous potentials in the dentate gyrus of temporal slices. Aperiodic waves with frequency and duration comparable to those of dentate spikes were commonplace and found to be locally generated. Subsequent experiments indicated that the dentate waves (DWs) can propagate across much of the granule cell layer and precede large sharp wave like potentials in field CA3.

METHODS

Slice preparation

Slices were prepared from young adult male Sprague-Dawley rats, ~4 wk of age. Animals were anesthetized with halothane and killed 4 wk of age. Animals were anesthetized with halothane and killed.
by decapitation following procedures set forth in a protocol approved by the University of California Institutional Animal Care and Use Committee. The brain was quickly removed and soaked in icy cold artificial cerebrospinal fluid (ACSF) of the following composition (in mM): 124 NaCl, 3 KCl, 1.25 KH2PO4, 5 MgSO4, 3.4 CaCl2, 10 d-glucose, and 26 NaHCO3. Slices were cut using a vibrating tissue slicer (VT1000, Leica, Bannockburn, IL) at a thickness of 350 μm. Tissue blocks containing the hippocampus and surrounding cortical and subcortical regions were positioned in such a way as to obtain slices that were roughly perpendicular to the longitudinal axis of the hippocampus and contained the hippocampal formation and adjacent tissue. Slices were obtained from the ventral (temporal) portion of hippocampus, approximately two-thirds of the distance from the septal pole.

**Field potential recording**

Immediately after cutting, slices were transferred to an interface chamber and allowed to recover for ~1 h prior to commencement of field recording. ACSF used for recording differed slightly from dissection ACSF and was of the following composition (in mM): 124 NaCl, 3 KCl, 1.25 KH2PO4, 1 MgSO4, 3 CaCl2, 10 NaHCO3, and 26 NaHCO3. Slices were cut using a vibrating tissue slicer (VT1000, Leica, Bannockburn, IL) at a thickness of 350 μm. Tissue blocks containing the hippocampus and surrounding cortical and subcortical regions were positioned in such a way as to obtain slices that were roughly perpendicular to the longitudinal axis of the hippocampus and contained the hippocampal formation and adjacent tissue. Slices were obtained from the ventral (temporal) portion of hippocampus, approximately two-thirds of the distance from the septal pole.

**Intracellular recording**

Whole cell recordings were made with 3–5 MΩ glass pipettes filled with solution of the following composition (in mM): 130 CsCl, 0.2 EGTA, 8 NaCl, 2 ATP, 0.3 GTP, and 10 HEPES (pH 7.35, 290–300 mosM). The liquid junction potential of the pipette solution was ~6 mV with respect to the Ringer solution. Holding potentials were ~90 mV. Recordings were obtained using a patch amplifier (AxoPatch-200A, Axon Instruments, Burlingame, CA) with a four-pole low-pass Bessel filter at 2 kHz. Other recording conditions were identical to those described above for field potential recording.

**Dentate wave detection**

For detection of DWs, the second derivative \(d2(t)\) of the recorded data \(v(t)\) was estimated at time \(k\) using data points \(v(k)\) and its two neighbors \(v(k - h)\) and \(v(k + h)\): \(d2(k) = -2v(k) + v(k-h) + v(k+h)\), where \(h\) is a parameter that was set according to the half-width of the population spikes to be detected. A simple threshold was then determined to select the second derivative values positive enough to be classified as a population spike. Additionally, only those potentials ≥40 μV in size and 20–120 ms in duration were classified as DWs. Accuracy of this detection method was verified by visual examination.

**Measurements**

Results are reported as mean ± SD and shown in figures as mean ± SE. One-way repeated measure ANOVA analyses were performed to assess statistical significance, unless otherwise indicated. Prior to computation of cross-correlation, traces were band-pass filtered from 0.1 to 200 Hz and notch-filtered at 28–32, 55–65, and 115–125 Hz to remove 60-Hz noise and associated harmonics. Cross-correlation analyses were performed using MATLAB (MathWorks, Natick, MA).

**Drugs and reagents**

CNQX, physostigmine, picrotoxin, and carbenoxolone were purchased from Sigma-Aldrich (St. Louis, MO). All compounds were dissolved in ACSF prepared on the day of the experiment.

**Entorhinal cortex lesion**

For entorhinal cortex ablation, rats were anesthetized with xylazine (10 mg/kg) and ketamine (50 mg/kg). An electrolytic lesion was placed in the entorhinal cortex under stereotaxic guidance using anodal current and insulated stainless steel wire, as described previously (Guthrie et al. 1995). Animals were killed, and ipsilateral hippocampi were used for electrophysiological experiments at 5–8 days after lesion, as described above. After electrophysiological testing, slices were fixed in 4% paraformaldehyde for 2–4 days, cryoprotected in 20% sucrose, and sectioned (30 μm) parallel to the broad face of the slice using a freezing microtome. Sections were processed by the Fink-Heimer technique for silver impregnation of axonal degeneration (Lynch et al. 1973; Steward 1992) to assess the extent of entorhinal lesions.

**Results**

**Distribution of DWs and their relationship to sharp waves**

Figure 1A shows a 3-s-long field recording from s. granulosa of the dentate gyrus in a slice prepared from temporal hippocampus. Spontaneous, negative-going potentials occurring at a frequency of ~1 Hz and several hundred microvolts in amplitude are evident. Mean frequency of DWs for a group of slices from 33 rats was 0.74 ± 0.43 (SD) Hz, a value comparable to that reported for dentate spikes in vivo (Bragin et al. 1995; Bramham 1998; Karlsson and Blumberg 2004; Penttonen et al. 1997). DWs were highly variable, with a mean amplitude of 0.114 ± 0.055 mV and a half-width of 26.3 ± 7.8 ms. Dentate spikes in vivo have durations on the same order of magnitude but, as might be expected, are usually larger. Closer inspection revealed that waves recorded in the granule cell layer were consistently associated with multiple spike action potentials, especially on the initial descending phase of the wave (Fig. 1, B and C). The latter observation suggests that DWs are excitatory events and, in agreement with this, spontaneous depolarizing currents occurred in synchrony with the extracellular waves, as evidenced by simultaneous intracellular and extracellular recordings (Fig. 1D). The waves were not restricted to any particular subregion but instead were evident across the full curve of the dentate gyrus. Moreover, simultaneous recordings showed that the waves were temporally coupled in the inner and outer blades of the dentate gyrus (Fig. 1, E and F). Correlation coefficients exceeded 0.60 in 63% of the 935 3-s-long records that were analyzed for a group of seven slices obtained from five rats. Negative-going potentials in the outer blade usually (91%) preceded temporally related activity in the inner blade in the subset of recordings with correlation coefficients > 0.60; the average delay in these instances was 7.8 ± 5.6 ms (median: 6.8 ms). Dentate spikes in the outer leaf also tended to be larger than corresponding spikes in the inner leaf, although this difference did not reach statistical significance (Fig. 1F).
The size of the DWs and their presence throughout the dentate gyrus indicate that a significant portion of the granule cell population, and therefore a comparable proportion of the mossy fibers, was active during each wave. From this it would be anticipated that the dentate events would be correlated with the activity of CA3 pyramidal cells. Figure 1 shows simultaneous field recordings from the granule cell layer and the CA3b pyramidal cell layer. The events seen in the latter region correspond to the previously described hippocampal slice sharp waves (SPWs); as is evident, the DW aligned with a large SPW. Smaller SPWs did not correspond with dentate events. Averaged results for over 400 DWs are summarized in Fig. 1H. A large SPW accompanied the DW in almost every case with the average peak of the CA3b events occurring 3.8 ms later than the peak of the dentate event. In all, most SPWs occur spontaneously but some larger SPWs appear to be triggered by DWs.

**Origins of DWs**

Laminar profile analyses showed that DWs formed a dipole with its positive end in the dentate outer molecular layer and its negative end in the dentate inner molecular layer.
negative pole in the granule cell layer and/or inner molecular layer (Fig. 2). Although their dendritic arbors are oriented in opposite directions, similar laminar profiles were obtained in the internal and external blades of the dentate gyrus. This, together with the phase reversal in each case, establishes that DWs were locally generated (i.e., not due to volume conduction from outside the dentate). With their maximal amplitude near the granule cell layer and phase reversal in the outer third of the molecular layer, these laminar profiles resemble those for one of the two types of dentate spikes recorded in vivo (i.e., DS1 of Bragin et al. 1995). However, the waves recorded here exhibited opposite polarity to the in vivo spikes. That is, DWs were negative-going in the hilus and inner molecular layer, whereas DS1 spikes in vivo are positive-going in these regions. These discrepancies could be due to a number of technical differences (e.g., 3-dimensional fields in vivo vs. essentially 2-dimensional fields in vitro) but could also indicate that the slice events are a simplified version of the dentate spikes recorded from behaving animals. In any event, the differences in laminar profiles prompt the use of a separate term for the slice potentials (i.e., DWs).

Two questions regarding the origins of spontaneous DWs emerge from the above observations: 1) what is the source of the depolarizing bias needed to trigger the events and 2) how do the waves become synchronized across the considerable extent of the dentate gyrus? Efforts to address the first of these issues began with compounds that block the AMPA-type glutamate receptors that mediate excitatory input to the granule cell layer and/or inner molecular layer (Fig. 2). Although their dendritic arbors are oriented in opposite directions, similar laminar profiles were obtained in the internal and external blades of the dentate gyrus. This, together with the phase reversal in the dendritic fields in each case, establishes that DWs were locally generated (i.e., not due to volume conduction from outside the dentate). With their maximal amplitude near the granule cell layer and phase reversal in the outer third of the molecular layer, these laminar profiles resemble those for one of the two types of dentate spikes recorded in vivo (i.e., DS1 of Bragin et al. 1995). However, the waves recorded here exhibited opposite polarity to the in vivo spikes. That is, DWs were negative-going in the hilus and inner molecular layer, whereas DS1 spikes in vivo are positive-going in these regions. These discrepancies could be due to a number of technical differences (e.g., 3-dimensional fields in vivo vs. essentially 2-dimensional fields in vitro) but could also indicate that the slice events are a simplified version of the dentate spikes recorded from behaving animals. In any event, the differences in laminar profiles prompt the use of a separate term for the slice potentials (i.e., DWs).

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cells (Fig. 3). The antagonist CNQX caused spike frequency to decrease from 0.61 ± 0.34 to 0.08 ± 0.18 events/s (n = 3 rats; P < 0.05; Fig. 3A). Individual DW area, measured for the few waves that did arise, also dropped significantly after CNQX infusion (P < 0.02; Fig. 3B). As CNQX did not differentially affect activity in the inner leaf versus outer leaf of the dentate, the results presented are an average of the two areas. These data point to the conclusion that excitatory synaptic input to the granule cells mediated by AMPA-type glutamate receptors provided the requisite depolarization to initiate a dentate wave.

The perforant path provides the major excitatory input to the dentate gyrus and lesions to its origins in the entorhinal cortex are reported to eliminate dentate spikes in vivo (Bragin et al. 1995). Responses in the dentate gyrus molecular layer could be elicited by stimulation of the entorhinal cortex in slices that exhibited DWs (Fig. 4), raising the possibility that spontaneous activity in the dentate gyrus is driven by the entorhinal cortex. However, very high stimulation intensities were consistently required to evoke responses of this kind, and it is therefore possible that the responses resulted from stimulation current spreading to severed perforant path fibers closer to the recording site. Nevertheless, if it were indeed the case that the entorhinal cortex remained functionally connected to the dentate gyrus in these slices, cutting perforant path axons would be expected to reduce spontaneous spike-mediated excitatory input to the granule cells. However, separating the retrohippocampal area from the dentate gyrus with a knife cut to the slice did not significantly reduce the size or frequency of DWs (Fig. 5, A and B). While this rules out an essential role for synchronously evoked release from the perforant path as a driving force for dentate waves, it remains possible that spontaneous release from these afferent areas provides the depolarization needed for DW generation. This was tested in a group of slices prepared from rats in which the entorhinal cortex had been lesioned ≥5 days prior to the experiment, with the idea being that chronic lesions of the entorhinal cortex would eliminate miniature excitatory postsynaptic currents (mEPSCs) associated with spontaneous release of glutamate from perforant path terminals. Silver impregnation of terminal degeneration confirmed deafferentation of the outer molecular layer in both blades of the dentate gyrus (Fig. 5C). DW frequency was substantially lower in slices from five lesioned rats relative to an equal number of yoked controls: outer leaf: 0.23 ± 0.27 versus 0.71 ± 0.41 spikes/s (P < 0.04, 1-tailed t-test); inner leaf: 0.32 ± 0.27 versus 0.65 ± 0.25 spikes/s (P < 0.05, 1-tailed t-test; Fig. 5D). These effects accord with the hypothesis that spontaneous glutamate release from the perforant path contributes to spike generation, but it is noteworthy that the magnitude of the decreases in the partially deafferented slices was substantially smaller than that obtained with CNQX. This suggests that release from the dentate commissural/associational projections, the glutamatergic afferents of the granule cells remaining after entorhinal lesions, was sufficient to initiate DWs, albeit at a much reduced rate.

While removal of the perforant path reduced the frequency of DWs, it did not greatly affect their size. The average area of individual events, which takes into account both amplitude and duration, was comparable in control and deafferented slices (Fig. 5E). However, the correlation between activity recorded from the internal and external blades of the dentate gyrus was significantly diminished in the lesion group (P < 0.04; 2-tailed t-test; Fig. 5F). This is not unexpected as removal of a depolarizing bias from the perforant path would reduce the likelihood that any given group of granule cells would discharge in response to the arrival of a dentate population spike; thus the chances that propagation will fail at some point between two distant recording loci should be higher in the deafferented slices.

**DW propagation**

There is evidence that SPWs in field CA3 spread via the extremely dense associational projections between the pyramidal neurons in that region (Ciscsvari et al. 2000; Kubota et al. 2003). It is possible that the sparser associational projections of the dentate gyrus allow for similar synchronization of granule cell activity. However, the latter system is indirect in that it involves a synapse with cells that lie immediately beneath the granule cell layer in the polymorphic region of the dentate gyrus (Ribak et al. 1985); thus there is substantial distance and additional synaptic delay between a granule cell that is spiking and a second granule cell that receives (delayed) excitatory feedback. The distance is increased further by the trajectory of the dentate associational axons. These projections curve around the free end of the internal blade of the granule cell layer and then course along the long axis of the dentate gyrus within the inner molecular layer before terminating at a distance ranging from several hundred micrometers to 2–3 mm away.
from their cells of origin (Amaral and Witter 1989; Laurberg and Sorensen 1981; Swanson et al. 1978). As long projections are not likely to remain within the plane of a 350-μm-thick acute hippocampal slice, most of the di-synaptic associational projections will have been severed in the slices used in the present experiments. As expected from this, cuts through the associational system near the free end of the inner blade of granule cells did not significantly alter the frequency or size of DWs distal to the cut or reduce the temporal correlation between events in the two blades of the dentate (Fig. 6A).

An alternative explanation is that DWs are propagated electrotonically by gap junctions between granule cells (i.e., electrotonic conduction; MacVicar and Dudek 1982). This would predict that physical separation of the internal and external blades of the granule cell layer would eliminate correlated spiking activity on the two sides of the separation. Accord-
ingly, after confirming that DWs were present during a baseline period of recording, cuts extending through s. granulosum and the full molecular layer were placed at the crest of the dentate gyrus in a group of slices from three rats. As predicted, the correlation between recordings in the inner and outer blades of the dentate fell sharply after the transection and did not recover (0.64 average correlation coefficient for 10-min baseline; 0.25 average correlation coefficient for the period 50–60 min after cut). While there was no evident difference between DW frequency in the inner leaf and outer leaf during the 10-min baseline (0.76 ± 0.30 vs. 0.79 ± 0.29 spikes/s, respectively), DWs in the inner leaf were observed much less frequently than their outer leaf counterparts after the transection. At 50–60 min after cut, the rate of DWs in the inner leaf was significantly reduced to 0.26 ± 0.29 spikes/s (P < 0.03), while the rate in the outer leaf did not significantly change (0.78 ± 0.77 spikes/s at 60 min after cut). Since DWs occurred significantly less frequently in the inner leaf relative to the outer leaf after the transection, the reduced correlation between activity in the inner and outer blades may have merely been due to the reduced incidence of DWs in the inner leaf. For this reason, the correlation between the two areas was re-examined for the subset of recordings in which DWs did occur in the inner leaf and was still found to be lower than baseline (0.31 average correlation coefficient). The areas of individual DWs were slightly reduced compared with baseline at 1 h after transection in all three cases (on average, 67% of baseline area for inner leaf and 73% of baseline area for outer leaf), but these effects did not reach statistical significance. Representative traces from both blades of s. granulosum prior to and 1 h after transection are shown in Fig. 6B.

The electrotonic conduction hypothesis necessarily predicts that gap junction inhibitors will reduce the spread and hence the frequency of DWs. This was confirmed with the potent inhibitor carbenoxolone which, as shown in Fig. 7, A–D, decreased the rate of spontaneous activity in both blades of the dentate gyrus in slices from five rats. Incidence in the inner leaf fell from 0.66 ± 0.47 to 0.33 ± 0.33 events/s (P < 0.05) and in the outer leaf from 0.70 ± 0.31 to 0.31 ± 0.35 DW/s (P < 0.03) at 30–40 min after the start of infusion. Carbenoxolone also caused an ∼50–60% decrease in the size of the spikes in both areas (P < 0.05; Fig. 7E) and eliminated the correlation between activity in the inner and outer blades (r = 0.61 during 10-min baseline vs. 0.38 during 30–40 min after start of infusion; Fig. 7F). The selectivity of carbenoxolone with regard to gap junctions has been questioned (Rouach et al. 2003) and, in agreement with these arguments, the compound reduced the monosynaptic perforant path response in the dentate gyrus by ∼30%. Thus results with carbenoxolone confirm a basic prediction of the electrotonic hypothesis but cannot be interpreted beyond this.

Gap junction conduction is reportedly enhanced under alkaline conditions (Church and Baimbridge 1991; Schweitzer et al. 2000; Spray et al. 1981). Thus if dentate waves spread electrotonically through gap junctions, it would be expected that their production would be facilitated by increasing extracellular pH. Figure 8 shows data from a group of slices in which ACSF with a relatively high pH (∼7.6) was infused for a 30-min period following an initial baseline during which slices were bathed in normal ACSF with a pH of ∼7.3. The rate of DW occurrence increased from 0.47 ± 0.18 spikes/s during the 10-min baseline to 0.59 ± 0.20 spikes/s for the 20–30 min after infusion of the high pH ACSF began (n = 4; P < 0.02; Fig. 8A). DW area also increased significantly to 16.00 ± 6.44% above baseline measurements (P < 0.02) after extracellular pH was raised (Fig. 8B). These data lend additional support to the hypothesis that DW production involved electrical coupling via gap junctions.

Although it appears that electrical coupling between granule cells was responsible for the spread of excitation across the granule cell population during DWs, the results with CNQX described above indicate that excitatory synaptic transmission was also essential for DW production. If gap junctions were solely responsible for generation of DWs, lowering extracellular calcium concentration would be expected to increase DWs because high levels of calcium are reported to inhibit gap junction function (Lazrak and Peracchia 1993; Perez-Velazquez et al. 1994). However, this did not occur. It appears instead that DWs were maximal when calcium concentration was relatively high. DWs virtually disappeared when the concentration of calcium in the ACSF was lowered from 3 to 1–2 mM (Fig. 9). This finding would not be unexpected if, as hypothesized above, DW production relies on spontaneous release of glutamate from dentate afferents. That is, spontaneous release of neurotransmitter would be maximized by high levels of calcium, and DWs would be enhanced as a result. In support of this, drugs that depend on spontaneous release of neurotransmitter (e.g., acetylcholinesterase inhibitors) are most effective when calcium levels are ≥3 mM (Colgin and Lynch, unpublished observations).

![Figure 5](http://jn.physiology.org/DownloadedFrom/jn.org)
Activity within hippocampal slices is suppressed by cholinergic Regulation by cholinergic and GABAergic synapses

Previous studies have shown that endogenously generated activity within hippocampal slices is suppressed by cholinergic afferents arising in the septum (Kubota et al. 2003). Tests of whether this also holds for DWs were carried out by infusing the acetylcholinesterase inhibitor physostigmine to enhance the effects of spontaneous release at cholinergic synapses in a group of five slices. As shown in Fig. 10A, physostigmine (5 μM) had a small and slow-to-develop effect on the frequency of DWs; the mean rate went from 1.15 ± 0.67 events/s during a 10-min baseline to 0.86 ± 0.65 events/s at 60–70 min after the start of drug infusion (P < 0.05). The effects of physostigmine on spike size were more rapid and reliable. The average area of the DWs began to decrease within 10 min of infusion and by 30 min reached a minimum value that was 65 ± 26% of the starting baseline (P < 0.04; Fig. 10B). There were no evident differential effects of the drug on inner versus outer blades of the dentate. Example recordings obtained before and after physostigmine infusion are shown in Fig. 10C.

Antagonists of GABA_A receptors given at low doses increase SPWs in vivo (Buzsaki 1986; Suzuki and Smith 1988), but it is not known if GABAergic transmission affects dentate spike generation. The next experiment was conducted to test if inhibitory transmission was involved in DW production in slices (Fig. 11). The GABA_A antagonist picrotoxin (PTX) reliably decreased the rate of DW occurrence from 0.98 ± 0.80 events/s during a 10-min baseline to 0.15 ± 0.24 DWs/s during the period 20–30 min following the start of PTX infusion (n = 5; P < 0.03; Fig. 11A). DW area was also significantly decreased to 14.87 ± 22.54% of baseline values (P < 0.001) by the end of the PTX application period (Fig. 11B). These results suggest that initiation of the depolarizing dentate wave depends on 1) a given level of membrane polarization, 2) low levels of spiking by the granule cells, or 3) interactions between granule cells and interneurons beyond those involving the first two points. These possibilities are not exclusive.

**DISCUSSION**

This study describes the presence of spontaneous dentate waves in slices prepared from temporal hippocampus. The slice activity had frequency and waveform characteristics comparable to those reported for dentate spikes in freely moving rats during awake immobile behaviors and slow-wave sleep (Bragin et al. 1995; Bramham 1998; Karlsson and Blumberg 2004; Penttonen et al. 1997). The nearly monophasic slice potentials were accompanied by depolarizing currents and action potentials, which is also consistent with in vivo reports (Penttonen et al. 1997). Moreover, the occurrence of dentate waves in vitro in this study was greatly reduced by prior ablation of the entorhinal cortex, and dentate spikes in vivo have also been reported to be eliminated after entorhinal cortex lesions (Bragin et al. 1995). Still, several important differences exist between the activity reported here and previous reports of dentate spikes in vivo. As described above, the laminar profiles are not identical, although this could be due to technical differences between slice versus intact recording. Perhaps more importantly, SPWs were never observed within 200 ms after dentate spikes in simultaneous recordings from the dentate gyrus and CA1 pyramidal cell layer of awake rats (Bragin et al. 1995). This contrasts sharply with the present finding that spontaneous waves in the dentate gyrus of slices were consistently followed by SPWs in CA3. It should be noted that dentate spikes in vivo were recorded from the dentate gyrus of

**FIG. 6.** A: severing the associational axons near the outer edge of the inner leaf did not prevent generation of spontaneous DWs. Example records are shown for the dentate inner leaf (top) and outer leaf (bottom) during baseline conditions (left) and ~1 h after the scalpel cut (right). Calibration bars: 500 ms, 0.1 mV. B: physically cutting the connection between the inner and outer leaves of the DG reduced spontaneous waves in s. granulosum, particularly in the inner leaf. Example recordings from the inner leaf during the initial control period (top left) and ~60 min after the microdissection (top right) are shown. DWs were minimal in the latter. Representative traces from the outer leaf during an initial control period (bottom left) and ~1 h after the cut are shown (bottom right). In the surgically isolated outer blade, DWs continued to arise at a fairly high rate. Note that while activity was correlated in the 2 regions during the control period, little to no dentate waves were observed to co-occur in the inner and outer blades postcut. Scale bars: 500 ms, 0.05 mV. C: photomicrograph of a section from ventral hippocampus is shown to illustrate the cuts made for the experiments depicted in A and B. Approximate location of the cut made through the associational axons at the free end of the inner leaf is shown (data shown in A) and indicated by A. Approximate location of the cut made to physically separate the inner and outer blades of the dentate gyrus (data shown in B) is shown and marked with a B.

**Regulation by cholinergic and GABAergic synapses**

Previous studies have shown that endogenously generated activity within hippocampal slices is suppressed by cholinergic afferents arising in the septum (Kubota et al. 2003). Tests of
the dorsal (i.e., septal) hippocampus, whereas the presently described activity was recorded from the dentate gyrus of slices prepared from ventral (i.e., temporal) hippocampus. Thus it is possible that the in vivo/in vitro discrepancies may be related to anatomical and/or functional differences between ventral and dorsal hippocampus (Witter and Groenewegen 1984; Moser and Moser 1998). In any case, further investigation is required to determine if and how the in vitro waves correspond to spontaneous dentate spikes in vivo.

Similar activity has been reported to occur spontaneously in the dentate gyrus granule cell layer of slices from mouse hippocampus that exhibit sharp wave-ripple complexes in the pyramidal cell fields (Maier et al. 2003). Activity of this kind has not been described previously for rat hippocampal slices, possibly in part because slices are usually prepared from the more dorsal portion of hippocampus. Detection of spontaneous waves in the dentate is further complicated by their small size. The average amplitude of the events described here is ~0.1

**FIG. 7.** The gap junction antagonist, carbenoxolone (100 μM), suppressed spontaneous DWs in s. granulosum. A and B: DWs occurred significantly less frequently in the inner (A) and outer (B) leaves of the DG following a 30-min application of carbenoxolone in slices taken from 5 animals. C: sample recordings from the inner leaf prior to (top) and at the end of (bottom) the drug application period. D: simultaneous recording from the outer leaf from the same time periods shown in C. A DW is visible in the bottom, which was recorded 30 min after the start of carbenoxolone infusion. The drug’s effect did not reach its maximum until ~30 min later. (Calibration for C and D: 500 ms, 0.1 mV). E: the normalized area of DWs was decreased by application of the gap junction inhibitor. F: correlation between activity in the inner and outer blades declined substantially during and for some time after carbenoxolone application.
waves are dependent on glutamatergic transmission because they were virtually eliminated by the AMPA receptor antagonist CNQX. By exclusion, these results point to the conclusion that spontaneous release of glutamate from severed excitatory afferents in the molecular layer provides the depolarization needed to initiate DWs. The markedly reduced population activity in slices from rats in which the perforant path afferents to the dentate had been removed is in accord with this hypothesis. Furthermore, DWs were maximal at a relatively high concentration of extracellular calcium, a condition that increases spontaneous neurotransmitter release.

DWs were found along the entire arc of the dentate gyrus, with near synchrony commonly observed between electrodes separated by several millimeters. The entorhinal cortex could drive both areas by sending synchronized inputs to the full medio-lateral extent of the dentate gyrus but, as noted above, knife sections through the perforant path did not affect DWs. This points to the surprising conclusion that the waves can propagate across sizeable distances within the dentate gyrus. It is difficult to envision how this rapid spread of activity occurs in the slice preparation. The commissural-associational projections of the dentate could in principle propagate activity, but the disynaptic and longitudinally oriented circuitry (Amaral and Witter 1989; Laurberg and Sorensen 1981; Swanson et al. 1978) is unlikely to be preserved within a slice. Moreover, the waves were often initiated in the outer leaf and were unaffected by cuts made to the associational axons at the free end of the inner leaf, findings that do not support a major contribution from the associational pathway. This leaves electrical coupling via gap junctions between granule cells (Durand et al. 1983; MacVicar and Dudek 1982; Schmitz et al. 2001; Yamamoto et al. 1989) as the likely substrate of propagation. Synchronization of activity in the granule cell population via electrotonic conduction has been previously proposed to underlie pathological field bursts (Schweitzer et al. 2000) and transmission of high-frequency spikelets (Schmitz et al. 2001) in the dentate gyrus. In this study, separating the blades of the granule cell layer blocked coordinated activity between cells on either side of the cut while the gap junction inhibitor carbenoxolone suppressed DWs throughout the dentate gyrus. Additionally, gap junction conductance is increased by alkalinization (Church and Baimbridge 1991; Schweitzer et al. 2000; Spray et al. 1981), and DWs were enhanced when the ACSF pH was raised from 7.3 to 7.6.

The electrotonic hypothesis for propagation could also help explain the initiation of the spikes. That is, electrically coupled cells would provide the recruitment needed to convert spiking in a few cells into a population event of sufficient magnitude to generate a field potential. By this argument, spontaneous transmitter release would, with some low probability, cause nearby granule cells to spike within a narrow time window; these cells would then, by electrotonic conduction, spread the action potential depolarization to neighboring cells, generating a population spike event. Once initiated, this wave would propagate electrotonically. According to this hypothesis, the huge numbers of tightly packed granule cells, each with a dense population of excitatory synaptic inputs, offsets the low probability that spontaneous release will activate a sufficient number of neighboring neurons to create a population event. This could be achieved more easily if gap junctions were located between granule cell axon initial segments, a recently proposed idea that

![Image](https://example.com/image.png)
is supported by physiological and anatomical data (Schmitz et al. 2001).

The DWs were tightly coupled to large SPWs in field CA3 with a time delay of about 4 ms. These arrangements strongly suggest that discharges along a significant extent of the dentate gyrus (i.e., involving a large number of granule cells) caused a greater than normal collection of pyramidal neurons to fire in near synchrony and thereby generate a larger than normal SPW. Cutting the mossy fibers does not eliminate CA3 SPWs (unpublished observations), indicating that these waves are an autonomous pattern generated by spontaneous action potentials that become coordinated into a population event, which is then propagated by the CA3 associational system. However, a more detailed analysis of SPWs in the presence and absence of mossy fiber input will be needed before it can be concluded that DWs do not alter the pattern or mean size of the CA3 SPW.
events. At this point, it can only be said that the DWs alter the pattern of spontaneous population events in the adjacent field CA3.

It has been known for some time that SPWs, with few exceptions, do not co-occur with theta rhythm (Buzsaki et al. 1983; Suzuki and Smith 1987). This antagonism appears to be related, at least in part, to cholinergic activation. Walking-induced theta rhythm is abolished and replaced by SPWs in rats injected with the nonselective muscarinic antagonists atropine, while physostigmine completely suppresses SPWs under all behavioral conditions (Buzsaki et al. 1983). The present studies show that physostigmine also depresses spontaneous dentate waves in slices. It is noteworthy in this regard that enhancement of cholinergic transmission with physostigmine relies on endogenous acetylcholine release from cholinergic (i.e., septal) terminals in the dentate gyrus. Thus the same machinery that is responsible for theta rhythm generation causes the suppression

FIG. 11. Blockade of GABA<sub>A</sub> receptors eliminated DWs in a group of 5 slices. A: occurrence of DWs declined sharply following infusion of 10 µM picrotoxin (PTX). B: normalized area of DWs was similarly reduced. C: example recordings from s. granulosum during a baseline period (top) and at the end of PTX application (bottom). Calibration: 500 ms, 0.1 mV.
of DWs. Previous work established that physostigmine at the concentrations used here reduces transmitter release from the perforant path (Colgin et al. 2003). While this effect of physostigmine on dentate afferents could account for the suppression of DWs, physostigmine reduced the amplitude of the waves with small effects on frequency while removal of the perforant path had large effects on frequency but not amplitude. It is possible that a combination of cholinergic disinhibition of granule cells combined with reduced spontaneous release results in the observed pattern of depressed but still frequent DWs. However, axon terminals of basket and chandelier cells in the dentate gyrus lack the m2-type muscarinic receptors thought to be involved in acetylcholine-mediated suppression of GABA release (Hajos et al. 1998). Another possible explanation is that acetylcholine activates intracellular signaling cascades that lead to the closure of gap junctions, as has been reported to occur with other neuromodulators (i.e., serotonin, dopamine, norepinephrine; Rorig and Sutor 1996), and through this route, reduces DWs.

That the GABA_α antagonist picrotoxin markedly reduced the size and frequency of DWs was unexpected since the antagonist increases firing frequency and thus would be expected to increase the likelihood of a population event. There is a class of interneurons that fire preferentially during dentate spikes in vivo, the hilar interneuron innervating commissural and associational pathway terminal field (HICAP) interneurons (Han et al. 1993; Penttonen et al. 1997; Sik et al. 1997). HICAP interneurons show maximal probability of discharge immediately prior to the peak of extracellular dentate spikes, so it is possible that their activation is important for dentate spike generation in vivo and also for DW production in slices. However, the axon collateral system of these interneurons courses a substantial distance (several millimeters) along the septotemporal axis of the dentate gyrus (Sik et al. 1997) and thus would not be expected to be preserved in a slice. There is also evidence to suggest that GABA_α activation increases excitability of pyramidal cell axons in hippocampus (Traub et al. 2003), raising the possibility that similar actions on granule cell axons promote the spread of dentate wave activity via gap junctions.

What, if any, functions are served by endogenous DWs? SPWs have been hypothesized to promote the encoding of long-term potentiation (Buzsáki et al. 1987b; King et al. 1999), but recent work shows that long-term potentiation (LTP) induced by theta burst stimulation does not consolidate in their presence (Colgin et al. 2004). It is well established that brief periods of low-frequency afferent activity can reverse recently encoded LTP (Barrionuevo et al. 1980; Larson et al. 1993; Staubli and Scafidi 1999; Straube and Frey 2003); this suggests the possibility that dentate waves enhance endogenous SPW oscillations in CA3 that perform a function of this type. Since cholinergic inputs suppress both the dentate waves and SPWs, it would appear that the proposed LTP-reversing process could only operate during behavioral states in which the cholinergic septo-hippocampal projections are relatively quiescent. Much evidence indicates that this occurs during waking in periods in which locomotor movement and interactions with the environment are minimal (Dudar et al. 1979; King et al. 1998; Leung and Vanderwolf 1980; Vanderwolf 1988). This accords well with the ‘awake immobility’ state in which dentate spikes and SPWs are found in vivo (Buzsáki 1986; Penttonen et al. 1997).

The above arguments lead to the hypothesis that episodes of low versus high levels of septal activity set up dentate gyrus states that in many respects have opposite consequences. High levels of cholinergic activity reduce perforant path glutamate release, but this can be overcome with frequency facilitation (Colgin et al. 2003), resulting in a dentate gyrus that is selectively responsive to rhythmically patterned excitatory input. It is intriguing in this regard that the activation of ascending cholinergic projections generates cortical rhythms (theta, beta, gamma) with periods appropriate to, as well as patterns of activity (theta bursting) that incorporate, frequency facilitation (Stewart et al. 1992). Conversely, low levels of septal afferent activity would be expected to release the block on perforant path transmission, generating a depolarizing bias that allows the dentate gyrus to respond to aperiodic inputs and to generate dentate waves. The consequence of high septal activity will be cholinergic rhythms within the hippocampal formation thought to be needed for information processing (beta, gamma) or memory encoding (theta bursting), while that of low activity will be a pattern that reduces recently induced changes in synaptic strength. Thus depending on the behavior of the animal and the activity of the ascending cholinergic projections, the hippocampus will switch between processing, encoding, or erasing modes.

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References


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