9–16 Hz Oscillation Precedes Secondary Generalization of Seizures in the Rat Tetanus Toxin Model of Epilepsy

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INTRODUCTION

Epileptic activity may produce abnormal motor behaviors including generalized seizures. Secondary generalization occurs when epileptic activity starts focally in the cortex and spreads to involve both hemispheres and results in a motor convulsion. This contrasts with primary generalization where epileptic discharges are recorded from both cerebral hemispheres at seizure onset. What causes focal epileptic activity to spread to motor centers and produce a secondarily generalized seizure is unclear. Previously interest concentrated on anatomic sites as initiators of generalized seizures or critical points within neuronal circuits e.g., area tempestas (Piredda and Gale 1985), substantia nigra (Iadarola and Gale 1982), cingulate gyrus (Hawkins and Mellanby 1987), or perirhinal cortex (McIntyre et al. 1993). The properties of the seizure discharge leading up to secondary generalization have received less attention. This contrasts with some types of primary generalized seizure where regular field potential oscillations are part of the definition of the seizure type e.g., 3-Hz spike and wave discharges in childhood absence epilepsy (ILAE Commission 1989).

Epileptic discharges are characterized electrophysiologically by pathologically synchronous (hypersynchronous) firing of large neuronal populations. Synchronized discharges, often of an order of magnitude smaller, occur in normal animals during behaviors such as sensory processing (Engel et al. 1991; Free- man 1978; Gray et al. 1989), sleep (Contreras and Steriade 1996; McCormick and Bal 1997; Steriade et al. 1993), and the performance of motor skills that require attention (Murthy and Fetz 1992). Typically, hippocampal oscillations have been associated with normal motor behaviors or sleep (Bragin et al. 1995; Buzsáki et al. 1992; Vanderwolf 1969; Ylinen et al. 1995). It may be that physiological mechanisms of network synchronization play key roles in the pathological hypersynchrony of epileptic activity.

We used the rat tetanus toxin model of focal epilepsy, which resembles human temporal lobe epilepsy, to study a pathological motor behavior. We report that secondary generalization of seizures is preceded by a 9–16 Hz field potential oscillation which is synchronized across both dorsal hippocampi.

METHODS

Implantation of recording electrodes

Male Sprague–Dawley rats (280–400 g, Harlan-Olac, Bicester, UK) were anesthetized with 4% halothane in 700 ml · min⁻¹ oxygen and 300 ml · min⁻¹ nitrous oxide and maintained with 1–2% halothane in 700 ml · min⁻¹ oxygen during stereotaxic surgery. A monopolar stimulating electrode (Teflon-coated stainless steel wire, bare wire 0.125 μm diam, Medwire, New York) was put through a burr hole into the left ventral commissure; 0.5 mm caudal, 1.1 mm lateral to bregma, and 3.7 mm below the neocortical surface. Bipolar recording electrodes made from twisted Teflon-coated stainless steel wire (tips separated by 250–350 μm vertically) were used to minimize interference from volume conduction. Electrodes for connection to the noninverting amplifier input were placed into cell body layers of CA3 and either CA1 or dentate gyrus of both cerebral hemispheres (electrodes for the inverting input were located in stratum radiatum for CA regions and stratum moleculare for the dentate area) using the evoked response produced by ventral commissural stimulation as a guide (Finnerty and Jefferys 1993). 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).

Phosphate buffered saline (1 μl) either without (controls) or with 12 mouse LD₅₀ (mLD₅₀) tetanus toxin (Wellcome Foundation Research Laboratories, Beckenham, Kent, UK) was injected, using a Hamilton 7101N syringe, into the right hippocampus 3.5 mm lateral and 2.7 mm posterior to bregma over 1 min. The injection needle was left in place for an additional 5 min to prevent toxin refluxing back up the needle track (Hawkins and Mellanby 1987). This procedure causes minimal pathology. A dental cement headstage was constructed to protect the recording contact points. Panalog (combination topical antibiotic, Ciba-Geigy) was applied to the wound edge to prevent infection tracking under the headstage. Animals were housed separately postoperatively with free access to food and water, allowed 24 to 48 h to recover.
recover, and handled gently to familiarize them with the recording procedure.

Recording in vivo

Recording started 3–6 days postoperatively (before the onset of spontaneous epilepticiform discharges). The headstage contacts were connected, via counterbalanced wires and a slip ring (IDM Electronics, Reading, UK), to a preamplifier (D169, input impedance 100 MΩ, gain ×1, Digitimer, Welwyn Garden City, UK) whose output fed into differential amplifiers (Digitimer D160, band-pass 0.5 Hz–3 kHz, and DC-3 kHz for 6 seizures). The amplified EEG signal was stored on FM tape (Racal V Store, Racal, Southampton, UK, band-pass DC–3.25 kHz). All animals were filmed continuously during EEG recording sessions using a Panasonic infrared camera and NEC 30 U-matic video recorder with time lapse and a clock that was synchronized with the tape recorder clock. EEG was recorded for a minimum of 1 h/day on at least 3 weekdays/wk over a period of 4 wk.

Seizures manifested themselves as a variety of stereotyped behaviors closely resembling those during the development of kindling (Racine 1972b). We used these behaviors to classify seizures as nongeneralized or secondary generalized (motor fit). Nongeneralized behaviors include immobility, vibrissal twitching, and gentle head noddling. Secondary generalized seizures were differentiated from nongeneralized seizures by rearing. Additional behaviors included forelimb myoclonus and falling. We defined the onset of secondary generalization to be when both forepaws first left the ground and we used our time-lapse video recordings to calculate the time after seizure onset that secondary generalization occurred. Stage IV electrographic activity was defined to start after stage III when recordings from one hippocampus showed a 9–16 Hz oscillation >1 s (see RESULTS).

After completion of the recording protocol, each animal was killed with an overdose of halothane. The brain was dissectioned out, fixed in formalin, and embedded in wax before staining 10– with an overdose of halothane. The brain was dissected out, fixed in formaldehyde, embedded in paraffin, and stained with cresyl fast violet or hematoxylin and eosin to confirm the electrode sites, including their laminar location and specifically the location of the noninverted electrode site in the cell body layer.

Ventral commissure/fimbria lesions

Anesthesia, surgery, and recovery were as described above except for the additional procedure of cutting the ventral commissure/fimbria. After we had implanted the recording electrodes, a brief current pulse was applied to each CA3 electrode to ensure that an evoked response in contralateral CA3 could be recorded. The ventral commissure stimulation electrode, which was sited on the left side of the midline, was replaced with a shard of razor blade located at identical rostro-caudal coordinates. The ventral commissure/fimbria, along with a small part of the caudal corpus callosum, was cut by lowering the razor blade 4.0 mm below the cortical surface. This persistently abolished the evoked response in the contralateral hippocampus. Tetanus toxin was injected into the right hippocampus in two animals and the left hippocampus in one. Buffer was injected into the right hippocampus of one control animal and the left hippocampus in the other control animal. The surgery was completed as usual.

Data analysis

Selected recordings were digitized (1401, Cambridge Electronic Design, Cambridge, UK) and analyzed using SPIKE2 (Cambridge Electronic Design). Epochs of 1–3 s were used to compare activity in different hippocampal subregions. We used an automated search routine to detect population spikes >0.3 mV in amplitude (Cambridge Electronic Design). The time of the population spike was taken to be the time at the minimum of the spike.

Analysis of synchronization of the electrographic activity in the two hippocampi used cross-correlations of the waveforms or of the times of the population spikes [treated in the same way as single units (Engel et al. 1990)], using the SPIKE2 software (Cambridge Electronic Design). Fitting damped cosine waves (Gabor function) to the cross-correlograms allowed quantification of the relationship between the oscillations at the two sites (Engel et al. 1990). We used the fits to estimate the amplitude of the modulation, its frequency, and the phase difference, φ, of population spike firing in right and left CA1. The number of population spikes varied between data epochs. Therefore we normalized the modulation amplitude with respect to the offset of the Gabor function from the baseline (Engel et al. 1990). The normalized modulation amplitude could be compared across different data epochs.

Demonstrating that neuronal firing at two sites oscillates at the same frequency gives no information on whether the firing is synchronous. The delay, or phase lag, between the sites is a second important variable. Here we used the phase angle, φ, extracted from the Gabor fits, as an equivalent of time and used it to calculate a measure of synchronization that we refer to as the “synchronization index.” This is a composite measure of the strength of the correlation and the phase lag between the two sites. It is defined as the product of the normalized modulation amplitude and (cos φ + 1)/2. Both the normalized modulation amplitude and [(cos φ + 1)/2] vary continuously between zero and one. Thus the synchronization index also varies between zero and one. A synchronization index of zero indicates perfect synchrony with strong correlation and zero phase lag. A synchronization index of zero designates asynchrony of the population spikes with the normalized modulation amplitude equal to zero, and/or the epileptic bursts 180° out of phase with each other. All statistics are quoted as mean ± SE unless noted.

RESULTS

Unilateral intrahippocampal injection of tetanus toxin results in a chronic epilepsy syndrome resembling temporal lobe epilepsy (Hawkins and Mellanby 1987). We used this focal epilepsy model to study secondary generalization of seizures. Recording electrodes were implanted into CA3 and CA1 (CA3/CA1) of both dorsal hippocampi in 10 toxin-injected and 4 control animals. A further four toxin-injected and two control rats had electrodes implanted into CA3 and the dentate granule cell layer (CA3/dentate) of both dorsal hippocampi. A total of 88 spontaneous seizures were recorded from the ten animals with CA3/CA1 recording electrodes and 93 spontaneous seizures from the 4 animals with CA3/dentate electrodes. No rats developed status epilepticus.

Secondary generalization

The proportion of generalized seizures was 63% and nongeneralized seizures 37% (n = 179, 14 rats). All rats had both seizure types except for two animals where we recorded generalized seizures only. Nongeneralized seizures were most common during the early part of the seizure syndrome (Fig. 1). We measured the time from seizure onset to generalization (identified by the time during the seizure when both forepaws were first lifted of the ground) to determine whether there was a constant latency to secondary generalization. When we examined the seizure syndromes of individual rats we found that the latency to seizure generalization was consistent throughout the seizure syndrome [latency to generalization of first recorded generalized seizure = 20.4 ± 2.9 s, latency to generalization of last recorded generalized seizure = 20.0 ± 2.6 s; P = 0.93, paired t-test, n = 5 rats]. However, the mean latency to generalization varied significantly between animals [n = 47...
Seizure stage classification

We explored whether the properties of discharges in the hippocampi during seizures had any bearing on how fast focal seizures became secondarily generalized. The hippocampal discharge appeared to evolve during individual seizures but not to progress with repeated seizures. Progressive changes in discharge waveform, spike frequency, and duration of successive afterdischarges have been reported during kindling (Racine 1972a,b). However, we did not record similar changes during spontaneous seizures in the tetanus toxin model. We divided the seizure discharges we recorded into five stages to facilitate further analysis. Seizure discharges comprise subunits that resemble interictal spikes. We refer to the envelope of these subunits as field postsynaptic potentials, although the envelope will contain a contribution from intrinsic currents. These field postsynaptic potentials form slow (<30 Hz) oscillations during seizures and it is the frequency of these slow oscillations that provide the basis for our classification. We use Roman numerals to avoid confusion with Racine’s behavioral seizure classification for kindling (Racine 1972b).

STAGE I. Beginning of the seizure. The hippocampal discharge may start in two ways (Fig. 2, Aa and Ab). The seizure onset was sudden in 67% (59 of 88) of seizures. A prolonged field postsynaptic potential was recorded in CA3 followed by irregular field postsynaptic potentials and rapid progression to stage II activity (Fig. 2A). The remaining 33% (29 of 88) of seizures started with 2–3 Hz field postsynaptic potentials (Fig. 2B). These rapidly evolved to polyspike-like discharges comprising 10 Hz field postsynaptic potentials.

STAGE II. Seizures which started suddenly developed high-frequency regular field postsynaptic potentials (Fig. 2Aa). Seizures which started with 2–3 Hz field postsynaptic potentials developed longer polyspike-like discharges (Fig. 2B). Field postsynaptic potential frequency in both types of activity was in the 10–30 Hz range, commonly 15–20 Hz.

STAGE III. High-frequency (stage II) EEG activity became discontinuous (Fig. 2A). Discharges which resembled polyspikes were separated by a “flat” EEG trace. The mean interburst interval was 241 ± 11 ms (n = 19 seizures recorded from 5 animals). The field postsynaptic potential frequency was slightly slower than stage II, in the range of 12–20 Hz.

STAGE IV. The discharges described in stage III lengthened or became continuous (Fig. 2A). The field postsynaptic potential frequency slowed to 9–16 Hz and became more regular (Fig. 2A, c and e). This stage played a key role in secondary generalization (see Stage IV activity precedes secondary generalization).

STAGE V. Late seizure stage. Epileptiform activity often stopped completely after stage IV. However, in 32% of seizures, rhythmic slow-field potential oscillations restarted after a quiet period of up to 30 s. Small-field postsynaptic potentials appeared first and evolved over seconds into interictal-like bursts (Fig. 2C). These bursts resembled the clonic
discharges commonly seen toward the end of a seizure (Ayala et al. 1970; Matsumoto and Ajmone Marsan 1964). As the interictal-like bursts became longer they occurred more regularly, commonly at 2–5 Hz, and the small field postsynaptic potentials disappeared. Alternatively, the stage V interictal type bursts evolved directly from stage IV without a quiet phase. Stage V discharges continued for up to 2 min.

Seizure duration

Recruitment of motor centers involved in secondary generalization of seizures may become more likely the longer the seizure discharge continued. This would imply that generalized seizures last longer than nongeneralized seizures on average. A frequency plot of seizure duration had a bimodal distribution for the nongeneralized seizures and a skewed unimodal distribution for the generalized seizures (n = 88 seizures, data not shown). Stage V activity resembled the clonic bursts that occur as neurons hyperpolarize after higher frequency discharges earlier in the seizure (Ayala et al. 1970; Matsumoto and Ajmone Marsan 1964) and normally was the final stage of generalized seizures. We measured the duration of hippocampal discharges excluding stage V to assess whether generalized seizures lasted longer. The mean duration for generalized seizures was 48.4 ± 2.0 s and for nongeneralized seizures was 46.2 ± 4.2 s (Fig. 3A). These results were not statistically different (Mann–Whitney Rank sum test, P = 0.27). We concluded that secondary generalization was independent of the duration of seizure discharges.

Hippocampus leading at seizure onset

Seizure discharges may be first recorded from the uninjected or injected hippocampus after unilateral tetanus toxin injection (unpublished observations). Therefore we analyzed 77 seizures to determine whether there was a correlation between the hippocampus leading at seizure onset and secondary generalization. Generalized seizures originated from the injected hippocampus and uninjected hippocampus in 73.5 and 26.5% of fits, respectively. For nongeneralized seizures these figures were 73.3 and 26.7%, respectively. The proportions of generalized and nongeneralized seizures arising from the injected and uninjected hippocampi are not significantly different (confidence intervals for population proportions, P > 0.25). Thus secondary generalization is independent of the hippocampus leading at seizure onset.

We explored whether the frequency of discharges in the dorsal hippocampi played a role in secondary generalization of seizures. Of 31 nongeneralized seizures, 29 progressed to stage III or less. Two nongeneralized seizures reached stage IV but stopped suddenly 17 and 20 s after seizure onset. In contrast, we found that 95% (n = 42 seizures from 5 rats) of generalized seizures progressed to stage IV. These data show that nongeneralized seizures reached stage IV significantly less often (P < 0.001, Fisher exact test).

During stage IV (Fig. 4A) there was a strong cross-correlation between the 9–16 Hz (Fig. 4B) discharges recorded in left CA1 and right CA1 (Fig. 4C) and also between those recorded from right and left CA3 (not shown). Furthermore, population spikes in right and left CA1, which reflect volleys of action potentials in the major afferents from the hippocampi, occurred together in bursts at the same frequency (Fig. 4D). This contrasts with discharges during the preceding stage III (Fig. 5A). Although discharge frequencies in stages III and IV can be similar (Fig. 5, A and B), the cross-correlation of discharge waveforms in the right and left hippocampi was much weaker in stage III (Fig. 5C) and CA1 population spikes were less synchronized. The latter is illustrated in Fig. 5D where the cross-correlogram of population spikes in right and left CA1 during stage III shows a modest sinusoidal modulation, but the firing is ∼180° out of phase, i.e., population spikes in right CA1 were not synchronous with those in left CA1.

We quantified the firing relationship between the hippocampi by fitting damped cosine waves to cross-correlograms of population spikes in right and left CA1 and calculating the normalized modulation amplitude (Engel et al. 1990) and the phase difference between population spike firing in left and right CA1 (Figs. 4D and 5D). We found an oscillatory component to the cross-correlogram of CA1 population spikes with a mean frequency of 12.3 ± 1 Hz (n = 7). There was a substantial, and statistically significant, increase in normalized modulation amplitude from 0.37 ± 0.10 during stage III to 0.88 ± 0.06 during stage IV in the seconds before secondary generalization (P = 0.002, paired t-test, n = 8). The mean of the absolute phase difference between right and left CA1 population spikes during stage III was 1.33 ± 0.39 rad and this decreased to 0.84 ± 0.22 rad during stage IV (P = 0.35, paired t-test, n = 8). We quantified the degree of synchronization between population spikes in right and left CA1 by calculating the synchronization index (see METHODS). We found that stage IV activity was associated with a marked increase in synchronization of CA1 population spikes across the dorsal hippocampi (synchronization index stage III = 0.29 ± 0.11, stage IV = 0.68 ± 0.07; P = 0.004, paired t-test, n = 8).

Our recordings from the dentate gyrus during seizures allowed us to study inputs to the hippocampi. Again, a 9–16 Hz oscillation was recorded from both left and right dentate gyrus during stage IV activity (Fig. 6, A and B). The waveforms of epileptic discharges in the right and left dentate gyrus were strongly correlated (Fig. 6C) and population spikes tended to fire together (Fig. 6D).

Stage IV activity precedes secondary generalization

Our data suggested that there was synchronization, on a time scale of milliseconds, of stage IV discharges between the right
and left hippocampi in those seizures that became secondarily generalized. We measured the time difference between the onset of stage IV activity and secondary generalization to assess whether stage IV activity started before secondary generalization. We found that stage IV activity preceded secondary generalization in 98% \((n = 40)\) of generalized seizures (40 of 42 seizures; Fig. 7). The median time from the onset of stage IV activity to secondary generalization was 4 s (IQR, 3–11 s). Stage IV activity continued after the seizures became secondarily generalized. The proportion of seizures that became secondarily generalized after the onset of stage IV activity is 0.93 (39 of 42 seizures). We concluded that the occurrence of stage IV activity during a seizure is a strong predictor of secondary generalization.

**Ventral commissure lesions**

Although stage IV activity synchronized between the two hippocampi rapidly, it was not clear whether secondary generalization required stage IV activity in both hippocampi. Therefore we cut the ventral commissure/fimbria in five rats (Fig. 8A). Unilateral intrahippocampal tetanus toxin was injected in three rats. The remaining two rats were injected with buffer and acted as controls.

Recordings in buffer-injected rats with ventral commissure lesions were not completely normal. A 25–100 ms duration “spike” occurred ipsilateral and contralateral to the ventral commissure (Fig. 8B). The spikes were recorded intermittently and on occasions formed doublets or triplets but never occurred repetitively at a frequency of \(<3\) Hz for several seconds as seen at the beginning of a seizure. Electrographically, a low amplitude field potential oscillation in CA3 preceded the CA1 waveform by 2–4 ms. In contrast, the CA1 field potential oscillation was very large, up to 7 mV. The CA1 spike could have either a positive- or negative-going envelope (presumably reflecting the relative locations of the electrodes and the synaptic currents responsible) with small amplitude negative oscillations superimposed, consistent with population
spikes. Similar EEG spikes have been reported previously following lesions of the ventral commissure and fornix (Buzsáki et al. 1989). We did not detect any behavioral response associated with spikes.

Interictal spikes, polyspikes, and seizures were recorded following lesioning of the ventral commissure in the rats injected with tetanus toxin in addition to the spikes seen in buffer-injected animals. The forms of epileptiform activity and motor behavior were qualitatively the same as previously described for rats with intact ventral commissures. The percentage of seizures that started in the injected and uninjected hippocampi of lesioned animals (injected 82%, uninjected 18%, n = 11) were not significantly different from the results for rats with intact ventral commissures (P = 0.72, Fisher Exact test). However, lesioning the ventral commissure was associated with a significant decrease in the proportion of seizures that generalized (29% generalized, 71% nongeneralized, n = 14) compared with the proportion of generalized seizures found in nonlesioned rats (χ² = 5.13, P = 0.02).

The temporal relationships of field postsynaptic potentials in CA3 and CA1 within each hippocampus were unchanged but propagation of epileptic discharges across the transected ventral commissure was blocked (Fig. 8C). We studied the progression of the hippocampal field potentials during seizures paying particular attention to stage IV oscillations. Stage IV activity occurred in either hippocampus but not simultaneously (Fig. 8D) and could switch between hippocampi during a seizure. Stage IV activity occurred in all generalized seizures. Stage IV oscillations also occurred in nongeneralized seizures, much less commonly than during generalized seizures, but more often than in nongeneralized seizures in nonlesioned rats. Of 10 nongeneralized seizures recorded from 3 lesioned rats, 4 seizures showed stage IV activity. This is a significantly greater frequency than that in nonlesioned rats where only 6% (2/31) of nongeneralized seizures developed stage IV activity and they stopped abruptly (P = 0.02, Fisher exact test). We concluded that stage IV activity was more likely to be followed by secondary generalization when the output from both dorsal hippocampi was synchronous.

**DISCUSSION**

We used the tetanus toxin model of epilepsy to study secondary generalization of focal seizures. Our major conclusions were as follows: 1) secondary generalization was preceded by a 9–16 Hz oscillation of the epileptic discharge waveform in the dentate gyrus, CA3, and CA1 subregions of both dorsal hippocampal formations; 2) the 9–16 Hz oscillation was accompanied by synchronization of the outputs from both dorsal hippocampi; and 3) disruption of the connection between the dorsal hippocampi, which prevents synchronization of epileptic discharges across right and left dorsal hippocampi, reduced the probability of 9–16 Hz oscillations being followed by secondary generalization of seizures.
Seizure stage classification

We described a five-stage classification of EEG activity during spontaneous seizures in freely moving rats previously injected with tetanus toxin. This contrasts with the division of seizure discharges, which were induced by the acute cortical application of penicillin in anesthetized animals, into tonic and clonic phases (Ayala et al. 1970; Matsumoto and Ajmone Marsan 1964). We found that there was an orderly evolution of the hippocampal EEG during seizures in our freely moving rats which was more complex than described by the tonic-clonic classification.

Other epileptiform models do not fit the tonic-clonic classification either. For instance, tetanic stimulation was followed by a primary afterdischarge comprising a slow oscillation at 2–12 Hz and faster oscillations at 30–120 Hz and 200–400 Hz (Bragin et al. 1997; Leung 1987). After the primary afterdischarge terminated, the EEG became flat for several minutes before the development of a secondary afterdischarge. The main frequency of the slow rhythmic component of the primary afterdischarge described by Bragin et al. (1997) was 2–6 Hz, slightly slower than that for stages II–IV of spontaneous seizures in the tetanus toxin model of epilepsy.

Stage IV activity

Our recordings showed that a 9–16 Hz oscillation in hippocampal field potentials (stage IV) preceded secondary generalization of focal temporal lobe seizures. Acute experiments in vivo revealed that stimulation of hippocampal inputs at 8–12 Hz produces marked frequency potentiation of hippocampal responses (Andersen and Lømo 1967). Thus the
burst frequency during seizures experienced by our freely moving rats converged on a frequency range expected to augment seizure discharges in postsynaptic sites.

The frequencies of the major oscillations recorded from the normal hippocampal formation of awake rats are in the $\theta$ (4–8 Hz) and $\gamma$ (30–80 Hz) ranges (Bragin et al. 1995; Vanderwolf 1969). These oscillations have bandwidths on either side of the burst frequencies (9–20 Hz) where most power is concentrated during seizures. Combined experiments and computer modeling of epileptic discharges generated by hippocampal slices in vitro bathed in the GABA$_A$ antagonist, picrotoxin, suggests that polyspike-like discharges in CA3 are produced by a combination of prolonged depolarization, which is synaptically mediated, and activation of intrinsic voltage-dependent calcium currents (Traub et al. 1993). The frequency of bursts during these discharges can be high for short periods at the beginning of discharges but rarely exceeds 8 Hz for prolonged periods when hippocampal slices are bathed in either bicuculline or picrotoxin alone (Borck and Jefferys 1999; Hablitz 1984; Miles et al. 1984; Swann et al. 1993; Traub et al. 1993). However, addition of picrotoxin and the metabotropic glutamate receptor (mGluR) agonist, (1S,3R)-1-aminocyclopentane-1,3 dicarboxylic acid (ACPD), to hippocampal slices prepared from juvenile rats results in spontaneous epileptiform discharges in CA3 comprising bursts with frequencies up to 30 Hz (Merlin et al. 1995; Taylor et al. 1995). Similar discharges may be evoked by a brief tetanus. A comparable situation may occur during spontaneous seizures in tetanus toxin injected rats, where there is a relatively selective loss of inhibition (Empson and Jefferys 1993; Whittington and Jefferys 1994). Synchronised bursts of inputs, the equivalent of a brief tetanus in vitro, would initiate discharges. The increasing duration of discharges seen during stage III of seizures may be caused by an increase in activation of mGluRs as the seizure progresses.

Oscillations in the 10–25 Hz range have been described in other subregions of hippocampal slices in vitro. Tetanic stimulation of CA1 is followed by population spikes that are initially at $\gamma$ (30–90 Hz) frequencies but slow to the $\beta$ (10–25 Hz) range before terminating (Bracci et al. 1999; Whittington et al. 1997). However, the duration and morphology of these discharges differs from the 9–16 Hz oscillation we recorded before secondary generalization, suggesting that different mechanisms underpin the two types of discharge. An alternative possibility is that recruitment of extrahippocampal sites into the neuronal network generating the seizure contributed to the emergence of stage IV activity.

**Ventral commissure/fimbria lesions**

Histology revealed that the lesions to the ventral commissure also involved the fimbria. This complicates interpretation of the effects of the lesion because the fimbria carry GABAergic connections to the hippocampus. Ventral commissure/fimbria lesions...
(Freund and Antal 1988) and neuromodulatory inputs such as the cholinergic pathway from the septum the dorsal hippocampus (Swanson and Cowan 1977). It could be argued that loss of neuromodulatory inputs to the hippocampus ipsilateral to the lesion affected neuronal excitability in a manner which prevented recruitment of that hippocampus into the seizure. We believe that this probably is not the case. First, lesioning the fimbria results in interictal spikes, suggesting that the lesion is proconvulsant (Buzsáki et al. 1989). Second, we recorded seizures originating in the injected hippocampus even if the ipsilateral ventral commissure/fimbria were lesioned.

Seizure discharges were recorded in the uninjected hippocampus when the ventral commissure, the major link between the dorsal hippocampi, was cut. We cannot be definitive about the provenance of this epileptic activity because our recordings did not include extrahippocampal sites. However, one possibility that needs to be considered is that it was driven by seizure activity at subcortical sites (Gale 1992).

Synchronization of CA1 firing

Synchronization occurs at several levels during seizures. First, population spikes require tight local synchrony of neuronal firing. Second, during stage IV activity there is increased synchrony of firing between right and left CA1, the major outputs of the hippocampi. The dorsal hippocampal project to entorhinal cortex and perirhinal cortex, in part via the subiculum (Witter et al. 1989). Multi-site recordings during kindling of the dorsal hippocampus suggest that seizures originating in the dorsal hippocampi propagate to motor centers via the amygdala, piriform cortex, and perirhinal cortex (McIntyre and Kelly 1993). Although the perirhinal cortex has a high threshold for afterdischarges, once they are evoked there is rapid spread of epileptic activity to motor centers (McIntyre et al. 1993). It is likely that secondary generalization of seizures in the hippocampal tetanus toxin model of epilepsy occurs via the same pathways.

We believe that the increase in synchrony and frequency of stage IV discharges set up optimal conditions for recruitment of sites postsynaptic to the hippocampi into the epileptic discharge and therefore secondary generalization of seizures. First, the frequency of bursts is in the range producing frequency potentiation of hippocampal outputs (Andersen and Lomo 1967). Second, synchronization of convergent inputs increases the likelihood that postsynaptic neurons will fire (Stevens and Zador 1998).

Bilateral synchrony of stage IV activity was lost after lesioning the ventral commissure. This was associated with a reduction in, but not an abolition of, secondarily generalized seizures. These results suggest that the larger the hippocampal network involved in stage IV activity the greater the probability of secondary generalization of seizures.

We conclude that the oscillatory properties of epileptic discharges in the hippocampi do play a role in secondary generalization of focal seizures in the tetanus toxin model of temporal lobe epilepsy. Secondary generalization represents an example of a pathological motor behavior associated with a hippocampal field potential oscillation. Understanding what causes the 9–16 Hz oscillation to emerge in vivo may open avenues to prevent its occurrence and offer the possibility of therapeutic benefit to epilepsy patients.

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