Neocortical Very Fast Oscillations (Ripples, 80–200 Hz) During Seizures: Intracellular Correlates

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Grenier, François, Igor Timofeev, and Mircea Steriade. Neocortical very fast oscillations (ripples, 80–200 Hz) during seizures: intracellular correlates. J Neurophysiol 89: 841–852, 2003; 10.1152/jn.00420.2002. Multi-site field potential and intracellular recordings from various neocortical areas were used to study very fast oscillations or ripples (80–200 Hz) during electrographic seizures in cats under ketamine-xylazine anesthesia. The animals displayed spontaneously occurring and electrically induced seizures comprising spike-wave complexes (2–3 Hz) and fast runs (10–20 Hz). Neocortical ripples had much higher amplitudes during seizures than during the slow oscillation preceding the onset of seizures. A series of experimental data from the present study supports the hypothesis that ripples are implicated in seizure initiation. Ripples were particularly strong at the onset of seizures and halothane, which antagonizes the occurrence of ripples, also blocked seizures. The firing of electrophysiologically defined cellular types was phase-locked with ripples in simultaneously recorded field potentials. This indicates that ripples during paroxysmal events are associated with a coordination of firing in a majority of neocortical neurons. This was confirmed with dual intracellular recordings. Based on the amplitude that neocortical ripples reach during paroxysmal events, we propose a mechanism by which neocortical ripples during normal network activity could actively participate in the initiation of seizures on reaching a certain threshold amplitude. This mechanism involves a vicious feedback loop in which very fast oscillations in field potentials are a reflection of synchronous action potentials, and in turn these oscillations help generate and synchronize action potentials in adjacent neurons through electrical interactions.

INTRODUCTION

Since the early description of electrographic patterns defining different types of epileptic seizures, a number of studies have refined our knowledge of the neurophysiological events occurring during paroxysmal events. Some seizures, resembling those seen in the Lennox-Gastaut syndrome, generally consist of spike-wave (SW) complexes at 2–3 Hz and fast runs at 10–20 Hz (reviewed in Niedermeyer 1999). Other components of seizures and related electrographic events in the neocortex are faster oscillations, at 50–80 Hz (Allen et al. 1992) and 70–130 Hz (Fisher et al. 1992; Traub et al. 2001). In epileptic patients, the presence of neocortical very fast oscillations (70–130 Hz) at the onset of seizures has led to the proposal that they could be involved in their initiation (Fisher et al. 1992; Traub et al. 2001). In the hippocampal-entorhinal cortex axis, very fast oscillations (fast ripples, 250–500 Hz) have been linked to seizure initiation and epileptogenesis (Bra- gin et al. 1999a–c, 2002).

The initiation of seizures is an important topic because interfering with the mechanisms involved in the onset of paroxysms might constitute a therapeutic avenue against seizures. Data from intracellular studies on anesthetized animals suggest that some seizures arise in neocortex, based on the following experimental evidence. 1) Seizures consisting of SW complexes and fast runs are generated in neocortex even after thalamectomy (Steriade and Contreras 1998) and in isolated neocortical slabs (Timofeev et al. 1998). 2) During such seizures, the majority of thalamocortical neurons are hyperpolarized and display phasic inhibitory postsynaptic potentials (IPSPs) but do not fire rebound spike bursts (Pinault et al. 1998; Steriade and Contreras 1995; Timofeev et al. 1998). 3) Complex seizures, with relatively slow SW complexes and fast runs, evolve without discontinuity from the cortically generated slow sleep oscillation (Steriade et al. 1998a). The slow oscillation (generally 0.5–1 Hz) consists of an alternation between hyperpolarized and depolarized membrane potential (Steriade et al. 1993a). This sleep rhythm arises in neocortical networks as it survives thalamectomy (Steriade et al. 1993b) and is absent in the thalami of decorticated animals (Timofeev and Steriade 1996).

In a previous study, we have analyzed the presence of neocortical ripples (80–200 Hz) during natural states of vigilance and under some anesthetics and have shown that these oscillations coincide with increased neuronal depolarization and firing in all types of neocortical neurons (Grenier et al. 2001). In conjunction with the presence of an oscillation of similar frequency in the electroencephalogram (EEG) of epileptic patients at seizure onset (Fisher et al. 1992; Traub et al. 2001), two more factors suggest that these neocortical ripples could play a role in initiating seizures: their presence during the depolarizing phase of the slow oscillation, which is known to evolve into seizures, and the strong correlation between neuronal excitation and the intensity of neocortical ripples. In this paper, we present the first in vivo description of neocortical ripples during seizures using multi-site field and intracellular recordings. Our results support the hypothesis that these oscillations are involved in seizure initiation. Based on the neuronal correlates of ripples during nonparoxysmal (Grenier et al. 2001) and paroxysmal activities and on the intensity they reach in field potentials at the onset of seizures, we propose a

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mechanism by which neocortical ripples could actively take part in the initiation of seizures.

METHODS

Experiments were conducted on 105 adult cats that were acutely prepared under ketamine-xylazine anesthesia (10–15 and 2–3 mg/kg im, respectively; n = 95) or barbiturate anesthesia (pentobarbital sodium, 35 mg/kg ip; n = 10). The animals were paralyzed with gallamine triethiodide after the EEG showed typical signs of deep general anesthesia, essentially consisting of a slow oscillation (0.5–1 Hz), which is similar under ketamine-xylazine anesthesia (Contreras and Steriade 1995) and during natural slow-wave sleep in chronically implanted animals (Steriade et al. 1996, 2001). Supplementary doses of anesthetics were administered at the slightest changes toward anesthetic levels. The cats were ventilated artificially with the control of end-tidal CO_2 at 3.5–3.7%. In some experiments (n = 8), the effect of halothane was tested by administration through the artificial ventilation at a concentration of 0.5–2%. The body temperature was maintained at 37–38°C, and the heart rate was ~90–100 beats/min. For intracellular recordings, stability was ensured by the drainage of cisterna magna, hip suspension, bilateral pneumothorax, and by filling the hole made for recordings with a solution of 4% agar.

Single and dual intracellular recordings from suprasylvian association areas 4, 5, and 7 were performed using glass micropipettes filled with a solution of 3 M potassium acetate (KAc) or one pipette filled with KAc and the other with potassium chloride (KCl). A high-impedance amplifier with active bridge circuitry was used to record the membrane potential (V_m) and inject current into the neurons. Field potentials were recorded in the vicinity of impaled neurons and also from more distant sites, using bipolar coaxial electrodes, with the ring (pial surface) and the tip (cortical depth) separated by 0.8 mm. In 16 cats, arrays of seven or eight electrodes, ~1.5 mm apart, were inserted along the suprasylvian gyrus (see Fig. 3). Glass micropipettes were also used to record field potentials. Intracellular and field potential signals were recorded on an eight-channel tape recorder with a band-pass of 0–9 kHz. They were also recorded with a 16-channel vision data-acquisition system from Nicolet, at a sampling of 10 or 20 kHz. At the end of experiments, the cats were given a lethal dose of pentobarbital.

Data analysis

Ripple cycles used for computing the wave-triggered averages (WTAs) and the peri-event histograms (PEHs) had at least four times higher amplitudes than the SD of the whole EEG filtered trace (between 80 and 200 Hz). In PEHs of firing related to ripples, the depth-negative peak of ripples was chosen as zero time. In these PEHs, the ripple trace at top is an average of 10 individual cycles.

RESULTS

Spontaneous and evoked neocortical seizures

Under ketamine-xylazine anesthesia, seizures occurred spontaneously (n = 1384) and/or were triggered by electrical stimulation (n = 372). These seizures consisted of SW or polyspike-wave (PSW) complexes at 2–3 Hz and fast runs at 10–20 Hz (Steriade et al. 1998a). We encountered two general modes of occurrence of spontaneous seizures: they arose from the slow-wave sleep (n = 321; as in Figs. 1 and 2) or they occurred repetitively (n = 1063), one seizure starting from the postictal depression of the preceding one, like the condition of status epilepticus (as in Fig. 3). Spontaneous seizures occurred in 27% of cats under ketamine-xylazine anesthesia (26 of 95), a value similar to that found in our previous studies (Steriade et al. 1998a). Seizures were evoked by trains of 10–25 electrical stimuli at 100 Hz repeated every second in the neocortex, a pattern that resembles the spontaneous occurrence of ripples at the start of seizures.

Ripples in neocortex during seizures

Ripples were observed in field potential recordings in the majority of seizures (n = 1287, 93%). During seizures, and especially at their onset, depth-negative EEG “spikes” were crowned with very fast oscillations that were more obvious after filtering between 80 and 200 Hz (Fig. 1, A and B, right). Neocortical ripples during the slow sleep-like oscillation were described in a previous paper (Grenier et al. 2001). They occurred during the EEG depth-negative phase (Fig. 1B, left;
also Fig. 4) that corresponds to neuronal depolarization. Because of their relatively small amplitude, they did not lead to a peak in fast Fourier transforms (FFTs) during the slow oscillation (Fig. 1C, left, thin trace). During seizures too, ripples occurred during the depth-negative phase of field potentials, in this case EEG spikes, but with much higher amplitude than during the slow oscillation (Fig. 1B). Their appearance over an EEG spike had the general pattern of a waxing-and-waning sequence. Within one EEG spike, there could be from 3 to 30 cycles [13 ± 2 (SE) cycles, 10 different animals]. In contrast to the slow oscillation, FFTs of EEG recordings during seizures (Fig. 1C, left, thick trace) showed a strong peak at ~100–130 Hz (117 ± 3 Hz, mean frequency at the peak ± SE). This occurred when ripples were present throughout seizures rather than just at their onset. Their frequency during seizures, calculated directly from the recordings, was 118 ± 6 Hz (range: 91–148 Hz, 10 different animals). Our decision to filter EEG traces between 80 and 200 Hz to single out these oscillations is based on these values.

Neocortical ripples are usually stronger at, but not restricted to, seizure onset

Ripples often displayed their highest amplitude at the onset of seizures. In a sample of seizures taken from all cats, 15 of 25 seizures evolving from the slow oscillation (60%, example in Fig. 1, top) and 19 of 25 recurring seizures (76%, example in Fig. 11) had this pattern, which was revealed in filtered traces. Sometimes, recordings with macroelectrodes did not show this pattern, while local field potential recorded with a micropipette or cellular recordings did (see for example Fig. 11, DC field and glial cell recordings vs. EEG). Ripples were not restricted to the onset of seizures. They could also be present throughout seizures and even on the last EEG spike. The only oscillations that displayed the striking pattern of strongest amplitude at the onset of seizures were within the 80- to 200-Hz frequency range.

When seizures evolved from the slow oscillation, strong ripples occurred just before the transition between normal and paroxysmal activity (Fig. 2). The last cycle of the slow oscillation before the seizure (Fig. 2A) and the first paroxysmal EEG spike (Fig. 2B) were very similar at their onset. However, before the EEG spike reached the negative peak level of the slow oscillation, ripples appeared over it and were present until the paroxysmal event reached its full extent. Thus ripples of strong amplitude were present right at the transition point (marked by arrows, Fig. 2B) between normal and paroxysmal activities. This indicates that strong ripples are not strictly dependent on the paroxysmal events but can precede their

FIG. 2. Neocortical ripples are present at the transition between normal and paroxysmal EEG spikes. Field potential recording from area 5. Spontaneous seizures evolving from the slow oscillation. Top: an epoch is shown along with the filtered trace between 80 and 200 Hz. The 2 underlined parts are expanded below. A: a depth-negative component of the slow oscillation reaching around ~3 mV, with the depth-positive phase being set at 0 mV. B: the 1st EEG spike of the seizure. Note that ripples appear in this case (B) at about the field level reached by the nonparoxysmal (A) negativity and that ~15 cycles of ripples occur before the negativity reaches values of seizure EEG spikes (~14 mV). Based on the amplitude of the field potentials, the section between the 2 arrows may be considered as the transition between nonparoxysmal and paroxysmal negativities.
onset. The moment of occurrence of these ripples suggests that they are involved in the evolution of a normal field potential event into a paroxysmal one.

**Neocortical ripples are present at seizure onset in foci where the paroxysmal event is initiated**

With multi-site recordings during recurring seizures \((n = 4)\), ripples were present from seizure onset within the site where paroxysmal activity started. Arrays of seven or eight electrodes were inserted along the antero-posterior axis of the suprasylvian gyrus \((n = 16; 4\) cats displayed seizures under these conditions). The site where the first EEG spike occurred changed from seizure to seizure, and the time of appearance of ripples at seizure onset (Fig. 3, ---, middle and bottom) followed closely the onset of the first EEG spike in different sites (Fig. 3, —, middle and bottom). Ripples were present from the onset of the progressively growing, first (precursor) EEG spikes (Fig. 3, left: EEG 5, and right: EEG 2), whereas they appeared with some delay over the abruptly rising follower EEG spikes (Fig. 3, left: EEG 2, and right: EEG 5). Similar results were obtained in the three other animals.

**Presence of ripples correspond to neuronal depolarization during both the slow oscillation and seizures**

Neocortical ripples during the slow oscillation correspond to neuronal depolarization (Grenier et al. 2001). This correlation was also present during seizures, with both field ripples and neuronal depolarization being stronger than during the slow oscillation. An example of a seizure evolving from the slow oscillation is illustrated in Fig. 4. Stronger ripples, revealed by the filtered trace, were present during the seizure than during the slow oscillation, and, as well, the neuron was more depolarized. There was a clear relation between the mean \(V_m\) of the neuron and the maximal amplitude of the field ripples (Fig. 4, bottom). All recorded neurons behaved in this manner. In the depicted case, the maximal amplitude of ripples was about three times higher during seizures than the slow oscillation.

**Halothane blocks ripples and seizures**

Very fast oscillations are abolished by various compounds both in vitro (Draguhn et al. 1998) and in vivo (Jones et al. 2000; Ylinen et al. 1995). These compounds have as common characteristic the fact that they block gap junctions. Consis-

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**FIG. 3. Paroxysmal EEG spikes start from ripples in sites where seizures are initiated. Depth field potential recordings from an array of 7 electrodes separated by 1.5 mm each along the antero-posterior axis of the left suprasylvian gyrus, plus 1 electrode in the contralateral gyrus (see scheme at top left). Seizures occurred spontaneously. Top right: recordings during 2 seizures. Middle: the expanded onsets of both seizures, A (1st seizure) and B (2nd seizure), along with the filtered trace (80–200 Hz). The onset of the first EEG spike is tentatively indicated (—) in panels with EEG traces, and the onset of ripples in panels with filtered traces (- - - line). This shows that ripples appear first in sites in which EEG spikes appear first. Bottom: EEG and filtered traces from 2 sites (2 and 5) are further expanded, revealing that the EEG spikes start with ripples in early sites (possibly the seizure focus), while in other sites (possibly receiving excitation from the seizure focus), ripples appear while the EEG spike is already started. This suggests that ripples are involved in the generation of the EEG spike itself in sites from which seizures originate.**
ently with these results, we have previously found that halothane strongly reduced the occurrence of neocortical ripples during the slow sleep-like oscillation (Grenier et al. 2001). In the present experiments, halothane administration diminished ripples and blocked the occurrence of seizures \((n = 4\) animals, 10 different administrations). Recurring seizures ceased to occur within a minute of halothane administration, which lasted for 1 min (Fig. 5). They returned some minutes \((12 \pm 4\) (SE) min) after stopping halothane administration (in 1 case, seizures did not return at all). The intensity of ripples was much stronger during seizures than during the period in which they were diminished or ceased to occur (Fig. 5, bottom). Before the seizures returned after cessation of halothane, the amplitude of ripples progressively increased. Seizures restarted after ripples’ amplitude reached a level comparable to that observed during the previous periods of seizures before halothane administration. This correlation suggests that the amplitudes of ripples and the capacity of the network to generate seizures are related.

Our neuronal database consisted of 570 intracellularly recorded neurons, with the following proportions: regular spiking (RS), 56\% \((n = 317)\); intrinsically bursting (IB), 9\% \((n = 52)\); fast rhythmic bursting (FRB), 23\% \((n = 132)\); and fast spiking (FS), 12\% \((n = 69)\) (Connors and Gutnick 1990; Gray and McCormick 1996; Steriade et al. 1998b). As seizures occurred in 27\% of animals, only a proportion of these neurons were recorded during seizures and 10 cells of each type were selected for further analyses.

We describe first the characteristics of RS, IB, and FRB neurons’ behavior in relation with ripples in field potentials during seizures. This behavior can be divided in two main categories.

First, neurons could fire at frequencies similar to, or lower than, ripple frequency. In those cases, the action potentials had
a strong tendency to be in phase with the negative peak of a ripple cycle. The phase-locked firing with negative peaks of ripples in field potentials was confirmed by PEHs (Fig. 6A for a RS cell). Phase-locked repetitive firing with ripples resulted in a peak in interspike interval histograms between 8 and 11 ms, corresponding to the period of ripples (Fig. 6A). Neurons that did not fire on most cycles of ripples could display sub- or supra-threshold membrane potential fluctuations within ripple frequency (Fig. 6B). In general, these fluctuations were in opposite phase to the field ripples (Fig. 6B, WTA).

Second, other neurons could fire at frequencies higher than that of ripples. In those cases (some IB and FRB cells), the major peak in the interspike interval histogram was ~3.5 ms (Fig. 7 for an IB cell). PEHs revealed that the action potentials did not occur completely independently from the ripples, as some action potentials of the spike bursts tended to occur in phase with the depth-negative peak of ripples (Fig. 7, bottom left and PEH).

These behaviors were expressed in different proportions by different neuronal classes. RS cells, which form the majority of neocortical neurons (Connors and Gutnick 1990), either displayed $V_m$ fluctuations with few spikes (7 of 10 analyzed cells; see Fig. 6B) or fired phase-locked with most ripple cycles (3 of 10; Fig. 6A). Some IB cells fired at high frequencies with a relation between some spikes and ripple negative peaks (4 of 10 analyzed cells; Fig. 7), while the remaining behaved as described for RS neurons. The majority of FRB cells displayed phase-locked firing with most ripple cycles (7 of 10 neurons), while the remaining three neurons preferentially fired at higher frequencies.

These data indicate that the firing of a majority of neocortical neurons during paroxysmal events with ripples is correlated because of their preferential firing in phase with the negative peak of these oscillations. This was confirmed with dual intracellular recordings made during seizures with pipettes located close to each other (300–700 μm). Field potentials were recorded with one of the pipette before or after the intracellular recording (Fig. 8). The relation between ripples in field potential and neuronal firing was strong (Fig. 8, bottom left PEH). When a second cell was impaled, neuronal firing between the two cells was strongly correlated when ripples were present in the EEG (middle), as revealed by PEH (bottom right).

**Fast-spiking neurons and ripples**

If ripples are involved in the initiation of seizures, their mechanism of generation could be important in understanding...
how seizures are generated. Neocortical FS cells activity and GABA_A-mediated IPSPs were found to be important for ripple patterning during the slow sleep-like oscillation (Grenier et al. 2001) as was previously found for hippocampal ripples (Ylinen et al. 1995). However, the increased amplitude of these oscillations during seizures compared with the slow oscillation (Fig. 1) raise the possibility of different mechanisms being involved during normal and paroxysmal activities.

During the slow oscillation, FS cells fired in a preferred phase of the field ripples, \( \sim 2.5 \) ms before the depth-negative peak (Fig. 9, left) (see also Grenier et al. 2001, Fig 8). The cells were identified as FS on the basis of their action potential (<0.5 ms at half-amplitude) and their response to depolarizing current pulses, namely tonic firing without frequency adaptation. In contrast to the behavior of FS cells during the slow oscillation, all 10 analyzed FS cells during seizures fired at higher frequencies than those of ripples on most EEG spikes (Fig. 9, right). During this high-frequency firing, there was no clear modulation of firing by ripples (see PEH during seizure).

**Recording with Cl^-filled pipettes does not modify the relation between ripples and neurons during seizures**

During ripples in the slow oscillation, the firing of RS cells recorded with KCl was shifted by 3.5 ms in comparison with RS cells recorded with KAc. This indicates, along with the phase-locked firing of FS cells, that Cl^-dependence inhibition plays a role in the patterning of neuronal firing during ripples in the slow oscillation (Grenier et al. 2001). However, the results from FS cell activity during seizures suggest this may not be the case during paroxysmal events. Dual intracellular recordings with one of the two cells recorded with KAc and the other with KCl showed that, during paroxysmal EEG spikes, neurons recorded with KCl were more depolarized than the KAc-recorded ones by an average of 10 mV. This was caused by the shift toward more depolarized values of the chloride reversal potential after chloride infusion through the recording pipette (Timofeev et al. 2002). In contrast to what was observed during the slow oscillation, recording with KCl did not change the relation between intracellular activities and the ripples. In all the couples that we recorded during seizures with ripples (1 KAc and 1 KCl, \( n = 5 \)), intracellular activities of neurons recorded with KCl showed a relation to the field ripples that was very similar to that of neurons recorded with KAc. This was clear on a cycle-to-cycle basis (Fig. 10, middle), and was also made clear by computing cross-correlations between the two intracellular recordings. In the depicted case, the average phase shift was \( \sim 1 \) ms.

**Ripple oscillations are present in glial cells during seizures**

Seizure related neuronal events during EEG spikes are characterized by strong activation of synaptic and intrinsic conductances (reviewed in McCormick and Contreras 2001) and possibly field interactions (Faber and Korn 1989; Grenier and Steriade 2001; Jefferys 1995). To reveal a possible role for field interactions, we performed intracellular recordings of glial cells during seizures (n = 14; Fig. 11). Glial cells may be roughly considered as a control for cellular membranes without the whole battery of synaptic and intrinsic conductances that characterize neuronal membranes. This is a simplification because glial cells have been shown to possess neurotransmitters receptors (Verhratsky and Steinhauser 2000). Ripple oscillations were seen in glial cells and could reach values of a few mV (Fig. 11, middle). This suggests that, whatever mechanisms may be involved in the generation of ripples during paroxysmal events, their presence in field potentials during seizures might be sufficient to influence the potential of polarized membranes. Dual micropipette recordings (DC field potential recording followed by glial cell recording, in conjunction with intracellular recording of another glial cell) revealed that local field ripples were reflected in phase in glial cells. Cross-correlations between field and glial cells located \( \sim 500 \) \( \mu \)m apart laterally and at similar depth revealed phase lags of \( \sim 2 \) ms. When a glial cell was impaled with the same pipette, the cross-correlation between the two glial cells was almost identical to the field-glial cell correlation. This suggests an absence of phase lag between ripples in field potentials and glial cells in the same location and contrasted with neuronal recordings in which \( V_m \) fluctuations were mainly in opposite phase to field ripples. It also suggests an active involvement of
neurons and a more passive one for glial cells in relation to field ripples.

**DISCUSSION**

There are five main results in this study. **First**, our findings corroborate recent EEG data showing the association of strong neocortical very fast oscillations (80–200 Hz) with seizure onset. **Second**, we have provided evidence that neocortical ripples are not only associated with paroxysmal events such as EEG spikes but are present at their onset. This suggests that they are involved in the transition to paroxysmal events. **Third**, we have described the neuronal bases of neocortical ripples during seizures and shown that they involve coordinated firing in a majority of neurons. **Fourth**, we have shown that the mechanism of ripples’ generation is different during normal and paroxysmal events. **Fifth**, we have presented data supporting a mechanism for the involvement of neocortical ripples in seizure initiation.

**Our results and human EEG studies**

Our results are consistent with the reports of very fast oscillations at the start of seizures in foci from EEG of epileptic patients (Fisher et al. 1992, Traub et al. 2001). They support the hypothesis that these oscillations are involved in seizure initiation. We will propose a mechanism for that involvement. Our studies reveal that these neocortical ripples are also present during normal activities, but that they reach their highest amplitude during seizures. The similarities between the oscillations described here and those from human EEG studies suggest that they are the same phenomenon.

**Neocortical ripples as a possible trigger for seizures**

Our results show a strong association between neocortical ripples and the onset of seizures. As neocortical ripples are linked with neuronal depolarization during nonparoxysmal activities such as the slow sleep-like oscillation (Fig. 4) (see also Grenier et al. 2001), they could simply be a consequence of the strong neuronal depolarization occurring during seizures. Then an important question toward the understanding of seizure initiation is to determine if ripples are just associated with the events underlying seizures or if they take an active part in the transition to seizures. During repetitive seizures, in the focus where individual paroxysms appeared first (precursor site), ripples did not appear after the first EEG spike had already started, as they should if they were conditional on the occur-

![Fig. 8](https://journals.physiology.org/doi/fig/10.1152/jn.00565.2002) Neuronal firing may be correlated during ripples in seizures. Field potential and intracellular recordings from area 7. Spontaneous recurring seizures. Two pipettes for intracellular recordings were used (lateral distance <50 μm). Traces were filtered between 80 and 200 Hz. In A, field potential was recorded through pipette 1; then in B, a neuron was impaled with the same pipette, while a neuron was recorded with the pipette 2 the whole time. **Top:** an epoch with field and neuronal recording (A) and 2 epochs with dual intracellular recordings (B). **Middle:** underlined parts are expanded, revealing a relation between ripples and neuronal firing (A1), and correlated firing between the 2 neurons during ripples (B, 1 and 2). The close relations between ripples and spikes (C1), and between spikes from the 2 neurons (C2) were revealed by PEHs. The action potentials used in C2 were those that occurred when there were ripples in the field potential recordings.
rence of the EEG spike. Instead, the first EEG spike slowly built up with ripples present from its onset (Fig. 2). In contrast, foci that followed the precursor site displayed more abruptly rising EEG spikes with ripples on top of them. This suggests that ripples could be dependent on neuronal depolarization during EEG spikes in secondary sites but that they are involved from the onset in the first EEG spike within the primary site.

Seizures in secondary sites could depend on excitatory projections from the primary site.

When seizures evolved from the slow oscillation, field potential recordings revealed that ripples were present at the level where the EEG negativity developed from that of a nonparoxysmal event to a full-blown paroxysmal negativity (Fig. 2). This set of data (Figs. 2 and 3) suggests that strong neocortical ripples are not merely conditional on the presence of paroxysmal events but that they precede them. This is also confirmed by their presence, with smaller amplitude, during nonparoxysmal activities (Figs. 1 and 4).

A role for ripples in seizure generation is consistent with the fact that, in the corticothalamic network, both ripples and seizures are neocortical in origin. Thus seizures can be recorded in the cortex of athalamic cats (Steriade and Contreras 1998) and in isolated cortical slabs (Timofeev et al. 1998) that are devoid of long-range intra- and extra-cortical connections (Timofeev et al. 2000), and ripples are also present in such slabs (Grenier et al. 2001).

Correlation between the amplitude of neocortical ripples and propensity to seizures

Our results with halothane are consistent with the antagonism of very fast oscillations (Draguhn et al. 1998; Grenier et al. 2001; Jones et al. 2000; Ylinen et al. 1995) and the disturbance of paroxysmal network activities (Amzica and Massimini 2000; Perez Velazquez and Carlen 2000; Perez Velazquez et al., 1994) by gap junction blockers. This suggests that gap junctions are involved in neocortical ripples and seizures. There is a more important point to this result. We have previously shown that halothane diminishes neocortical ripples during the slow sleep-like oscillation, a nonparoxysmal network activity. The arrest of seizures with halothane shows that a manipulation that decreases ripples, even in nonparoxysmal
events, reduces at the same time the likelihood of seizures. This correlation is consistent with a role for neocortical ripples in seizure initiation.

There are other examples of correlation between propensity to seizure and the conditions in which neocortical ripples are ampler. In our experiments, seizures were much more frequent in cats under ketamine-xylazine anesthesia than during chronic experiments and as well ripples were ampler during ketamine-xylazine anesthesia than natural states of vigilance. Spontaneous seizures rarely occur under barbiturate anesthesia, and neocortical ripples are very weak in this condition. It is also known that some types of seizures appear more readily during somnolence and light sleep than during activated EEG states (Kellaway 1985; Steriade 1974). As well, ripples are ampler during slow-wave sleep than during waking and rapid-eye-movement sleep (Grenier et al. 2001). Thus the amplitude of neocortical ripples seems correlated with an increased propensity to seizures.

Very fast oscillations and seizures

Very fast oscillations (250–500 Hz, termed fast ripples) have been proposed to be involved in epileptogenesis in the hippocampal-entorhinal axis (Bragin et al. 1999a–c, 2002). Although these and our results fit into the general theme of the involvement of very fast oscillations in seizure generation, they occur in a different structure and probably involve a different mechanism. The fast ripples were proposed to reflect the pathological synchronous bursting of neurons, so these oscillations are reflections of synchronous action potentials. The synchronizing mechanism in this case is proposed to be pathological connections between these neurons. This leads to the synchronous bursting that is the synaptic trigger for seizures.

It has also been proposed that axo-axonal connections between principal neocortical cells are responsible for the very fast oscillations at the onset of seizures and thus play an important role in the initiation of seizures (Traub et al. 2001). This proposition is consistent with our results and would explain the antagonism of neocortical ripples by halothane. Results supporting the existence of such connections have recently been published for hippocampal neurons (Schmitz et al. 2001). This proposition was based on data from hippocampal slices. A new result from the present study is the amplitude (≤5 mV between crest and trough) that neocortical ripples reach in the neocortex in vivo during seizures. Our data and the recent results on the influence of field potentials on the activity of neocortical neurons during seizures (Grenier and Steriade 2001) lead us to propose a complementary mechanism for the initiation of seizures from neocortical ripples.

FIG. 11. Ripple oscillations are present in glial cells during seizures. Field potential and intracellular recordings from area 7. Spontaneous recurring seizures. Two pipettes for intracellular recordings were used (lateral distance <700 μm). Field potential was recorded through pipette 1 before a glial cell was entered, while a glial cell was recorded with the pipette 2 for the whole epoch. Top: 2 full seizures. Each trace was filtered between 80 and 200 Hz. Although the EEG did not show the pattern of stronger ripples at the onset of seizure, this pattern was present in the local field potential and the glial cells. Middle: underlined onset of seizures are expanded, revealing clear ripples in field and glial membrane potentials. Cross-correlations between field and glial cell (bottom left) and the 2 glial cells (bottom middle) were computed. An overlay of the 2 correlations reveals that they are almost identical. This indicates that local field potential and ripples are strongly correlated and in phase.
**Neuronal correlates of neocortical ripples during normal and paroxysmal events**

In our study of neocortical ripples during natural states of vigilance and anesthetized states, we found that firing of all neuronal types was phase-locked with ripples. FS neurons fired ~2.5 ms before the negative peak of ripples, while the firing of other neurons (FRB, RS, and IB) was more centered on the negative peak. The major difference for neocortical ripples outside seizures is that FS neurons almost always fired at high frequencies (200–600 Hz) with no relation with ripples. Because the activity of FS cells is important in the generation of ripples outside seizures, this would indicate that another mechanism for ripples is at play during seizures. An important result from the present study is that neuronal firing is coordinated among a majority of neocortical neurons during seizures when ripples are present in the field. This was revealed by single (Figs. 6 and 7) and by dual (Fig. 8) intracellular recordings. This implies that neurons do not fire independently on the depolarizing pressure of paroxysmal events but that this firing is structured around field ripples when they are present.

**Field ripples as a reflection of synchronous action potentials**

Because action potentials are short-duration strong amplitude events, it is generally accepted that under physiological conditions they do not contribute much to the generation of field potentials. However, when action potentials occur highly synchronously, field events have been partially ascribed to action potentials. The earliest component of cortical evoked field potentials arise from synchronous discharges of afferent thalamocortical fibers (Morin and Steriade 1981). In rats, fast ripples (250–600 Hz) have been proposed to be the extracellular reflection of highly synchronous bursting in hippocampal neurons (Bragin et al. 1999a,b). We propose that ripples in seizures could be a reflection of synchronous action potentials.

**Ripples as a possible autoregenerative process**

In a recent study, we have shown that field potentials influence the activity of neocortical neurons during paroxysmal activities by decreasing the intracellular firing threshold of neurons (Grenier and Steriade 2001). Based on the rapid and ample fluctuations of field potentials during neocortical ripples in seizures (≤5 mV between crest and trough, see field 1 in Fig. 11), we suggest that field ripples may induce a synchronizing of action potentials. This means that, on reaching a certain threshold amplitude, neocortical ripples could help generate themselves, as has been proposed in modeling studies of hippocampal very fast oscillations (Traub et al. 1985). Direct interactions between neurons through their electrical activities have already been proposed to play an important role in synchronizing and/or inducing action potentials in adjacent neurons in certain conditions (Braucci et al. 1999; Haas and Jefferys 1984; Krnjević et al. 1986; Taylor and Dudek 1984b; reviewed in Faber and Korn 1989; Jefferys 1995). The presence of ripples in glial cells is a strong indication that field potentials fluctuations can influence polarized membranes (Fig. 11).

We propose the following: field ripples are a reflection of synchronous action potentials and strong field ripples help generate and synchronize action potentials. If we combine these assumptions, we see the possibility of an autoregenerative loop: ripples help generate and synchronize action potentials, and synchronous action potentials generate ripples. Neocortical ripples during nonseizure states depend on IPSPs. Other mechanism, such as axo-axonal gap junctions between principal cells (Traub et al. 2001), can also be involved in generating ripples.

**Scenario for a possible involvement of neocortical ripples in seizure initiation**

We propose the following scenario for events leading to the initiation of seizures from neocortical ripples. During the slow sleep-like oscillation, ripples vary in amplitude from cycle to cycle of the slow oscillation (Fig. 4) (Grenier et al. 2001) and depend mostly on GABA_A-mediated IPSPs for their patterning. As these ripples are linked to neuronal depolarization and increased firing, we suggest that if this activity is ample enough and ripples reach a certain amplitude, the influence of the field ripples on neuronal transmembrane potential becomes strong enough to recruit nonfiring neurons close to firing threshold into action potentials. Firing of these neurons contributes to further depolarize their target neurons closer to threshold (because most neurons with RS, IB, and FRB firing patterns are excitatory), and their action potentials contribute to the field ripples themselves. The general idea is that there is a threshold level above which the effect of field potential ripples on neurons takes over and produces a regenerative recruiting activity, generating the first local EEG “spike,” which then excites neighboring loci through synaptic and possibly field interactions (Grenier and Steriade 2001). This process should be highly explosive in accordance with the strong increase in neocortical ripples right at the start of seizures. In this proposition, ripples are not an epiphenomenon reflecting neuronal events but are an active part of the mechanisms leading up to seizures. Their presence during nonparoxysmal activities, whatever its signification, is a factor that can lead to seizures if they reach the threshold at which the autoregenerative process takes place. This proposition is consistent with the block of seizures by halothane. In this case, halothane would block seizures primarily because it reduces the amplitude of neocortical ripples and thus prevents them from reaching the critical threshold that triggers seizures. After seizures are initiated, they could depend on other sustaining mechanisms because ripples are not always present up to the end of seizures. In this hypothesis, any other mechanism contributing to neuronal synchrony would produce or reinforce the action potential synchrony necessary to generate strong enough field ripples.

A role for gap junctions (either axo-axonal or otherwise) between principal cells in generating this synchrony could explain the blockage of seizures by halothane. Other neocortical gap junctions could also be involved in this scenario. It has been shown that electrical coupling can synchronize the firing of FS cells when they are close to firing threshold (Galarreta and Hestrin 1999; Gibson et al. 1999). We have observed “spikelets” in our recordings of FS cells in vivo that are similar to those ascribed to gap junctions couplings in the in vitro studies mentioned in the preceding text (unpublished observations). As ripples during the slow oscillation are associated with neuronal depolarization, correlated firing in FS cells mediated by gap junctions could be the basis of the phase-locking of FS firing and thus lead to the generation of
synchronous IPSPs in principal neurons, giving rise to neocortical ripples during nonparoxysmal activities. It would be when these nonparoxysmal ripples reach a certain threshold that field interactions would come into play through the proposed feedback loop leading to seizures.

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