Investigation of the Neuronal Aggregate Generating Seizures in the Rat Tetanus Toxin Model of Epilepsy

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A key question in epilepsy is the organization and size of the neuronal networks necessary for generating seizures. Hypotheses include: a single focal neuronal network drives seizure discharges across the brain, which may or may not be identical with the circuits that generate interictal spikes; or multiple neuronal networks link together in re-entrant loops or other long-range networks. It remains unclear whether any of these hypotheses apply to spontaneous seizures in freely moving animals. We used the tetanus toxin chronic model of epilepsy to test the different predictions made by each hypothesis about the propagation and interaction of epileptic discharges during seizures. Seizures could start in either the injected or noninjected dorsal hippocampus, suggesting that seizures have multifocal onsets in the tetanus toxin model. During seizures, individual bursts propagated in either direction, both between the right and left dorsal hippocampi, and between CA3 and the dentate gyrus in the same hippocampus. These findings argue against one site “driving” seizures or seizures propagating around a limbic loop. Specifically, the side leading each burst switched a median of three times during the first 20 s of a seizure. Analysis of bursts during seizures suggested that the network at each recording site acted like a neuronal oscillator. Coupling of population spikes in right and left CA3 increased during the early part of seizures, but the cross-correlation of their whole-discharge waveforms changed little over the same period. Furthermore, the polarity of the phase difference between population spikes did not follow the phase difference for complete discharges. We concluded that the neuronal aggregate necessary for seizures in our animals comprises multiple spatially distributed neuronal networks and that the increased synchrony of the output (population spike firing) of these networks during the early part of seizures may contribute to seizure generation.

INTRODUCTION

Three types of epileptic discharges have been identified during electroencephalogram (EEG) recording in localization-related (focal) epilepsy: the interictal spike, polyspike, and seizure. Experiments on brain slices maintained in vitro combined with computer modeling have shown that synaptic connections between CA3 pyramidal cells and their intrinsic conductances are sufficient to generate interictal spikes (Traub and Wong 1982) or polyspike discharges (Traub et al. 1993) in the hippocampus. It remains unresolved whether similar spatially localized neuronal populations can also generate seizures (Jefferys 1998). This is important because only seizure discharges lead to behavioral fits in vivo. Hence, understanding what network features distinguish brief interictal or polyspike discharges from seizures is a crucial issue. Several proposals have been made. One hypothesis is that seizures can be “driven” by one spatially localized neuronal network when it is large enough (Borck and Jefferys 1999; Dichter and Spencer 1969; Dichter et al. 1973; Lothman et al. 1991; Traub et al. 1996). An extension of this idea is that two neuronal networks are necessary for seizures. One area initiates epileptic discharges, but a second separate neuronal network is necessary to “amplify” those discharges into seizures (Köhling et al. 1999; Lothman et al. 1991; Siegel et al. 1990). Highly epileptogenic brain regions such as the area tempestas (Piredda and Gale 1985) or entorhinal cortex (Jones and Heinemann 1988; Nagao et al. 1996; Walther et al. 1986) could play this role.

The crucial question is whether hypotheses based on in vitro or acute in vivo models of epilepsy can explain spontaneous seizures in freely moving animals (Dichter and Ayala 1987). Focal seizures in humans can start in different parts of the brain in the same patient (Spencer and Spencer 1994). Similar multifocal onsets to seizures have been described in a chronic animal model of epilepsy (Bertram 1997). Thus seizures may be the product of multiple spatially localized neuronal networks that are each capable of driving seizures. Alternatively, seizures could be generated by several localized neuronal populations linked together in a loop such as the entorhinal cortex-hippocampal loop (Paré et al. 1992; Stringer and Lothman 1992). A different interpretation is that multifocal seizure onsets reflect a diffusely hyperexcitable network. This suggestion led to the proposal that limbic seizures occur when epileptic discharges spread to the thalamus because the diffuse thalamic projections synchronize epileptic activity across the network (Bertram et al. 1998).

The hypotheses discussed in the preceding text make distinct predictions about the propagation of epileptic discharges during the early part of seizures. For instance, if one site drives seizures, then this site should lead postsynaptic sites during seizures by a phase difference that is determined by the time lagging of population spikes in right and left CA3 increased during the early part of seizures, but the cross-correlation of their whole-discharge waveforms changed little over the same period. Furthermore, the polarity of the phase difference between population spikes did not follow the phase difference for complete discharges. We concluded that the neuronal aggregate necessary for seizures in our animals comprises multiple spatially distributed neuronal networks and that the increased synchrony of the output (population spike firing) of these networks during the early part of seizures may contribute to seizure generation.
ment applies to epileptic discharges propagating around a loop; the phase difference between two sites should be determined by the time for epileptic discharges to propagate from one site to another. Because the pathways by which epileptic discharges spread during seizures are not completely known, propagation has commonly been inferred by mathematical methods such as: coherence/phase analysis (Brazier 1972; Gersch and Goddard 1970; Gotman 1983), mutual information in signals (Mars and Lopes da Silva 1983), and linear and nonlinear regression (Fernandes de Lima et al. 1990). These methods typically use data epochs lasting seconds and make assumptions about the stationarity of the EEG signal and the relationship between EEG signals. We have adopted a different, simpler, strategy that allows us to follow propagation at defined times within seizures in addition to using conventional signal analysis methods. We applied our method to the chronic model of epilepsy that follows unilateral intrahippocampal injection of tetanus toxin (Finnerty and Jefferys 2000; Hawkins and Mellanby 1987). Here, we study spontaneous focal seizures in those freely moving rats to test predictions made by hypotheses of seizure generation. Preliminary data have been published in abstract form (Finnerty and Jefferys 1993b).

METHODS

Implantation of recording electrodes and recording protocol

We have described the implantation and recording process in detail elsewhere (Finnerty and Jefferys 2000; Finnerty et al. 2001). Male Sprague-Dawley rats (280–400 g) were anesthetized with halothane. Bipolar recording electrodes (twisted Teflon-coated stainless steel wire with the tips separated 250–350 μm along the axis of the wires) were placed into CA3 and either CA1 or dentate granule cell body layers of both cerebral hemispheres, using the evoked response produced by ventral commissural stimulation as a guide (Finnerty and Jefferys 1993a). Coordinates were: CA3, 2.7 mm posterior and 3.3 mm lateral to bregma; CA1, 3.1 mm posterior and 2.9 mm lateral; and dentate gyrus, 3.3 mm lateral and 3.1 mm posterior (Pellegrino et al. 1979). One microliter phosphate buffered saline either without (controls) or with 4–5 ng (12 mouse LD50) tetanus toxin (gift from Wellcome Foundation Research Laboratories, Beckenham, Kent, UK) was injected into the right hippocampus 3.5 mm lateral and 2.7 mm posterior to bregma. Animals were housed separately postoperatively with free access to food and water, allowed 24–48 h to recover, and then handled gently to familiarize them with the recording procedure.

Recording started 3–6 days postoperatively. The headstage contacts were connected, via counterbalanced wires, a slip ring and preamplifier to differential amplifiers (Digitimer D160, band-pass: 0.5 Hz to 3 kHz, and DC −3 kHz for 6 seizures). The amplified EEG signal was stored on FM tape (Racal V Store, Racal, Southampton, UK bandpass DC −3.25 kHz). Selected recordings were digitized (1401, Cambridge Electronic Design, Cambridge, UK) and analyzed using SPIKE2 (Cambridge Electronic Design). All animals were filmed continuously during EEG recording sessions using a Panasonic infra-red camera and NEC 30 U-matic video recorder with time lapse and a clock that was synchronized with the tape recorder clock. After completion of the recording protocol, the animal was killed with an overdose of halothane. The brain was dissected out, fixed in formalin, and embedded in wax prior to staining 10-μm parasagittal sections with cresyl fast violet or hematoxylin and eosin to confirm the electrode sites.

Recordings in vivo

The bipolar recording electrodes were designed to enable differential recording of the cell body potential with respect to a dendritic layer potential. This removed the contribution of volume conduction of distantly generated epileptic discharges to our recordings.

We were concerned that our recordings of seizures might be modified by our bipolar recording electrodes. Hence, we recorded evoked field potentials and interictal spike discharges with glass microelectrodes and bipolar electrodes at the same time as a control. This was done using hippocampal slices in vitro to ensure accurate placement of the electrodes. The evoked potentials and epileptic discharges comprise an envelope, which we refer to as a field postsynaptic potential, with superimposed population spike(s). We found that bipolar recording could alter the polarity of the field postsynaptic potential depending on the site of the synaptic sink with respect to the recording electrodes but that this resulted in a negligible difference (0.2 ms) in latency of the field postsynaptic potential. Population spikes, in contrast, were always negative going (data not shown).

Recording strategy

Our experiments required the recorded neuronal populations to be far enough apart so that activity propagated with a latency of a few milliseconds because this would allow clear separation of the neuronal population initiating the discharge from the neuronal population following. Several problems had to be solved. 1) The injected tetanus toxin spreads 1–1.5 mm mediolaterally along the hippocampal fissure. Hence, the recording electrode may be at some distance from the site where epileptic discharges originate. 2) An extrahippocampal site could drive discharges in the dorsal hippocampi. 3) Field postsynaptic potentials result from synaptic inputs originating locally and distantly.

Mapping experiments using evoked potentials indicated that the functional connectivity of the dorsal hippocampi corresponded to the anatomical projections (Finnerty and Jefferys 1993a). We used the direct measurements of latency differences made in the mapping experiments to devise a simple strategy for studying propagation of epileptic discharges in those animals implanted with electrodes in CA3 and CA1. Consider recordings from CA3 of both dorsal hippocampi using our coordinates (Fig. 1A). Epileptiform activity beginning in the injected hippocampus 600 μm from the ipsilateral CA3 recording spreads along the CA3 recurrent collaterals at ~0.1 m/s (Miles et al. 1988) and thus takes 6 ms to reach the recording electrode. Because of the more rapid conduction velocity in the ventral commissures, the latency of the contralateral CA3 discharge is also 6 ms. Therefore recordings would not resolve which hippocampus was the “pacemaker.” This problem was solved by implanting recording electrodes into CA3 and CA1 of both hippocampi (Fig. 1B). In the tetanus toxin model, epileptiform activity starts in the CA3 b/c region of the hippocampal slice (Jefferys 1989). Therefore we implanted recording electrodes into homotopic CA3 b/c points of both dorsal hippocampi in vivo (Fig. 1C). The maximal functional projection from both CA3 sites to ipsilateral and contralateral CA1 overlap (Finnerty and Jefferys 1993a). This led us to conclude that we could determine which hippocampus led by making two measurements. First, the difference in onset of epileptiform activity between CA3 traces should be 0–6 ms (this assumes 1 electrode is within ~600 μm of the focus). The second measurements used the findings that the latency of epileptiform activity in CA1 is 2–3 ms ipsilateral to the focus and 5–6 ms in contralateral CA1. Therefore there should be 2–to 4-ms difference in onset of epileptiform activity between the CA1 traces. These estimates provide minimum latency differences that occur when there is maximal activation; weaker inputs evoke responses with longer latencies.

The local circuitry at each recording site could potentially make a large contribution to the waveform of epileptic discharges during seizures. To prevent this from interfering with the interpretation of propagation of discharges, we stipulated that the direction of propagation could only be measured when the discharges were preceded by 50 ms of “flat” EEG.
We implanted bipolar electrodes into CA3 and dentate granule cells to study local discharges and the input of the entorhinal cortex to the hippocampal formation. Stimulation of the perforant pathway/dentate granule cells evokes a response in CA3 with a latency of 2.5 ms using our coordinates (Finnerty 1993).

**Population spike analysis**

The rationale behind our analysis of population spikes has been described in greater detail elsewhere (Engel et al. 1990; Finnerty and Jefferys 2000). We used an automated search routine in SPIKE2 (Cambridge Electronic Design) to detect population spikes in amplitude during epochs lasting 1–2 s. The time of the population spike was taken to be the time at the minimum of the spike. We fitted damped cosine waves (Gabor function) to the cross-correlograms (Engel et al. 1990) of population spikes in right and left CA3 and used the fits to estimate the frequency of the modulation, its amplitude and the phase difference, $\phi$, of population spike firing in right and left CA3. The amplitude of the modulation was normalized with respect to the offset of the Gabor function from the baseline to allow for the varying number of population spikes in differing data epochs.

**RESULTS**

Unilateral intrahippocampal injection of tetanus toxin resulted in chronic epilepsy. Seizures manifested themselves as a variety of stereotyped behaviors closely resembling those described during the development of kindling (Racine 1972). We implanted recording electrodes into CA3 and CA1 (CA3/CA1) in 10 toxin-injected and 4 control animals and into CA3 and the dentate granule cell layer (CA3/dentate) in 4 toxin-injected and 2 control rats. A total of 88 spontaneous seizures were recorded from the 10 animals with CA3/CA1 recording electrodes and 93 spontaneous seizures from the 4 with CA3/dentate electrodes. Seizures were first observed 7–10 days postoperatively in 10 (71%) animals. In the remaining four animals, seizures began on days 4, 6, 22, and 23. The maximal seizure frequency occurred within 2 days of the first seizure in 86% of epileptic rats. No rats developed status epilepticus. No epileptic activity was recorded from buffer-injected rats.

Unilateral intrahippocampal injection of tetanus toxin results in three types of electrographic activity (Finnerty et al. 2001). Interictal spikes last $\leq 100$ ms and comprised an envelope, which we refer to as a field postsynaptic potential, with superimposed population spikes. Polyspikes last 0.4–2 s and resemble a series of interictal spikes conjoined. Finally, prolonged epileptic discharges, which we refer to as seizures, lasted tens of seconds and were always associated with an observable fit. Seizures were composed of a series of discharges that we refer to as bursts. The bursts resembled interictal spikes or polyspikes separated by flat baseline (Fig. 2). The frequency of the interictal-like discharges during bursts varied, particularly during the early part of seizures. However, there was a tight
Relationship between dentate and CA3 discharges

We recorded from the dentate gyrus and CA3, in another cohort of rats, to determine whether activity propagated from caudal regions, such as the entorhinal cortex, to CA3 via the dentate gyrus. Interpretation of dentate and CA3 phase relationships is complicated by the short latency of CA3 responses to excitation from dentate granule cells via the mossy fibers and the existence of a direct entorhinal cortex projection to CA3 in addition to the disynaptic projection via dentate granule cells (Yeckel and Berger 1990).

Phase reversals

If seizures were driven by single pacemaker site or a “central synchronizer” (Bertram et al. 1998) then inter-hippocampal phase relationships should be constant during the early part of each seizure. However, we found that this was not the case in our animals. The absolute values of the phase lags clustered around the 0–6 ms range for CA3 traces and 2–4 ms for CA1 traces we described in METHODS. Using our method for defining the direction of propagation at the onset of bursts, we found that the leading hippocampal CA3 region could switch from the injected to the uninjected hippocampus or vice versa. We refer to this as a phase reversal (Fig. 4). We counted the phase reversals in the first 20 s of seizures recorded with CA3/CA1 electrodes to assess how frequently phase reversals occurred and whether there was a difference between seizures originating from the injected and uninjected hippocampi (Fig. 4, B and C). Phase reversals were found in 75.7% seizures (n = 74, 8 rats). The median number of phase reversals during the first 20 s of each seizure was 3 for seizures originating in the injected hippocampus and 2 for seizures originating in un.injected hippocampus (not statistically different, Mann-Whitney rank sum test, $P = 0.513$). Thus the number of phase reversals in the initial part of the seizure was independent of the hippocampus leading at seizure onset. The existence of phase reversals led us to conclude that seizures were not driven by epileptic activity at one site or required a central synchronizer (Bertram et al. 1998).

Seizure onset in tetanus toxin injected rats

We measured the times of onset of seizures recorded in CA3 and CA1 to assess whether seizure onset was restricted to the injected hippocampus. Seizure onset was defined as the time when the first component (field postsynaptic potential or population spike) of the first burst in a seizure left the baseline. We found that either the injected or the uninjected hippocampal CA3 region could lead at the beginning of seizures (injected = 72.8%, uninjected = 27.2%; $n = 81, 9$ rats; Fig. 3). All animals had ictal onsets in both the injected hippocampus and uninjected hippocampus except for one rat where only two seizures were recorded. We concluded that seizures could start in either dorsal hippocampus in our animals.

Phase reversals

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Two patterns of temporal relationship between the granule cell and CA3 field postsynaptic potentials emerged. Both patterns occurred with equal frequency and were found in all rats. In the first type, the granule cell field postsynaptic potential preceded the ipsilateral CA3 field postsynaptic potential by a maximum of 2 ms or lagged by \( \pm 1 \) ms (Fig. 5, left hippocampal traces). This time range is consistent with the entorhinal cortex exciting the hippocampus via the dentate gyrus as discussed in the preceding text. In particular, the maximum latency difference was consistent with dentate to CA3 mapping experiments (Finnerty 1993). In the second type, the CA3 field postsynaptic potential preceded the granule cell field postsynaptic potential by 2.5–4 ms (Fig. 5, right hippocampal traces). This was unexpected, but near identical phase relationships have been described during afterdischarges evoked by tetanic stimulation of the perforant pathway or ventral commissure (Bragin et al. 1997). This may be due to CA3 activating the contralateral granule cells via dentate hilus mossy cells or alternatively by a direct synaptic connection from CA3 to ipsilateral dentate granule cells. The latter is supported by two findings. First, a sparse pathway from CA3 to ipsilateral granule cells exists (Li et al. 1994). Second, we have recorded epileptic discharges in CA3 and dentate gyrus with similar temporal relationships in hippocampal slices prepared from rats previously injected with tetanus toxin (Whittington et al. 1994).

We concluded that some of our dentate/CA3 recordings of seizures were consistent with epileptic discharges propagating from the entorhinal cortex to the hippocampal formation. The entorhinal cortex may, therefore, be part of the neuronal aggregate that generates seizures in the tetanus toxin model of epilepsy. This is consistent with other epilepsy models in vitro where the entorhinal cortex generates prolonged epileptic discharges (Walther et al. 1986). However, the observation that CA3 could lead the dentate, combined with our finding of phase reversals in the CA3/CA1 recorded seizures suggested that the spread of epileptic activity during spontaneous seizures is more complex than simple propagation around a limbic loop.

Population spike firing

Our study of phase relationships gave information on the propagation of epileptic activity at the beginning of each burst within a seizure but not about the interaction between the hippocampi during individual bursts. Therefore we used the population spikes generated during bursts to explore the relationships of outputs from CA3 hippocampal subregions during seizures. Bursts in CA3 commonly start with a prolonged field postsynaptic potential on which are superimposed multiple high-frequency population spikes (Fig. 6A). This first field postsynaptic potential differs from other field postsynaptic potentials in the burst. Therefore we excluded it from further analysis of the population spike cross-correlogram (Fig. 6E).

Autocorrelations and power spectra of the waveform of bursts demonstrated their oscillatory nature (Fig. 6, B and C). Cross-correlation of the burst waveforms in right CA3 and left CA3 during 1- to 2-s epochs within 10 s of seizure onset revealed a correlation (mean, 0.35 \pm 0.03, \( n = 9 \) seizures from 4 rats) that peaked around 0 ms (mean, 0.8 \pm 3.3 ms, \( n = 9 \) seizures from 4 rats) and was modulated at the frequency of field postsynaptic potentials during the burst (Fig. 6D). We assessed whether population spike firing in right CA3 and left CA3 was modulated in the same way by fitting damped cosines (Gabor functions) to their cross-correlogram from the same epochs we used for cross-correlation of burst waveforms and calculated the normalized modulation amplitude (Engel et al. 1990) (Fig. 6E). We found that population spike firing was modulated at the same frequency as the cross-correlogram of the burst waveform (Fig. 6E). The mean normalized modulation amplitude was 0.22 \pm 0.05 (\( n = 9 \) seizures from 4 rats). We concluded that right and left CA3 acted as coupled oscillators during bursts.

In approximately two-thirds of seizures, the early part of the seizure was dominated by bursts comprising oscillations at \( \sim 20 \) Hz. These bursts tend to be long, allowing study of the progression of oscillatory firing in right and left CA3 during the early part of seizures (Fig. 7). We used the amplitude of the sinusoidal modulation of the cross-correlograms to quantify the strength of coupling of CA3 discharges and to assess how coupling changed during seizures. Cross-correlograms of CA3 discharge waveforms became more clearly sinusoidal as seizures progressed (Fig. 7, compare Bi and Ci), but the peak cross-correlation changed little over a 6-s interval starting \( \sim 5 \) s into the seizure (increase in waveform cross-correlation = 0.05 \pm 0.07, \( n = 4 \); \( P = 0.26 \), paired \( t \)-test; Fig. 7Di). In contrast, there was an increase in the normalized modulation amplitude of the cross-correlogram of population spikes in right and left CA3 over the same time period (increase = 0.31 \pm 0.04, \( n = 4 \); \( P < 0.001 \), paired \( t \)-test; Fig. 7, Bi, Ci, and Di).

Comparison of the phase lag of the discharge waveform and phase lag of the modulated population spike firing showed that...
these did not always have the same polarity. In the example illustrated in Fig. 7E, the polarity of the phase lag of the discharge waveform is constant over the period studied. The phase lag of the modulated population spikes, however, changes polarity twice. The neuronal oscillators in CA3 of the injected and uninjected hippocampi are coupled by neuronal firing, which propagates along monosynaptic pathways running in the ventral commissure (Fernandes de Lima et al. 1990; Finnerty and Jefferys 2000; Swanson and Cowan 1977). The finding that the phase of the modulated population spike firing can have the opposite polarity to the phase of the discharge waveform could result from circuitry intrinsic to each CA3 region dominating the discharge waveform and/or from each CA3 region receiving substantial inputs from other areas such as the entorhinal cortex as suggested by our recordings in the dentate gyrus and CA3.

**DISCUSSION**

We investigated the neuronal aggregate that generates seizures in the tetanus toxin model of epilepsy (Finnerty and Jefferys 2000; Hawkins and Mellanby 1987). Our major findings were 1) seizures could start in either the injected or noninjected hippocampus. 2) The direction of propagation of bursts during seizures was not unidirectional. This argued against one site driving seizures or seizures propagating around a limbic loop. And 3) neuronal networks in the dorsal hippocampi behaved like oscillators that were weakly coupled
together during the early part of seizures. Population spike firing showed a progressive increase in synchrony but in the absence of any change in correlation of epileptic discharge waveforms. This suggested that the output of both dorsal hippocampi was synchronized even though one dorsal hippocampus was not reliably driving the other throughout bursts.

**Neuronal aggregate generating seizures**

Neither the minimum size nor the organization of the neuronal aggregate necessary to generate a seizure is known (Lothman et al. 1991). Brief epileptic discharges such as interictal spikes can be generated by segments of in vitro hippocampal slices containing an estimated 1,000 CA3 pyramidal neurons (Miles et al. 1984). Larger blocks of tissue produced by surgical isolation of an island of hippocampus in vivo generate acute “electrographic seizures” after topical application of the GABA_A antagonist, penicillin (Dichter et al. 1973). GABA_A receptor-mediated inhibition is also reduced in the tetanus toxin model (Empson and Jefferys 1993). However, hippocampal slices prepared from the dorsal hippocampi of these chronically epileptic rats generate interictal spikes or polyspikes but no electrographic seizures. These data suggest that the limited circuitry preserved in these slices is sufficient for brief epileptiform discharges but not seizures. Slices prepared from the ventral hippocampus can generate more prolonged discharges when disinhibited (Borck and Jefferys 1999; Traub et al. 1996). This led to the proposal that a network comprising several thousand neurons may be sufficient to generate certain types of seizure. However, the longer discharges in vitro differ electrographically from the spontaneous seizures we recorded from the tetanus toxin model in vivo, suggesting that there are fundamental differences between the two types of discharge.

**Phase reversals**

We believe that it is unlikely that either a third extrahippocampal site or a combination of sites linked together drives epileptic discharges in both dorsal hippocampi. There are a limited number of well-defined excitatory pathways to the dorsal hippocampi. The ventral commissural pathway linking the dorsal hippocampi is one of the most prominent (Amaral and Witter 1989; Swanson and Cowan 1977). There is no anatomical evidence for an excitatory pathway from an extrahippocampal site that would have a similar effect when linked together. However, if the known pathway linking the dorsal hippocampi is cut, the temporal relationship between discharges in right and left hippocampi are lost (Finney and Jefferys 2000), which should not occur if there were other site(s) driving the discharges.

We imposed stringent requirements for counting phase reversals (50 ms of flat EEG prior to the measurements plus both the CA3 and CA1 phase lags must be within the limits described in Methods and must have reversed). There were CA1 phase lags of 0–2 ms, outside the 2- to 4 ms range specified in Methods. We believe that these shorter time lags arise when CA1 sites are excited by CA3 sites with submaximal projection (Amaral and Witter 1989); our previous mapping experiments indicated that CA3–CA1 latencies lengthen by several milliseconds under these situations. This suggests that we have probably underestimated the number of phase reversals occurring during the early part of seizures.

**Propagation of discharges during seizures**

Results from epilepsy models in which tetanic stimulation was used to evoke afterdischarges in the entorhinal cortex and hippocampus led to the suggestion that afterdischarges propagate through a re-entrant loop involving the entorhinal cortex and hippocampal formation (Paré et al. 1992; Stringer and Lothman 1992). However, the existence of re-entrant loops has been questioned. A study of the phase relationships of population spikes in the entorhinal cortex and hippocampal formation during evoked afterdischarges found no evidence of sequential activation of structures in an entorhinal cortex–hippocampal loop (Bragin et al. 1997). Similarly recordings from both dorsal hippocampi showed no net phase difference between CA1 and dentate gyrus EEG when whole afterdischarges were analyzed (Fernandes de Lima et al. 1990).

Our data suggest that this is not the whole story. Epileptic discharges do propagate in an orderly fashion, but the direction of propagation changes during seizures. The difference between our results and those of previous studies is probably due to two factors. First, local circuitry plays a major role in shaping individual bursts within each seizure and adds noise to the cross-correlation. Measuring the time of onset of each burst removes this component and provides a more precise analysis of the propagation of activity between the hippocampi. Second, the reversals of phase between the left and right hippocampi, revealed by our analysis, explain why there is no mean phase difference when cross-correlations are made over complete seizures.

**Oscillations and synchronization**

Our data suggested that seizures were generated by spatially localized neuronal networks that produced oscillatory discharges. We found increased coupling of population spike firing in the right and left hippocampi without a similar increase in coupling of the discharge waveform during the early part of seizures. This finding was unexpected. Oscillations in the gamma range (20–70 Hz) have been suggested to help establish synchrony of neuronal firing over long distances in the neocortex (Engel et al. 1992; König et al. 1995). This argument, when extrapolated to epileptic discharges, would suggest that discharges within this frequency range in the dorsal hippocampus should rapidly become synchronized during seizures. A simple explanation for our failure to find increased coupling of right and left CA3 burst waveforms is that they were dominated by the intense activation of local circuitry that occurs during epileptic discharges. However, the synaptic inputs from contralateral hippocampus may be sufficient to adjust the precise timing of population spike firing and, hence, cause the output of right and left CA3 to become synchronous. Further work is required to tease apart the exact cellular mechanisms. Theoretical studies involving realistic models of neurons and their connections (Traub and Miles 1991; Traub et al. 1993) could give insights into why coupling of population spikes increases during the early part of seizures, but the waveform correlation is unchanged.
Spatially distributed seizure generator

We believe that the most parsimonious explanation for our data are that the neuronal aggregate that generates seizures in our animals comprises multiple spatially localized neuronal networks that become coupled during seizures with attendant synchronous population spike firing to form a seizure generator. Our experiments do not chart all the brain structures that are involved in seizure generation. They do, however, implicate the dorsal hippocampi and entorhinal cortex in our animals. It is highly likely that multiple pathways link areas involved in the seizure generator with no specific pathway being necessary for seizure generation. For instance, cutting the ventral commissure to sever the reciprocal connections between right and left dorsal hippocampi does not abolish seizures (Finnerty and Jefferys 2000). The importance of synchronization of outputs at different sites within a seizure generator is that this will increase the likelihood that other areas will be recruited into the seizure generator and, hence, that a fit will occur.

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