Epileptiform Discharges With In-Vivo-Like Features in Slices of Rat Piriform Cortex With Longitudinal Association Fibers

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Demir, Rezan, Lewis B. Haberly, and Meyer B. Jackson. Epileptiform discharges with in-vivo-like features in slices of rat piriform cortex with longitudinal association fibers. J Neurophysiol 86: 2445–2460, 2001. Brain slices serve as useful models for the investigation of epilepsy. However, the preparation of brain slices disrupts circuitry and sever axons, thus complicating efforts to relate epileptiform activity in vitro to seizure activity in vivo. This issue is relevant to studies in transverse slices of the piriform cortex (PC), the preparation of which disrupts extensive rostral-caudal fiber systems. In these slices, epileptiform discharges propagate slowly and in a wavelike manner, whereas such discharges in vivo propagate more rapidly and jump abruptly between layers. The objective of the present study was to identify fiber systems responsible for these differences. PC slices were prepared by cutting along three different nearly orthogonal planes (transverse, parasagittal, and longitudinal), and epileptiform discharges were imaged with a voltage-sensitive fluorescent dye. Interictal-like epileptiform activity was enabled by either a kindling-like induction process or disinhibition with bicuculline. The pattern of discharge onset was very similar in slices cut in different planes. As described previously in transverse PC slices, discharges were initiated in the endopiriform nucleus (En) and adjoining regions in a two-stage process, starting with low-amplitude “plateau activity” at one site and leading to an accelerating depolarization and discharge onset at another nearby site. The similar pattern of onset in slices of various orientations indicates that the local circuitry and neuronal properties in and around the En, rather than long-range fibers, assume dominant roles in the initiation of epileptiform activity. Subtle variations in the onset site indicate that interneurons can fine tune the site of discharge onset. In contrast to the mode of onset, discharge propagation showed striking variations. In longitudinal slices, where rostral-caudal association fibers are best preserved, discharge propagation resembled in vivo seizure activity in the following respects: propagation was as rapid as in vivo and about two to three times faster than in other slices; discharges jumped abruptly between the En and PC; and discharges had large amplitudes in superficial layers of the PC. Cuts in longitudinal slices that partially separated the PC from the En eliminated these unique features. These results help clarify why epileptiform activity differs between in vitro and in vivo experiments and suggest that rostral-caudal pyramidal cell association fibers play a major role in the propagation of discharges in the intact brain. The longitudinal PC slice, which best preserves these fibers, is ideally suited for the study of their role.

INTRODUCTION

The piriform cortex (PC) is highly susceptible to seizure activity and is thought to play an important role in temporal lobe epilepsy (Croucher et al. 1988; De Curtis et al. 1994; Hoffman and Haberly 1989; McIntyre and Wong 1986; Piredda and Gale 1985; Stevens et al. 1988). Experiments in transverse slices prepared from the PC have shown that this seizure susceptibility is preserved in vitro (Hoffman and Haberly 1989; McIntyre and Wong 1986). In these slices, epileptiform discharges originate from a distinct focus that generally includes the endopiriform nucleus (En), situated at the deep boundary of the PC (Demir et al. 1998c; Hoffman and Haberly 1991, 1996). This is consistent with the finding that injections of drugs into the deep boundary of the PC have a profound impact on seizure activity (Croucher et al. 1988; Piredda and Gale 1985, 1986; Stevens et al. 1988). This similarity would suggest that transverse PC slices retain essential properties relevant to epileptogenesis. However, there are also important differences. Current source density analysis and intracellular recording in vivo indicate that superficial pyramidal cells in the PC can generate high-amplitude interictal-like epileptiform discharges and that discharges in the PC undergo abrupt transitions between layers (Haberly and Sutula 1992). By contrast, in transverse PC slices epileptiform discharges in the superficial layers are much smaller (Demir et al. 1998c), appear to be passively driven by the En, and propagate in a wavelike matter to superficial layers of the overlying PC and neighboring neocortex (Demir et al. 1999; Hoffman and Haberly 1991). It has been noted that epileptiform discharges generally spread through the intact brain much more rapidly than in slices (Albowitz and Kuhn 1995). This is also true for the PC: in vivo discharges propagate with a velocity of ~0.3 m/s (Ketchum and Haberly 1992), corresponding well with action potential conduction velocities in association fibers. In transverse PC slices, the conduction velocity is <0.2 m/s (Demir et al. 1998c; Hoffman and Haberly 1993), which is too slow for action potential conduction and indicates propagation by different mechanisms (Traub et al. 1987).

These differences between in vivo and in vitro studies of epilepsy must reflect some form of damage that occurs when brain slices are prepared. Many long-range axonal projections are severed during slice preparation, and the PC contains a number of fiber systems that could suffer this fate. The pyramidal cells of the PC project over considerable distances along the rostral-caudal axis (Haberly 1998; Haberly and Price 1978;
Johnson et al. 2000; Luskin and Price 1983), with trajectories that cross the plane of section of transverse slices. In the En, deep cells are connected by an extensive fiber system with a predominant rostrocaudal orientation (Behan and Haberly 1999).

The objective of the present study was to investigate the roles of these fiber systems in the generation and propagation of epileptiform discharges by preparing PC slices cut along different planes. Slices were cut along a rostrocaudal plane to improve the preservation of extensive rostrocaudal projections in the PC and En mentioned in the preceding text. Interictal-like epileptiform discharges were elicited by making use of two different in vitro models of epilepsy and were visualized using a voltage-sensitive fluorescent dye. In slices cut along parasagittal and longitudinal planes, discharge initiation followed the same basic sequence described previously in slices cut along a transverse plane (Demir et al. 1999) with relatively minor differences in the site of onset. However, dramatic differences were observed in the mode and velocity of propagation. Epileptiform discharges in longitudinal slices propagated rapidly, jumped abruptly between layers, and generated large depolarizations in the superficial layers of the PC. In these respects, discharge activity in longitudinal slices resembled discharge activity in the intact PC. These findings indicate that rostrocaudal association fibers preserved in longitudinal slices play an important role in the propagation of epileptiform discharges in the intact brain and suggest a circuit in which long-range association fibers and inhibitory neurons dictate the directions and set the timing for discharge initiation and spread. A preliminary account of this work has been presented (Demir et al. 1998b). Ictal-like activity in rostrocaudally oriented slices has also been examined (Demir et al. 1998a) and will be reported subsequently.

**METHODS**

**Piriform cortex slices**

Male Sprague-Dawley rats (175–250 g) were killed by decapitation while under CO₂-induced narcosis. The brain was removed and immersed in ice-cold physiological saline [which contained (in mM) 124 NaCl, 5 KCl, 26 NaHCO₃, 1.2 KH₂PO₄, 2.4 CaCl₂, 1.3 MgSO₄, and 10 glucose] bubbled with 95% O₂-5% CO₂ (carbogen). Slices of PC 350-μm thick were cut with a Vibratome in three different planes, defined as transverse, parasagittal, or longitudinal (Fig. 1A). Transverse slices, as described previously, were cut along a near coronal plane, perpendicular to the cortical surface (Demir et al. 1998c; Hoffman and Haberly 1989). Figure 1B shows a Nissl-stained transverse slice with - - - indicating the planes of section for parasagittal and longitudinal slices.

Previous imaging studies in transverse PC slices identified two sites where important forms of latent period activity were seen prior to discharge onset (Demir et al. 1998c, 1999, 2000). These sites are indicated by shaded outlines in the inset of Fig. 1B. Examples and descriptions of these forms of activity are presented in RESULTS (see Figs. 2 and 3). Plateau activity appears in the lateral boundary region of the En (lbr) and includes a part of layer III of the PC immediately adjacent to the En. Onset activity appears in the dorsal boundary region of the En (dbr) and includes part of the adjoining deep layers of neocortex. Parasagittal slices were cut parallel to the sagittal plane (Fig. 1B, - - - ) and selected to contain both the sites of plateau and onset activity (both lie in the same parasagittal plane; Fig. 1B, inset). These slices contained essentially the entire rostrocaudal extent of the En, all layers of the PC, and the lateral boundary region of the En (the site of plateau activity). Because we used slices cut through the center of the En, there was little if any of the onset site seen in other slices (the dorsal boundary region of the En). The reason for taking this plane approximately orthogonal to the cortical surface is that it optimized the preservation of both vertical and rostrocaudal components of association fibers.

At the end of an imaging experiment, slices were preserved by fixation in 4% formaldehyde, sectioned at 60 μm with a freezing microtome, and counterstained with cresyl violet for histological analysis.

**Epileptiform activity**

Two different in vitro models were used to generate interictal-like discharges, disinhibition and induction (Demir et al. 1998c). The disinhibition model involved blockade of GABA_A receptor-mediated inhibition by bath application of 5 μM bicuculline methiodide (Sigma, St. Louis, MO) while recordings were in progress. The induction model involved subjecting slices to a 60- to 90-min period of spontaneous bursting at 34°C by replacing 93% of the Cl⁻ in the bathing medium with isethionate. Slices were then returned to normal physiological saline for recordings. Slices treated in this manner showed a change in excitability that persisted for as long as slices were maintained (≤7 h) and is thought to be mediated by an NMDA receptor-dependent process (Hoffman and Haberly 1989; Stasheff et al. 1989).

Epileptiform discharges were triggered by electrical stimulation with 0.2-ms current pulses supplied by a stimulus isolator through a saline-filled glass micropipette.

**Voltage imaging**

The voltage-sensitive fluorescent dye RH414 (Molecular Probes, Eugene, OR) was used to image voltage. Slices were stained in RH414 (200 μM, in carbogen-bubbled physiological saline) for ~45 min. A 464-element photodiode-fiber optic camera (Chien and Pine 1991) attached to an epifluorescent microscope equipped with a Zeiss Fluar ×5 objective (NA = 0.25) and a 100-W tungsten-halogen light source was used to record fluorescence. The optical and electronic instrumentation have been described in detail previously (Demir et al. 1998c; Wu and Cohen 1993). Optical signals were high-pass filtered with a 500-ms time constant and low-pass filtered with a corner frequency of 500 Hz. Visual records of each slice were made with a CCD-camera using trans-illumination, and read into the computer with a frame-grabber.

**Data acquisition and analysis**

The computer program Neuroplex (RedShirtImaging, Fairfield, CT) was used for data acquisition and analysis. Imaging data were acquired at 0.944-ms per frame. Additional computer programs written in IDL (Research Systems, Boulder CO) served to make overlays of optical signals and intensity contours onto video images. As described previously (Demir et al. 1998c), the contours for the site of discharge onset indicate the earliest detector fields where the fluorescence change had surpassed 50~70% of its maximum amplitude. The contours for plateau activity were prepared manually by marking detector fields that clearly showed a sustained depolarization above the baseline noise (Demir et al. 1999). Color maps were prepared by scaling the fluorescence of each trace to its own range of fluorescence. This normalized signal was encoded as color for display as a sequence of images in which the spatiotemporal pattern of discharge development could be visualized. Propagation velocities were measured for epileptiform discharges by plotting the distance from site of onset versus time at which fluorescence at that site reached half its maximum change. Velocity was taken as the slope of the best-fitting line. These
velocities were only measured in discharges that advanced in a wave-like front (see RESULTS), so that the distance versus time plots were linear (e.g., Fig. 15 of Demir et al. 1998c).

**RESULTS**

To analyze the roles of rostrocaudally oriented fiber systems, two different planes of section were employed in which these systems are better preserved than in transverse slices: parasagittal slices cut parallel to the midplane and longitudinal slices cut rostrocaudally in a plane that was angled to lie approximately perpendicular to the cortical surface (see METHODS). In these slices, anatomical structures have different spatial relationships, as indicated in Fig. 1. In longitudinal slices, the En appears as an extended ribbon rather than the small spot seen in transverse slices; a long parallel expanse of overlying cortex comprises all three layers of the PC (Fig. 3A). In parasagittal slices, the En is obliquely cut so that it is larger than in transverse slices but considerably smaller than in longitudinal slices (Fig. 2A). Parasagittal slices have the advantage of preserving the dorsal boundary region between the En and neocortex, which is the site of onset in induced transverse slices. But because the PC tapers in width from caudal to rostral (Paxinos and Watson 1986), long association axons are not well preserved over long distances in these slices. In contrast to both transverse and parasagittal slices, longitudinal slices contain little if any of the dorsal boundary region of the En (dbr), where discharge onset was seen, and the lateral boundary region of the En (lbr), where plateau activity was seen (Demir et al. 1999, 2000).

**Discharge initiation in longitudinal and parasagittal slices**

Interictal-like epileptiform discharges were readily evoked in parasagittal and longitudinal slices, using either the induced or disinhibited models (Figs. 2 and 3). The initiation phase of discharges in these slices closely resembled the two-stage process described previously in transverse PC slices (Demir et al. 1999, 2000). When the stimulus current was slightly above threshold for an epileptiform discharge, a discharge appeared...

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**FIG. 1.** Different planes of section of piriform cortex (PC) slices. A: a sketch of the rat brain shows the transverse, parasagittal, and longitudinal planes. AON, anterior olfactory nucleus; APC, anterior piriform cortex; PPC, posterior piriform cortex. B: a Nissl-stained transverse PC slice shows some of the anatomical structures important in this study. En, endopiriform nucleus; Cl, claustrum; AI, agranular insular cortex; LOT, lateral olfactory tract; OT, olfactory tubercle. Roman numerals indicate layers of PC. - - -, the plane of section for parasagittal and longitudinal slices (modified from Paxinos and Watson 1986). Inset: simplified sketch to illustrate the dorsal boundary region of the En (dbr), where discharge onset was seen, and the lateral boundary region of the En (lbr), where plateau activity was seen (Demir et al. 1999, 2000).
with a latency of ~100 ms. During the latent period, we saw plateau activity and onset activity, each of which was spatially confined to distinct sites with little if any overlap. Plateau activity was initiated within a few milliseconds of electrical stimulation and remained roughly constant during the entire latent period prior to discharge onset (trace 2 in each part of Figs. 2C and 3C; Fig. 2C1, parasagittal-induced; Fig. 2C2, parasagittal-disinhibited; Fig. 3C1, longitudinal-induced; Fig. 3C2, longitudinal-disinhibited). As in transverse slices (Demir et al. 1999), plateau activity remained confined to a small region during the latent period without spreading. Onset activity began as a ramp-like, accelerating depolarization roughly halfway through the latent period, culminating with the onset of the discharge (trace 1 in each part of Figs. 2C and 3C). The time of half-maximal fluorescence change at the site of onset (- - -) makes the point that the discharges at the other sites follow the discharge at the site of onset. Only after onset did discharges spread through the entire slice (see Fig. 5). Near the site of stimulation and away from the sites of onset and plateau activity, a rapidly decaying local response could be seen immediately after stimulation (trace 3 in each part of Figs. 2C and 3C). Depending on the duration of the latent period, these local responses decayed to baseline or were still decaying when the discharge reached these sites. These local responses were graded with stimulus current and were similar to those seen in control slices not exhibiting epileptiform activity (Demir et al. 1998c, 1999). By contrast, epileptiform discharges were all or none. The discharge amplitude did not change as the stimulus current increased above threshold, but the latency to onset became shorter as described previously in transverse slices (Demir et al. 1998c; Hoffman and Haberly 1989).

These results show that in slices with three different orientations (transverse, parasagittal, and longitudinal) discharges are initiated in the same basic sequence of plateau activity and onset activity. This finding indicates that this two-stage mechanism of discharge generation is robust and independent of slice orientation. Thus long-range axonal projections, which should be different in each slice orientation, do not appear to play a role in discharge initiation. However, as detailed in the following text, longitudinal slices were unique in the spatial localization of onset activity and in the spatiotemporal pattern of the ensuing epileptiform discharge.

**Sites of onset and plateau activity in parasagittal slices**

In transverse PC slices, a slightly suprathreshold electrical stimulus applied anywhere in the slice evoked epileptiform discharges originating within the En in disinhibited slices and
at the dorsal boundary of the En in induced slices (Demir et al. 1998c). In induced slices, the onset site included part of layer VI of the adjoining neocortex. The site of onset in parasagittal slices was similar but not identical to that reported previously in transverse slices. Onset activity appeared at the dorsal boundary region of the En in induced slices but did not include an adjoining part of layer VI of the neocortex (n = 7; Fig. 2B1, light gray-filled contours). In disinhibited slices onset activity also occurred at the dorsal boundary region (n = 7; Fig. 2B2, light gray-filled contours), but from trial-to-trial in the same slice, onset activity was intermittently focused centrally within the En away from the dorsal boundary. Plateau activity in transverse slices also had a highly characteristic location in the lateral boundary region of the En with involvement of layer V of the neocortex in the induced model (Demir et al. 1998, 2000). In parasagittal slices, plateau activity was seen in the lateral boundary of the En in both induced (n = 7; Fig. 2B1) and disinhibited (n = 7; Fig. 2B2) slices (dark gray-filled contours) with no involvement of the neighboring neocortex in the induced model. The sites where onset and plateau activity was seen varied only slightly from trial-to-trial within the same slice or between different slices (variation ~150–300 μm). The sites of onset and plateau activity showed no systematic shifts in location as experiments were continued for ~7 h. This low variability stood in marked contrast to the high variability seen in longitudinal PC slices (next section).

Epileptiform discharges in parasagittal slices spread from the onset site to the overlying PC. This spread can also be seen in a sequence of color maps (Fig. 5B). As in transverse slices, the amplitude attenuated as the discharge spread to the superficial layers of the PC (trace 3 in Figs. 2C, 1 and 2, and 5B). This suggests that the preservation of excitatory fiber systems in the PC of parasagittal slices is not sufficiently improved over that in transverse slices to allow regenerative discharges to develop in the PC. Dramatically different results were obtained in longitudinal slices as will be detailed in the following text.

Sites of onset and plateau activity in longitudinal slices

Longitudinal PC slices contain the lateral boundary of the En, which was the site of plateau activity in transverse and parasagittal slices. They also contain the deep central portion of the En, which was the site of onset in disinhibited transverse slices (Demir et al. 1998c, 2000) and occasionally in disinhibited parasagittal slices (results in the preceding text). However, longitudinal slices contain little if any of the dorsal boundary

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**Fig. 3.** Epileptiform discharges in longitudinal PC slices. Nissl-staining after experiments revealed the anatomical structures in induced (A1) and disinhibited (A2) slices. The region selected for imaging in a central portion of the PC is outlined in black (abbreviations as in Fig. 1). Video pictures (B1, induced; B2, disinhibited) of imaged regions at higher magnification indicate the sites of discharge onset and plateau activity as light and dark gray-shaded regions, respectively. Traces (C1, induced; C2, disinhibited) from the indicated locations show onset activity (1), plateau activity (2), and responses in layer III (3). - - -, the time of half-maximal fluorescence change at the site of onset. Each trace represents an average of 4–6 neighboring photodetectors. Epileptiform discharges were evoked by electrical stimulation in layer Ib (250 μA, 200 μs, B1 and C1; 170 μA, 200 μs, B2 and C2) at sites indicated by the MM.
region of the En (Fig. 1B), which was the site of discharge onset in transverse and parasagittal slices in the induced model. The absence of this part of the En in longitudinal slices allows us to assess how this region contributes to discharge initiation.

As noted in the preceding text, in longitudinal slices, discharges were initiated with the same temporal sequence of plateau activity and onset activity. In longitudinal slices, onset activity could occur anywhere within a wide area that extended over much of the rostrocaudal extent of both the En and lateral boundary region. When slices were stimulated in the superficial layers of the PC, discharge onset occurred in the lateral boundary region of the En ($n = 11$; Fig. 3B1, light gray contour). The location of the discharge onset site within this large area was strongly dependent on the position of the stimulating pipette (Fig. 4A). The onset site...
also varied from trial to trial to a greater extent than in transverse or parasagittal slices, but the trial-to-trial variation was less than that seen when the stimulation site was varied. There were no systematic changes in onset site during experiments.

When the triggering stimulus was moved from the superficial layers of the PC to the En, onset activity moved from the lateral boundary region of the En to a deeper location within the En. For stimulation in both the PC and En, the onset site tended to be spatially removed from the stimulating pipette. Thus onset was more posterior with anterior stimulation (Fig. 4A, 2 and 3) and more anterior with posterior stimulation (Fig. 4A, 1 and 4).

In disinhibited longitudinal slices onset occurred deep in the En, close to the external capsule \((n = 6; \text{Fig. 3B2, light gray-filled contour})\). The site of onset was far less variable than in induced longitudinal slices and remained in the same part of the deep En regardless of where stimulation was applied (Fig. 4B, 1–4). In slices cut in other orientations, the disinhibited model generally showed onset within the En, suggesting that GABAA receptor-mediated inhibition can limit regenerative activity within this nucleus (Demir et al. 1998c).

We also examined the localization of plateau activity in longitudinal slices and saw that it consistently occurred in the lateral boundary region of the En with both the induced (Fig. 3B1, \(n = 6\)) and disinhibited (Fig. 3B2, \(n = 5\)) models (dark gray-filled contours). Plateau activity occurred at the same location as in transverse and parasagittal slices, and this suggests that the lateral boundary region of the En has intrinsic properties allowing it to generate a sustained depolarization regardless of the long range projections preserved in slices of different orientation. It was noted in the preceding text that for stimulation sites in the PC, onset activity in induced longitudinal slices was also seen in the lateral boundary region of the En. However, this region is quite extended in longitudinal slices (Fig. 3) so that plateau activity and onset activity can occur in different parts without significant spatial overlap. It was noted in the preceding text that moving the stimulation electrode from anterior to posterior sites resulted in a movement of the site of onset in the opposite direction (Fig. 4). A parallel shift was seen in the site of plateau activity, although this site was generally larger, so there was some overlap for different stimulation sites.

### Propagation patterns in different PC slices

In transverse slices, propagation was wavelike, spreading continuously away from the site of onset. This qualitative behavior is illustrated in Fig. 5A, with a sequence of images from a transverse slice in which fluorescence intensity is encoded as color (data set from Demir et al. 1998c). The first row in this figure shows the initial local response to an electrical stimulus in the overlying PC. This response died out early in the second row, ~50 ms after the stimulus. The discharge began in the fourth row following a latency of ~80 ms. The progressive growth of the yellow-red region in a transverse slice illustrates the wavelike spread of depolarization as the discharge moved by roughly constant spatial increments from frame to frame (Fig. 5A). In parasagittal slices, discharges propagated in the same basic way (Fig. 5B), although the

### Table 1. Discharge properties in parasagittal and longitudinal PC slices

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>Velocity, m/s [rostrocaudal (deep-superficial)]</th>
<th>((\Delta F/F)_{\text{lin}} \times 1000)</th>
<th>((\Delta F/F)<em>{\text{PC}}/\Delta F/F)</em>{\text{lin}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasagittal disinhibited</td>
<td>7</td>
<td>0.25 ± 0.05 (0.14 ± 0.05)</td>
<td>1.9 ± 0.3</td>
<td>0.25 ± 0.03</td>
</tr>
<tr>
<td>Parasagittal induced</td>
<td>5</td>
<td>0.19 ± 0.02 (0.15 ± 0.04)</td>
<td>2.3 ± 0.3</td>
<td>0.26 ± 0.07</td>
</tr>
<tr>
<td>Longitudinal disinhibited</td>
<td>6</td>
<td>0.20 ± 0.04</td>
<td>2.2 ± 0.3</td>
<td>0.82 ± 0.10</td>
</tr>
<tr>
<td>Longitudinal induced</td>
<td>18</td>
<td>0.39 ± 0.07</td>
<td>2.1 ± 0.1</td>
<td>0.86 ± 0.06</td>
</tr>
<tr>
<td>PC leads</td>
<td>6</td>
<td>0.56 ± 0.19*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>En leads</td>
<td>12</td>
<td>0.30 ± 0.04*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal induced (partial cut)</td>
<td>8</td>
<td>0.20 ± 0.04</td>
<td>1.4 ± 0.1</td>
<td>0.33 ± 0.08</td>
</tr>
</tbody>
</table>

Propagation velocities and fluorescence changes for epileptiform discharges in parasagittal and longitudinal slices under disinhibited and induced conditions. The last row is for induced longitudinal slices with partial cuts along the endopiriform nucleus-piriform cortex (En-PC) border (Fig. 9). In parasagittal slices, velocities were measured along the rostrocaudal and mediolateral (deep-superficial) axes. Deep-superficial velocities could not be measured in longitudinal slices because propagation was saltatory. \(\Delta F/F\) is the fluorescence change for the discharge divided by the resting light intensity at the same site. For each experiment, the values were computed for averaged signals from 7 hexagonally arranged photodiodes. * These two numbers were different, with \(P < 0.05\). Means ± SE.

**Regenerative discharges in the PC of longitudinal slices**

In marked contrast to transverse and parasagittal slices, discharge amplitudes were large in the superficial layers of the PC in longitudinal slices (trace 3, Fig. 3C, 1 and 2). Discharges propagated from the En to the overlying PC with relatively little decrement for both the induced and disinhibited models (compare traces 2 and 3 in Fig. 3C, 1 and 2; see also Figs. 6, and 7). In parasagittal slices, the depolarizations in the PC were much smaller than in the En (Fig. 2), and transverse slices were similar in this regard (Demir et al. 1998c). The amplitudes of fluorescence changes associated with discharges were averaged in the En and presented in Table 1 (along with the velocities, which will be discussed in the following text). These data show that under the various conditions employed in the present study, the normalized fluorescence changes in the En were all very similar. However, the ratios of fluorescence changes in the PC to those in the En (last column of Table 1) were much higher in longitudinal slices (0.82–0.86) than in parasagittal slices (0.24–0.25). These ratios illustrate how in parasagittal slices propagation along the deep to superficial trajectory is associated with far greater attenuation in amplitude than in longitudinal slices. The high-amplitudes of discharges in the PC of longitudinal slices are consistent with the interpretation that these slices contain a fiber system capable of supporting regenerative activity. This represents one of several distinct features of epileptiform activity in longitudinal slices that are similar to in vivo seizure activity.
spread along the rostrocaudal axis was somewhat more rapid than along the medial-lateral axis (Table 1). Wavelike propagation is commonly observed for epileptiform discharges in slices prepared from other brain regions, including the hippocampus (Miles et al. 1988) and neocortex (Golomb and Amitai 1997).

In longitudinal PC slices, epileptiform discharges spread with a fundamentally different spatiotemporal pattern. As noted in the preceding text, discharges first appeared either in the lateral boundary region of the En (Figs. 3B1 and 5C) or in deep portions of the En (Figs. 3B2 and 5D) under induced or disinhibited conditions, respectively. Following onset, epileptiform discharges spread in the rostrocaudal direction with velocities given in Table 1. In disinhibited slices, rostrocaudal spread first occurred in the En (Fig. 5D, 4th row). After a delay of ~10–20 ms, the discharge jumped abruptly in the lateral direction into the PC (Fig. 5D, 5th row, 2nd frame).

This pattern was seen in all six disinhibited longitudinal slices tested, but in only two-thirds (12 of 18) of the induced longitudinal slices. One-third of the induced slices showed a strikingly different discharge pattern in which the initial phase of rapid rostrocaudal spread was seen in the PC (Fig. 5C, 3rd row). After spread through the PC was complete, the discharge jumped abruptly into the En (Fig. 5C, 4th row). In general, discharges in longitudinal slices showed a rapid initial wave-like phase of spread in the rostrocaudal direction, and ~10–20 ms after this initial spread was complete, a second saltatory phase of spread in the medial-lateral direction. Within this general framework we identified two basic variations. 1) Discharges spread in a wavelike fashion through the En and then abruptly invaded the PC; these were classified as having an En → PC sequence and were seen in all disinhibited slices and most induced slices. 2) Discharges spread in a wavelike fashion through the PC and then abruptly invaded the En; these were classified as having a PC → En sequence and were seen in 6 of 18 induced longitudinal slices. We also noted that in three of the induced longitudinal slices with the En → PC sequence a second burst appeared in the En, so that the overall sequence was En → PC → En (Fig. 7B). These abrupt jumps between layers were a particularly striking and unique feature of discharges in longitudinal slices and were never seen in transverse or parasagittal PC slices. In vivo, similar abrupt transitions in the laminar distribution of membrane current have been described for kindled epileptiform discharges in the PC (Haberly and Sutula 1992). Thus the neural circuitry preserved when slices are prepared by cutting in the longitudinal plane supports a pattern of propagation closer to that seen in vivo.

Unique temporal relations in longitudinal slices

The color maps of Fig. 5, C (4th row) and D (5th row), illustrate the abrupt migration of discharges between the En and PC in longitudinal slices. Fluorescence traces from specific sites were selected so that we may examine these transitions more closely (Figs. 6 and 7). Figure 6 shows traces from an experiment like that of Fig. 5C (from an induced slice) where the discharge first spread through the PC and subsequently jumped to the En. Onset activity (Fig. 6A2) and plateau activity (Fig. 6A3) can be seen in these traces, although in this experiment the shorter latency to discharge onset made it difficult to see these forms of latent period activity clearly. Traces from deep layer III (trace 3), superficial layer III (trace 4) and layer I b (trace 5) show a nearly synchronous onset (- - -). The trace from the deep En (trace 1) clearly shows that the discharge at that location follows the discharge in the PC; fluorescence is only starting to rise (at the - - -), and this rise is much slower than that of the other traces. Thus the discharge in the deep En is temporally separated from the discharge in the rest of the slice. A second vertical line in Fig. 6A (· · ·) shows that the fluorescence peak in the En occurs at a time when the fluorescence is decaying in the PC. These traces thus show the same temporal behavior that can be seen in the sequence of color maps in Fig. 5C, in which red is seen first in the lateral part of the slice (end of row 3 and beginning of row 4) and then invades the medial part of the slice (end of row 4 and beginning of row 5).

Fluorescence from the lateral boundary of the En (Fig. 6A, trace 2) shows two components of depolarization, indicating that this region participates in both phases of the discharge. Superimposing a trace from the site of discharge onset with a trace from a location deep in the En (Fig. 6B1, traces 1 and 2 from Fig. 6A) shows that the second component of depolarization at the lateral boundary region of the En coincides with the discharge in the deep En. Superimposing a trace from the lateral boundary region of the En with a trace from the PC shows that the first component of the discharge in the lateral boundary region coincides with the discharge in the PC (Fig. 6B2, traces 2 and 4 from Fig. 6A).

Regardless of whether discharges first spread through the En or PC, the region that discharged later showed a slower rise that began shortly before or while the lead region peaked and continued as the discharge in the lead region began to decay. This can be seen by comparing trace 1 of Fig. 6A with the other traces of that figure. Figure 7 presents examples of this for discharges showing the En → PC sequence (as in Fig. 5D), which is opposite to the sequence shown in Figs. 5C and 6. As in Fig. 6, vertical lines were drawn to help visualize the temporal relations. These traces show that the depolarization in the PC is generally just beginning at the time when the discharge has nearly peaked in the En (Fig. 7, · · ·). The middle trace of Fig. 7B also shows a second burst in the En, in a slice where the sequence was En → PC → En. Mechanisms for the delays between these bursts, including the possible role of inhibitory synapses, are considered in the Discussion.

Discharge propagation velocities

Figs. 6 and 7 indicated that along an axis perpendicular to the cortical surface, discharges in longitudinal slices were
nearly synchronous within the En and nearly synchronous within the PC but underwent abrupt transitions between these two regions. By contrast, discharges spread in a wavelike fashion parallel to the cortical surface (Fig. 8). These fronts moved with a well-defined velocity, which was measured under various conditions and summarized in Table 1. In transverse PC slices, discharges propagated with a velocity of $\approx 0.1\text{–}0.2 \text{ m/s}$ (Demir et al. 1998c). In parasagittal slices, a somewhat faster velocity of $0.14\text{–}0.25 \text{ m/s}$ was seen (Table 1). The initial wave-like spread along the rostrocaudal axis in longitudinal slices allowed us to measure the velocity (see METHODS), but because of the abrupt way in which discharges moved between the En and PC in longitudinal slices, the velocity for spread in this direction was poorly defined and could not be determined. Figure 8 and Table 1 show that discharges propagated significantly faster in induced (0.39 m/s) compared with disinhibited (0.20 m/s) longitudinal slices. Indeed, the rostrocaudal velocity in induced longitudinal slices was much greater than under any other condition studied here.

Effect of partial separation of the En and PC

To determine the role played by En-PC interactions in the unique discharge patterns described in the preceding text in longitudinal slices, these two structures were partially separated by cutting along the lateral boundary of the En, starting

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**FIG. 6.** Temporal relations for discharges in induced longitudinal PC slices showing the PC $\rightarrow$ En sequence (for the En $\rightarrow$ PC sequence, see Fig. 7). A: traces show epileptiform discharges from the deep En (trace 1), the site of onset near the lateral boundary region of the En (trace 2), deep layer III of the PC (trace 3), superficial layer III of the PC (trace 4), and layer Ib of the PC (trace 5). Each trace represents an average of 6–7 neighboring detectors. $\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cd..
from the caudal end (Fig. 9A). Figure 9B1 shows the site of discharge onset in an induced longitudinal PC slice prior to making this cut. As noted in the preceding text, in induced longitudinal slices, onset occurred at the lateral boundary region of the En (Figs. 3B and 4A). The pattern of propagation in this slice is shown with color maps in Fig. 9B2. The local graded response to the electrical stimulus can be seen in the first two rows. The epileptiform discharge first appeared in the fourth row of this figure and spread through the superficial layers of the PC in ~10 ms. The subsequent jump to the central and deep En (fifth row) indicated that the (PC → En) sequence is similar to that shown in Fig. 5C.

After making a cut and partially separating the PC and En, the site of discharge onset and pattern of propagation changed. The local response to the stimulus spread within the PC (Fig. 9C2, 1st 2 rows), indicating that damage from the cut did not spread to this region. The site of discharge onset did not simply move to another part of the lateral boundary of the En, but rather moved to a deep portion of the En (close to the external capsule) (n = 6/8; Fig. 9C1). Interestingly, this site was below the cut, despite the fact that the stimulus was applied to a superficial site (layer II). Onset at this site may be enabled by damage to inhibitory inputs to the deep En (see DISCUSSION).

A small number of slices (2 of 8) showed discharges first appearing at more superficial locations in layer III (data not shown), and this too may reflect a reduction of inhibitory inputs, in this case to the PC. Although this was only seen in two experiments, one of the slices showed a PC → En sequence prior to cutting and the other showed an En → PC sequence. Thus one cannot relate this behavior (onset in the PC after cutting) to the prior character of the discharge in the intact slice.

Cutting longitudinal slices in this way dramatically reduced the spread of discharges to the overlying PC. A trace from a site in layer II opposite the cut showed a small fluorescence change associated with the epileptiform discharge (Fig. 10B, trace 5). As noted in the preceding text, prior to the cut discharge amplitudes in longitudinal slices were similar in the PC and En (the ratio was 0.86, Table 1), but after the cut, the ratio was reduced to 0.33, a value that was almost as small as that seen in parasagittal slices (0.26). The depolarization associated with the epileptiform discharge in the PC was smaller than the graded local response to the stimulus, and since the color maps were prepared by scaling to the maximum fluorescence at each site (see METHODS), they did not show the discharge very clearly in much of the PC (Fig. 9C2).

The velocity of discharge propagation in lesioned slices was reduced to 0.2 m/s (Table 1). A similar reduction of velocity was seen in coronal neocortical slices of guinea-pig following lesions (Albowitz and Kuhnt 1995). It is likely that the axons responsible for rapid conduction in intact longitudinal slices pass through the cut area in the lateral boundary region. In cut slices, discharges propagated in a wavelike fashion (Fig. 9C2) as in transverse and parasagittal slices. Thus partial separation of the En and PC eliminated the unique saltatory pattern of discharges jumping back and forth between the PC and En. This was illustrated with fluorescence traces from different sites. Prior to making the lesion, the discharge spread first to the PC, and a second component of depolarization followed in the En (Fig. 10A, traces 1–3). After cutting along the lateral boundary of the En, fluorescence traces revealed a wave-like spread from the En to the PC (Fig. 10B). This implies that saltatory propagation in longitudinal slices depends on projections through the lateral boundary region of the En in longitudinal slices. In summary, partial separation of the PC from the En by making a cut along the lateral boundary of the En eliminated the features of discharges unique to longitudinal slices, including large amplitudes in the PC, rapid velocities, and saltatory propagation.

**DISCUSSION**

This study investigated the generation and propagation of interictal-like epileptiform discharges in PC slices cut in three different planes. Using two different in vitro models of epilepsy, these experiments showed that discharges were generated in the same two-stage process described previously in transverse slices of PC (Demir et al. 1999). The first stage consisted of plateau activity and the second consisted of onset activity. Each of these forms of latent period activity was located at a distinct site within the deep piriform region. In slices cut in all three planes, the electrical activity during the latent period prior to discharge onset had essentially the same form and was located in the same general area within the deep piriform region. Thus fiber systems exhibiting differential vulnerability to cutting in different planes of section are not required for this highly characteristic discharge generation sequence. Although this basic sequence was observed in slices of all three orientations, the sites where latent period activity was observed differed in subtle ways between transverse and parasagittal slices and more significantly between longitudinal slices and slices of other orientations.

After initiation, discharges in parasagittal slices propagated away from the site of onset in a wave-like pattern similar to that described previously in transverse slices. By contrast,
discharges in longitudinal slices propagated in a qualitatively different way. The velocity of propagation in longitudinal slices was more than twice as fast and showed a strong directional preference. Furthermore discharges jumped abruptly between layers in a manner that was never seen in transverse or parasagittal slices. Finally, when discharges propagated to superficial layers, there was little attenuation in amplitude. Introducing cuts that partially separated the PC from the En eliminated these unique features of discharges in longitudinal slices. These unique features are characteristic of discharge activity in vivo. These findings thus indicate that longitudinal slices preserve fiber systems that play a role in the spread of epileptiform discharges in the intact brain. Fiber systems with predominantly longitudinal orientations are capable of serving as the anatomical substrate for these features, and such fiber systems are found in the PC (Haberly 1998; Johnson et al. 2000) and the En (Behan and Haberly 1999).

Onset activity and plateau activity

Previous studies in transverse PC slices showed that discharge onset occurred in the En and layer VI of the adjoining neocortex (Demir et al. 1998c). In these slices, the regions of adjacent neocortex were either agranular insula (Fig. 1A) or anterior perirhinal cortex depending on whether the slice was taken from an anterior or posterior part of the brain, respectively. In parasagittal slices, onset was seen within the En; but in these slices, the neocortex at the boundary with the En was orbital cortex. Longitudinal slices contain no neocortex but still show high susceptibility to epileptiform activity. Thus the initiation of discharges does not depend on proximity with specific neocortical hot spots. Longitudinal slices generally contained the center of the En and much less if any of the dorsal boundary region where onset was seen in other slices. In disinhibited longitudinal slices, discharge onset was seen in the deep En. In induced longitudinal slices, the onset site moved to the lateral boundary region of the En. Thus the site of discharge onset is somewhat fluid within the En and adjoining regions; discharge initiation does not require a unique subdivision of the En.

Although not required for discharge initiation, different parts of the En such as the dorsal boundary region may influence the site of onset. The greater variability in onset site in longitudinal slices suggests that the dorsal boundary region helps fix the site of discharge onset. The greater consistency of onset site in disinhibited longitudinal slices probably reflects the fact that these slices contain the central part of the En, where onset occurs in disinhibited slices cut in other orientations.

In contrast to the variable location of the onset site, plateau activity was more consistently seen in the lateral boundary region of the En. Again, the region of neocortex included in these slices made little difference. Intrinsic cell properties and local circuitry appear to play an important role in the generation of plateau activity, more so than interactions with regions outside the PC. Excitatory synapses between the plateau and onset sites play a critical role in the initiation of discharges...
(Demir et al. 1999). Since plateau activity precedes discharge onset in slices cut in three different nearly orthogonal planes, we can conclude that the fibers between these regions are well distributed without a preferred orientation. Furthermore, the finding that this sequence does not depend on pathways unique to one particular orientation increases the likelihood that it is relevant to seizure activity in the intact PC.

A striking feature of the PC is the presence of distinct foci where discharges are consistently initiated. Based on the consistent observation of plateau activity at the same location, the lateral boundary region of the En should be viewed as an anatomical subdivision endowed with cellular properties and local circuitry uniquely disposed for sustaining depolarizations that can serve as a precursor to epileptiform discharges. Distinctive neurochemical features have been demonstrated in this region (Domroese 1999) that may serve as molecular components of the machinery that generates plateau activity. The dense excitatory interconnections (Behan and Haberly 1999; Hoffman and Haberly 1993) and other features of the neural circuitry within the En (Domroese et al. 1997, 1998) may also be important components of the plateau activity-generating circuit.

**Comparison with in vivo epileptiform activity**

The propagation of epileptiform discharges varied dramatically depending on slice orientation. In transverse and parasagittal PC slices, discharges propagated in a wave-like fashion with velocities ranging from 0.1 to 0.25 m/s (Table 1) (Demir et al. 1998). Only the upper end of this range (>0.2 m/s) is consistent with action potential propagation. Similar slow velocities have been observed for discharge spread in hippocampus and neocortex (Albowitz and Kuhnt 1995; Miles et al. 1988), although small patches with higher velocities have been described (Wadman and Gutnick 1993). It is likely that these slow velocities reflect a propagation mechanism dependent on recurrent excitation (Golomb and Amitai 1997; Traub et al. 1987). In induced longitudinal PC slices, discharges propagated rostrocaudally at 0.39 m/s, and in a subpopulation of slices in which the discharge first invaded the PC, the velocity was 0.56 m/s. In vivo the velocities are in a comparable range, with 0.25 m/s in cat neocortex (Goldenson and Salazar 1986), 0.6 m/s in rat somatosensory cortex (London et al. 1989), and 0.3 m/s in rat PC (Ketchum and Haberly 1992). Thus the velocities in longitudinal slices exceed those seen in other PC slice preparations and fall within the range seen in vivo.

It is notable that in disinhibited longitudinal slices discharges propagated more slowly (0.2 m/s, Table 1). In these slices, discharges were initiated in the deep En and spread through the En first. The slower velocity in this region may reflect smaller diameter axons in the En (Behan and Haberly 1999). By contrast, in the induced model discharge onset was seen in the lateral boundary of the En, and in one-third of those slices, discharges spread through the PC first (Fig. 5C). The more rapid spread in these cases may reflect the early activation of PC pyramidal cells. This implies that association fibers, which project over most of the rostrocaudal extent of the PC (Haberly 1998), can under some conditions contribute to discharge spread in longitudinal slices, and when they do, the velocity is high.

In both disinhibited and induced longitudinal slices, discharges first spread within specific layers along the rostrocaudal axis and then, after 10- to 20-ms delays, jumped to other layers. These jumps resemble the abrupt shifts in laminar distribution seen in vivo (Haberly and Sutula 1992). The high
amplitudes of fluorescence signals in the PC of longitudinal slices (Table 1) are also closer to the in vivo situation, where current source density analysis has demonstrated prominent current sinks in superficial layers (Biella et al. 1996; Haberly and Sutula 1992). The much smaller depolarizations in the superficial layers of parasagittal and transverse PC slices suggest that shorter pyramidal cell axons (probably shorter than the 350 μm thickness of our slices) do not provide sufficient connectivity for regenerative activity comparable to that seen in vivo.

The similarities between epileptiform discharges in longitudinal slices and in vivo were lost after partial cuts were introduced between the En and PC. Discharge propagation became slow, abrupt jumps between layers disappeared, and amplitudes in the PC became smaller. Thus the unique features of discharges in longitudinal slices depend on fibers in the lateral boundary region of the En. This may include fibers projecting mediolaterally (between pyramidal cells and deep cells), but transverse slices preserve these fibers as well as longitudinal slices (both are cut perpendicular to the cortical surface). It is more likely that the key difference is the preservation of association fibers. The plane of section of longitudinal slices parallels the rostrocaudal trajectory of association fibers of PC pyramidal cells. This fiber system would be vulnerable to cuts at the En-PC border because many of these fibers have deep trajectories (Johnson et al. 2000).

Proposed circuitry

Many of these results can be interpreted in terms of well-documented excitatory pathways of the PC (Haberly 1998) (Fig. 11): excitatory synapses between deep cells in and around the En, reciprocal excitatory synapses between deep cells and pyramidal cells, and pyramidal cell association fibers. Positive feedback between deep cells generates plateau activity in the lateral boundary region of the En, and reciprocal excitatory interactions between the lateral boundary region and deeper regions lead to discharge onset. This discharge initiation sequence was seen in slices cut along any plane and was discussed in detail previously (Demir et al. 1999). In induced longitudinal slices where GABAergic inhibition remains active, inhibitory interneurons projecting from the site of plateau activity to deeper parts of the En may prevent discharge initiation at that site (Fig. 11B, blue circles). Weakening this pathway in longitudinal slices, either by disinhibition (Fig. 3B2) or with cuts in the lateral boundary region of the En (Fig. 9), thus permits discharge onset to occur deep in the En.

The PC and En each have strong intrinsic excitatory connections that allow each region to produce regenerative activity (Fig. 11B). There is also strong connectivity between the En and PC that allows regenerative activity to move from one region to another. In longitudinal slices, this movement occurs after a delay of ~10–20 ms, and the depolarization associated with spread between the En and PC has a slower rise. These slow rises cannot be explained by a dispersion of conduction velocities for axonal action potentials because the relevant distances of ~1 mm would be traversed in 5 ms by the slowest action potentials with velocities of 0.2 m/s but the rises take 10–20 ms. Thus the delay in discharge movement between layers and the gradual depolarization associated with these movements are likely to be caused by changes in excitability of the target region that occur as the discharge in the lead region continues. These delays could reflect a time during which transient voltage-gated K+ channels (A currents) inactivate. These rapidly activated K+ channels would oppose the development of a synchronous discharge. However, they inactivate with time constants in the range of 8–16 ms (for small depolarizations) (Banks et al. 1996), and this could account for the delay of discharge spread.

Another possibility is that inhibitory interneurons, which are extensively distributed in the PC and En (Ekstrand et al. 2001), delay the spread between these regions. When the sequence is En → PC, interneurons in the En could delay the spread of discharges to the PC, and when the sequence is PC → En, interneurons in the PC could perform a similar function (Fig. 11B, blue circles). The decay of this inhibition would then determine the timing of jumps between the En and PC. Stimulation in layer III activates a GABA_A receptor-mediated IPSC in PC pyramidal cells with a ~10-ms decay time constant. Furthermore, these inhibitory postsynaptic potentials (IPSPs) show strong paired-pulse depression with pulse intervals as brief as 10–20 ms (Kapur et al. 1997). If the inhibitory interneurons responsible for these IPSPs are activated at the beginning of a discharge in the En, they could delay discharge spread to the PC as observed.

Long association fibers between pyramidal cells are likely to play an important role in the rapid rostrocaudal spread of discharges, particularly when discharges lead in the PC. These fibers often descend deep into layer III of the PC and lateral boundary region (D.M.G. Johnson, K. R. Illig, M. Behand, and L. B. Haberly, unpublished observations) (Fig. 11B). This would prevent their preservation in parasagittal slices and make them vulnerable to the cuts used here to separate the En and PC. The stronger positive feedback between pyramidal cells can also account for the larger depolarization in superficial layers of the PC. The abrupt shifts between the En and PC suggest that the strong intrinsic fiber systems in each of these regions (illustrated with thick lines in Fig. 11B) allow discharges to occur within the PC and En with some autonomy. Reverberations between these two circuits could contribute to the generation of rhythmic discharges such as those seen during the clonic phase of seizures.

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