SPONTANEOUS FIELD POTENTIALS INFLUENCE THE ACTIVITY OF NEOCORTICAL NEURONS DURING PAROXYSMAL ACTIVITIES IN VIVO

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Abstract—Field-potential recordings with macroelectrodes, and extra- and intracellular potentials with micropipettes were used to determine the influence of spontaneous field potentials on the activity of neocortical neurons during seizures. In vivo experiments were carried out in cats under anesthesia. Strong negative field fluctuations of up to 20 mV were associated with electroencephalogram “spikes” during spontaneously occurring paroxysmal activities. During paroxysmal events, action potentials displayed an unexpected behavior: a more hyperpolarized firing threshold and smaller amplitude than during normal activity, as determined with intracellular recordings referenced to a distant ground. Considering the transmembrane potential (the difference between extra- and intracellular potential) qualified this observation: firing threshold determined from the transmembrane potential did not decrease, and smaller action-potential amplitude was associated with depolarized firing threshold. The hyperpolarization of intracellular firing threshold was thus related to the field potentials. Similar field-potential effects on neuronal activities were observed when the paroxysmal events included very fast oscillations or ripples (80–200 Hz) that represent rapid fluctuations of field potentials (up to 5 mV in <5 ms). Neuronal firing was phase-locked to those oscillations.

These results demonstrate that: (a) strong spontaneous field potentials influence neuronal behavior, and thus play an active role during paroxysmal activities; and (b) transmembrane potentials have to be used to accurately describe the behavior of neurons in conditions in which field potentials fluctuate strongly. Since neuronal activity is presumably the main generator of field potentials, and in return these potentials may increase neuronal excitability, we propose that this constitutes a positive feedback loop that is involved in the development and spread of paroxysmal activities, and that a similar feedback loop is involved in the generation of neocortical ripples. We propose a mechanism for seizure initiation involving these phenomena. © 2003 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: transmembrane potentials, field potentials, seizure initiation, firing threshold, neocortical ripples.

An accurate description of neuronal activities during paroxysmal events is essential to understand the mechanisms involved in electrographic seizures and interictal electroencephalogram (EEG) “spikes.” Neuronal interactions occur via different routes. The most extensively studied are synaptic interactions (reviewed in McCormick and Contreras, 2001; Steriade, 2003). Non-synaptic interactions during paroxysmal events have been studied mostly in in vitro conditions (reviewed in Jefferys, 1995; Dudek et al., 1998; Perez Velazquez and Carlen, 2000). Interactions between neurons through field effects have been shown to occur in hippocampal slices, where such effects can synchronize neuronal firing in the absence of synaptic transmission (Jefferys and Haas, 1982; Taylor and Dudek, 1982, 1984a,b), and also in the hippocampus in vivo (Krnjevic et al., 1986). An influence of applied (Chan et al., 1988) and evoked (Richardson et al., 1984) field potentials on the transmembrane potential of neurons has been described in in vitro preparations. Only in a few cases have field effects been demonstrated in non-paroxysmal physiological activities in the CNS (Furukawa and Furshpan, 1963; Korn and Axelrad, 1980; reviewed in Faber and Korn, 1989). An active involvement of field potentials in neocortical paroxysmal events has not been demonstrated yet. In general, it is still not clear to what extent field potentials actively participate in the shaping of neuronal activity or merely represent a passive sign of the ongoing synchronous electrical activities of cellular populations (Bullock, 1997).

Intracellular studies of neuronal activities in vivo are most often carried out with the reference electrode placed in an indifferent location, for example the neck muscles. In those cases, it is assumed that this location is isopotential to the extracellular environment. However, if extracellular potentials fluctuate in a significant manner, this supposition is no longer valid. In that case, the potential that is actually felt by membrane constituents, the transmembrane potential, can be different from the intracellular one (see Faber and Korn, 1989).

In the present study we assess the impact of field potentials on neocortical neuronal activities by examining action-potential characteristics. Fluctuations in neuronal firing threshold have been related to the recent history of voltage fluctuations of the neuronal membrane (Noble and Stein, 1966; Calvin, 1974; Schlue et al., 1974; Stafstrom et al., 1984; Azouz and Gray, 2000; Henze and Buzsáki, 2001). To our knowledge, there have been no studies that specifically examined the relation between threshold fluctuations and field-potential amplitudes. In view of what is
known about the properties of action-potential generation (Hodgkin and Huxley, 1952), a lower firing threshold should indicate the involvement of more Na$^+$ channels, and hence be associated also with higher amplitude of action potentials.

There are different components in paroxysmal activities: lower frequency components, such as spike-and-wave complexes (2-4 Hz), and fast runs (10-20 Hz) (reviewed in Niedermeyer, 1999), but also higher-frequency components (Allen et al., 1992; Fisher et al., 1992; Alarcon et al., 1995; Steriade and Amzica, 1994; Steriade and Contreras, 1995; Steriade et al., 1998; Bragin et al., 1999a,b; Grenier et al., 2000, 2003; Traub et al., 2001). Very fast oscillations (ripples, 80-200 Hz) have been proposed to play a role in seizure initiation in the neocortex (Fisher et al., 1992; Grenier et al., 2000, 2003; Traub et al., 2001) and hippocampus and entorhinal cortex (Bragin et al., 1999a,b,c, 2002).

Here we have examined in general the impact of field-potential fluctuations on neuronal activity during paroxysmal events, and looked in particular at the case of neocortical ripples. Our results suggest that field potentials are involved in the generation and spread of paroxysmal activities and that very fast field-potential oscillations are involved in the generation and synchronization of action potentials. They demonstrate the importance of considering field and transmembrane potentials for an accurate understanding and a complete description of neuronal activities.

**EXPERIMENTAL PROCEDURES**

*Intracellular and field-potential recordings*

Experiments were conducted on 95 adult cats under ketamine-xylazine (10-15 and 2-3 mg/kg i.m., respectively) anesthesia. The depth of anesthesia was continuously monitored by EEG. Additional doses of anesthetics were given at the slightest signs of brain activation (waves with lower amplitudes and higher frequencies). After the typical signs of deep anesthesia appeared on the EEG, the animals were paralyzed with gallamine triethiodide, artificially ventilated, and the end-tidal CO$_2$ was maintained at 3.5-3.7%. Heart rate was monitored (acceptable range was 90-110 beats/min) and body temperature was maintained at 37-39 °C. When the animals were fixed in a stereotaxic apparatus, all peri-event histograms (PEHs) had at least a peri-event histogram with a band pass of 0–9 Hz, later digitized at 20 kHz for off-line analysis. Signals were also recorded with a 16-channel Vision data acquisition system from Nicolet (Madison, WI, USA) at a sampling rate of 10 or 20 kHz.

At the end of experiments, the animals were given a lethal dose of pentobarbital (50 mg/kg i.v.).

**Transmembrane potential determination**

To determine the transmembrane potential, we recorded with two intracellular recording pipettes inserted in the neocortex very close to each other (lateral distance less than 500 µm). With one pipette we recorded focal field-potential activity either before or after an intracellular recording with the same pipette. The field recording from the same pipette was performed less than 10 µm away from the neuronal recording, as evaluated by the values from the microdrive. The other pipette, located at a similar depth, was used to record the field potential for the duration of the experiment. The transmembrane potential was obtained by subtracting the DC field trace from the neuronal trace. By comparing the signals in both field-potential recordings, the ratio of amplitude of the two signals was determined and their similarity controlled. This ratio was then used to calibrate the value of the DC recording from the field pipette before subtracting it from the neuronal recording.

Ripple cycles used for computing the wave-triggered averages (WTAs) and the peri-event histograms (PEHs) had at least a four-times higher amplitude than the standard deviation of the whole EEG-filtered trace (between 80 and 200 Hz). In PEHs of firing in relation to ripples, the depth-negative peak of ripples was chosen as time 0. In these PEHs, the ripple trace at top is an average of 10 individual cycles.

**Experiments in neocortical slabs**

Experiments were performed on 39 cats. The preparation of small isolated neocortical slabs (6 mm×10 mm) is fully described elsewhere (Timofeev et al., 2000).

**RESULTS**

Reduction of intracellular firing threshold during paroxysmal events

Paroxysmal activities occurred spontaneously in our experiments in about 25% of animals. They comprised interictal spikes (Figs. 1 and 2) and/or full-blown electrographic seizures (Figs. 3, 4, 5, 6). Intracellular recordings of neocortical neurons revealed that the firing threshold of action potentials could be more negative during the EEG spikes of paroxysmal events than outside them (Fig. 1, bottom left panel). Unexpectedly, these action potentials arising from a more hyperpolarized intracellular potential showed at the same time smaller amplitude than action potentials occurring outside EEG spikes. Because of the probable involvement of more Na$^+$ channels, it should be expected that action potentials generated form a more hyperpolarized level would be at least as ample, if not more, than others. To elucidate this phenomenon, neurons were held at different intracellular potential levels with steady current injections during spontaneous paroxysmal events (Fig. 1, top panel). The amplitude of action potentials was plotted as a function of intracellular firing threshold. Those occurring outside EEG spikes showed the expected relation between firing threshold and amplitude: more depolarized threshold associated with smaller action-potential ampli-
Fig. 1. Hyperpolarized firing threshold and reduced amplitude of action potentials with intracellular recordings during IIS. Field potential (AC) and intracellular recordings from neocortical area 4. Spontaneous seizures and IIS occurred in this animal. Two epochs with IIS are displayed in the top panel. The neuron was held at different Vm levels with steady current injections (left: 0 nA, right: +0.6 nA). Action potentials during IIS arose from more hyperpolarized intracellular potential, and had smaller amplitudes than action potentials generated outside of IIS. Action potentials marked by letters in the top panels are expanded at bottom left. Their firing threshold is indicated by arrows. The dotted line marks the firing threshold of the first action potential. The firing thresholds of action potential B and D were more hyperpolarized, while at the same time they were of smaller amplitude than A. Action potential C displayed the expected relation of having a more depolarized firing threshold and a smaller amplitude than A. In the bottom right plot, action-potential amplitude versus firing threshold was plotted for 25 action potentials occurring during IIS (empty circles) and outside IIS (filled circles) selected from different DC current levels. Both groups showed a decrease of amplitude with depolarized firing threshold, but the curve during IIS was shifted by about 12 mV to more hyperpolarized values. Equations for the fitting lines were of the form $y = mx + b$, and the values ($\pm$S.D.) were: outside IIS $m = -1.7 \pm 0.2$ mV, $b = -23 \pm 6$ mV, $r = -0.9$; during IIS $m = -1.9 \pm 0.3$ mV, $b = -55 \pm 14$ mV, $r = -0.85$. 
tude (Fig. 1, bottom right plot, filled circles). Action potentials occurring during EEG spikes revealed a similar relationship with similar slope (Fig. 1, bottom right plot, empty circles); however, their values did not fit within the same line as that for action potentials during normal activities. Indeed, action potentials fired from more hyperpolarized potentials during EEG spikes (Fig. 1, top panels and examples in bottom left panel, marked B and D) could have smaller amplitude than those fired outside of EEG spikes from more depolarized levels (marked A and C). The relation between amplitude and firing threshold was thus similar for normal and EEG-spike action potentials, except for a shift of about 12 mV in the depicted case of Fig. 1. Since a decrease in firing threshold during paroxysmal events could be an important factor in the generation and propagation of paroxysmal events, we examined what could be responsible for this shift.

**Field potentials in close proximity to neurons**

During interictal (Figs. 1 and 2) and ictal (Figs. 3–6) paroxysmal events, strong values of field potentials, often more negative than −10 mV (with zero potential fixed during normal activity), were recorded in the depth of the neocortex. The maximal amplitude reached by field potentials during EEG spikes was 17.7±2.3 mV (mean±S.D., range 15–21 mV, n=10 animals). Such values could be recorded a few microns away from neurons before or after they were recorded intracellularly. An example of a DC field-potential recording close to a neuron is shown in Fig. 2. The field potential was recorded for a few minutes through a micropipette, and then a neuron was impaled 6 μm below (three 2-μm-microdriver steps). During interictal spikes (IIS), the focal field potential reached values of around −10 mV. This shows that the extracellular potential just outside neurons strongly fluctuates during EEG spikes. This fluctuation was in the same range of values as the shift in the action-potential amplitude versus firing threshold curves (see Fig. 1).

The transmembrane potential, the difference between intra- and extracellular potential, is the potential that the neuronal membrane actually experiences. We hypothesized that the presence of focal-field potential fluctuations...
Fig. 3. Extracellular field potential influences firing threshold and action-potential amplitude during paroxysmal events. Intracellular and field-potential recordings from area 7. Intra- and extracellular DC recordings along with conventional AC EEG recording during paroxysmal activity under ketamine–xylazine are shown at top left. The two DC recordings were made from very close sites (same depth, ~0.2 mm lateral distance). The extracellular DC recording was subtracted from the intracellular trace to reveal the transmembrane potential (top right). Firing characteristics were computed for the intracellular (left) and transmembrane potential (right). Firing threshold for each action potential (n = 180) in an epoch was plotted as a function of the field potential (middle panels). Action-potential amplitude versus firing-threshold plots is shown in bottom panels.
Fig. 4. Extracellular field potential influences neuronal firing even when neurons are spontaneously hyperpolarized outside of EEG spikes. Intra- and extracellular DC recordings along with AC field during paroxysmal activity in area 7 are shown at top. The two DC recordings were made from very close sites (same depth, <0.4 mm lateral distance). Depolarizing current pulses were given to the neuron so that the $V_{m}$ outside of EEG spike was slightly superior to the $V_{m}$ during the EEG spike. The underlined part is expanded in the middle panel (only DC recordings). Increased firing in the neuron was accompanied by intracellular hyperpolarization during the EEG spike while the field became more negative. The influence of the field was revealed by hyperpolarized firing threshold and smaller action potentials. Determining the transmembrane potential revealed that increased firing was actually accompanied by a depolarized transmembrane potential (bottom panel).
Fig. 5. Field-potential impact on neuronal firing during fast oscillations. Fast rhythmic-bursting (FRB) cell identified by its response to depolarizing current pulses (top right insert). The top panel depicts a spontaneously occurring seizure with the field potential, the cell and the EEG trace filtered between 80 and 200 Hz. The underlined part is expanded in the middle panel, with the filtered trace amplified 10 times compared with the original field potential. The dotted line indicates the firing threshold for the first action potential. Effects of field potentials, as described before, are present: hyperpolarized firing threshold and reduced spike amplitude. The firing threshold of each action potential is plotted at bottom left, revealing the decrease during the EEG spike. The firing of the neuron was clearly linked to the ripples. This is illustrated by a PEH calculated from this seizure (bottom right, n=152 action potentials) showing that firing occurs preferentially during the depth negative peaks of the fast oscillations.
Fig. 6.

Intracellular potential

Estimated transmembrane potential

Peri-event histograms

DC field potential area 7 (0.90 mm)

-80 mV

Intra-cell area 7 (0.88 mm)

Counts

Firing probability

AP peak in relation to ripple cycle (ms)

AP firing threshold in relation to ripple cycle (ms)
could be the cause of the shift in intracellular firing threshold we observed, and decided to simultaneously record field potentials and intracellular neuronal electrical activity to determine the transmembrane potential.

Neuronal behavior with transmembrane potentials during paroxysmal events

Transmembrane potentials of neurons were determined with two similar recording micropipettes. One was used to record focal field activities during the whole experiment, while the other was used to record intracellularly, as well as field potentials before or after the intracellular recording (see Experimental Procedures). An example of a neuron recorded in this manner during an electrographic seizure of the polyspike-and-wave type of paroxysmal discharges is shown in Fig. 3. An inspection of the intracellular recording revealed fluctuations in the peak depolarization reached by action potentials. They reached a more depolarized level when the field potential was less negative (Fig. 3, top left panel, DC1), but at the same time arose from a more depolarized intracellular potential, similarly to what was described above (Fig. 1). Plotting the intracellular firing threshold versus the field potential revealed that the threshold fluctuations were directly related to the value of the field potential in their vicinity (Fig. 3, middle left plot). For all action potentials, regardless of the value of the field, plots of action-potential amplitude versus intracellular firing threshold indicated the same peculiar behavior as described above (Fig. 1): ampler action potentials could be fired from more depolarized intracellular potential (Fig. 3, bottom left plot). Similar plots, but with the transmembrane potential, revealed different and more expected relations. With the transmembrane potential, the firing-threshold fluctuations were not related to variations of the field potential (Fig. 3, middle right plot), and smaller action potentials were associated with more depolarized firing threshold (Fig. 3, bottom right plot). Taken together, these results show that the decrease in firing threshold with intracellular recordings is directly linked to the presence of the field outside the neuron. Moreover, when the transmembrane potential was taken into account, the relation between action-potential amplitude and firing threshold was more consistent with what is known about conductances involved in action potential generation (see Fig. 1, bottom right plot). This clearly confirms that the transmembrane potential is a more accurate descriptor of neuronal behavior than the intracellular potential.

The effects of the field were obvious when neurons fired spontaneously outside EEG spikes. Then, the peculiarities of firing thresholds and action-potential amplitudes between “normal” and “paroxysmal” action potentials were noticeable. Most neurons were steadily hyperpolarized during seizures and only fired during the paroxysmal depolarization shifts associated with EEG spikes. In those cases, EEG spikes were associated with straightforward neuronal depolarization and firing. The presence of a strong field negative fluctuation indicated that the actual transmembrane depolarization was even stronger during EEG spikes than revealed by intracellular recordings alone. This was confirmed by applying suprathreshold depolarizing current pulses to recorded neurons during paroxysmal events (see an example in Fig. 4). Such neurons spontaneously fired only during EEG spikes, during which they experienced a strong depolarization (top panel). When an EEG spike occurred during a current pulse, action potentials before and during this event could be compared (top panel, underlined epoch). Even though the neuron increased its firing rate during the EEG spike, it was at the same time hyperpolarized (Fig. 4, middle panel). Smaller action potentials were fired at a higher frequency and from a hyperpolarized intracellular firing threshold during the EEG spike (see dotted line). The field potential during the EEG spike was around ~10 mV at its peak. This suggested that the picture obtained with the transmembrane potential could be different. Indeed, high-frequency firing and smaller action potentials came from a more depolarized transmembrane potential (see dotted line). This shows that the strong depolarizations observed with intracellular recordings during EEG spikes are actually even stronger than they appear. It also leads to the unexpected conclusion that neuronal excitation and increased firing can actually take place while the intracellular potential becomes more negative.

Neocortical very fast oscillations during paroxysmal events as an example of field influence on neuronal firing

Some neocortical paroxysmal field-potential events include very fast oscillations (80–200 Hz), also termed ripples. These oscillations have been proposed to play a role in the initiation of seizures (Fisher et al., 1992; Grenier et al., 2000, 2003; Traub et al., 2001). Since ripples constitute field-potential fluctuations, we were interested to verify if they could have an impact on neuronal activity. During
some EEG spikes with ripples, indications of the impact of the field potential were present: hyperpolarized intracellular firing threshold and reduced action-potential amplitude (Fig. 5, middle panel and bottom left plot). Action potentials did not occur independently of the fast oscillations. Instead, they had a tendency to be phase-locked to the depth-negative phase of the ripples (Fig. 5, middle panel and bottom right PEH). Such results were obtained with macroelectrode recordings of field potentials. We also used two closely located pipettes to study the impact of these oscillations on neuronal activity (Fig. 6). Neocortical ripples could represent field-potential fluctuations of up to 5 mV occurring within less than 5 ms (Fig. 6, top panel). The firing of intracellularly recorded neurons was strongly phase locked with the negative peak of fast oscillations (Fig. 6, middle left panel). The firing probability of neurons fluctuated between different phases of ripples’ cycles. Action potentials started preferentially during the rising negativity of the ripples, which correspond to an increase in transmembrane depolarization. In the case depicted in Fig. 6, 50% of the action potentials were generated within a 1.5-ms window (between the dotted lines, Fig. 6 middle right plot), corresponding to 15% of the whole 10 ms cycle. Examining the transmembrane potential revealed that the negative peaks of these fast oscillations corresponded to depolarizing humps in the neuronal transmembrane potential (indicated by arrows in Fig. 6 middle panel). These results strongly suggest that the field ripples are involved in phase-locking the firing of neurons through depolarization of the transmembrane potential.

In slices, action potentials arising from a transient intracellular hyperpolarization is one of the signs of the involvement of field effects in their generation. When field potentials are taken into account, this intracellular hyperpolarization is shown to correspond to a transmembrane-potential depolarization (Taylor and Dudek, 1984b; Bracci et al., 1999). It is not clear, however, if such effects may occur in vivo in the neocortex (Jefferys, 1995). We observed a similar phenomenon in the isolated neocortical slab preparation. The slab is a portion of neocortex in which all neuronal connections with the rest of the brain have been interrupted (Timofeev et al., 2000). Neocortical slabs present silent periods interrupted by brief bursts of activity. In a few of these slabs, these bursts were clearly paroxysmal (Fig. 7). The amplitude of action potentials was strongly reduced during these bursts. The typical signs of field-potential influence were present: reduced amplitude associated with hyperpolarized firing threshold for action potentials. Short-lasting hyperpolarizations (Fig. 7, middle panel, underlined epoch A) and action potentials arising from these events (underlined epoch B) were present. Averages of similar transient hyperpolarizations (A, WTA) and of action potentials arising from such hyperpolarizations (B, spike-triggered average, STA) revealed that the hyperpolarizations reached a value of a little more than 1 mV. These hyperpolarizations and the action potentials arising from them are a strong indication that the field effects described in hippocampal slices occur also in vivo in the neocortex.

**DISCUSSION**

There are two main findings in this study: (a) the importance of transmembrane potentials for an accurate description of neuronal behavior during paroxysmal events, and (b) the influence of spontaneous field potentials on neuronal activity during paroxysmal events. We discuss these points and propose a general mechanism for seizure initiation involving neocortical ripples and field-potential influence on neuronal excitability.

**Importance of transmembrane potentials to accurately describe neuronal behavior**

We demonstrate in this study that field potentials are needed for a complete description and understanding of neuronal behavior, especially in conditions, such as paroxysmal events, during which field potentials strongly fluctuate. Although this point has been stressed over the years in some studies (see Faber and Korn, 1989), it is often neglected in the interpretation of results. However, our results clearly show that not considering field potentials may lead to erroneous conclusions, as follows. (a) The reduced firing threshold obtained with intracellular recordings during paroxysmal events may lead to the conclusion that a modification in the voltage sensitivity of Na⁺ channels takes place during paroxysmal events, and that this increases neuronal excitability. However, determining the transmembrane potential revealed that the negative deflections of the field potential were responsible for this phenomenon. (b) The paradox of the hyperpolarization of the intracellular firing threshold of neurons being accompanied by a reduced amplitude of action potentials (Fig. 1) is also resolved by considering the transmembrane instead of the intracellular potential (Fig. 3). Otherwise, the reduced amplitude could have been exclusively attributed to a shunt of the membrane due to a strong increase in membrane conductance during paroxysmal events (Neckelmann et al., 2000; Timofeev et al., 2002).

Thus, the best conditions for studying neuronal electrical activities are to record extracellular potentials along with intracellular ones. However, this is not always possible or practical. In in vivo conditions, it is an arduous task to bring two micropipettes close enough to actually record intracellularly with one pipette and the extracellular potential a few microns away with the other. Our results with the transmembrane potential (Figs. 3 and 4) support the validity of the method we used.

To our knowledge, our studies are the first in which fluctuations in intracellular firing threshold were observed between normal and paroxysmal events (see also Timofeev et al., 2002). Fluctuations of many millivolts in firing thresholds have been reported in a number of studies (recent examples in Azouz and Gray, 2000; Henze and Buzsáki, 2001). The firing threshold fluctuations were linked to the recent membrane-potential history of the neu-
Fig. 7. Signs of field effects in paroxysmal bursts in the neocortical slab. Field (AC) and intracellular recordings in a neocortical slab. The top panel depicts an epoch with spontaneous and evoked paroxysmal bursts of activity, including the filtered field trace between 80 and 200 Hz. The period marked by arrow is expanded in the middle panel. Transient hyperpolarizations were observed in association with the depth-negative peak of fast oscillations or ripples (underlined A). Ten such hyperpolarizations are overlaid (A) and averaged (WTA) below at left. Action potentials could arise from these hyperpolarizations (underlined B). Ten such action potentials are overlaid (B) and averaged (STA) below at right.
rons, and were of higher amplitudes than the expected field fluctuations (Henzé and Buzsáki, 2001).

Besides field-potential fluctuations, an alternate hypothesis could explain the fluctuations of firing threshold. Ectopic action potentials have been shown to occur during seizures (Noebels and Prince, 1978), and they can lead to action potentials being triggered from apparently different intracellular potential. However, ectopic action potentials should display more or less random firing-threshold fluctuations in relation to field potentials, and not the clear relation displayed in Fig. 3. This strongly suggests that the phenomenon observed was not the result of ectopic action-potential generation.

This study is not the first with intracellular recordings in vivo during seizures. It may be asked why such neuronal behaviors were not reported before. The importance of considering the field potential should apply for all cases. However, the effects that clearly demonstrate the importance of field potentials occur only in certain conditions, and depend on the range of values of intracellular and transmembrane potentials. If a neuron is strongly activated during an EEG spike, its intracellular potential will often go beyond firing threshold (see for example Fig. 13 in Steriade and Contreras, 1995; and Fig. 4 in Steriade et al., 1998). If the intracellular potential reaches $-50$ mV, than the reduced amplitude of action potentials is straightforwardly attributed to spike inactivation. The field potential is still present, and the neuron may actually experience a transmembrane depolarization of $-40$ mV (if the field value is $-10$ mV). However, the qualitative behavior is the same, increased firing threshold and spike inactivation, whether the field is considered or not. This is probably the reason why this was not reported before. If there is already a depolarized firing threshold, there is no reason to examine the point further to see if the action-potential amplitude corresponds to firing from $-50$ or $-40$ mV. What prompted us to consider the field are those cases, less numerous, in which the intracellular potential during EEG spikes is below the usual intracellular firing threshold. Then the presence of the field may lead to firing from intracellular potentials from which action potentials are not fired in non-paroxysmal conditions. In those cases, considering the field leads to a qualitatively different behavior, and its influence is obvious. Those cases may have occurred less often in other studies, or were considered as the exception and not analyzed in depth.

**Influence of spontaneous field potentials on neuronal activity during paroxysmal events**

From a physiological point of view, the most interesting result from this study is the demonstration of an important influence of field potentials on neuronal behavior. Field-potential negative deflections led to a decrease in intracellular firing threshold (Figs. 1 and 3). This decrease was clearly dependent on the presence of these field potentials because the transmembrane firing threshold was not affected (Fig. 3). However, some arguments may be raised against the extent of the impact of field potentials on neuronal activity. It could be argued that the field experienced by an individual neuron is mainly produced by this same neuron. Our results from depolarizing pulses to neurons during paroxysmal events demonstrate that this is not the case. In Fig. 4, the neuron clearly behaved as if it experienced a negative field in its vicinity (lower firing threshold associated with smaller action potentials) when the EEG spike occurred during a depolarizing current pulse. In that case, the neuron was intracellularly hyperpolarized during the EEG spike. So the neuron could not be the main source of the field negative deflection in its surroundings, since it was probably generating an outward current. The main generators of the field experienced by the recorded neuron had to be the synchronous activity of the cells in its vicinity.

Another argument is that the negative deflections of field potentials could be neutral toward the transmembrane potential. This implies that the field fluctuations could be instantly and completely compensated for by ionic fluxes between different parts of neurons or across their membrane. Recordings of glial cells during seizures suggest that some adjustments in intracellular potential do occur in polarized membranes together with the fast field-potential oscillations (unpublished observations). However, in the great majority of cases, recordings of intracellular potential of neurons during neocortical ripples did not show fluctuations compensating for the field-potential oscillations (Fig. 6). Otherwise, ripples in field potentials would not lead to depolarizing humps in neuronal transmembrane potentials, but instead would cancel out with the field. In contrast with glial cells, neurons usually displayed intracellular fluctuations in reversed phase with neocortical ripples.

**Neocortical ripples as an autoregenerative process**

We propose that field potentials modify the transmembrane potential of neurons and contribute to the spread of excitation during paroxysmal events. Our results with neocortical ripples during paroxysmal activities support an active effect of field potentials. Strongly phase-locked action potentials occur during the ripples, and at the same time these oscillations constitute strong fluctuations of extracellular potential (Fig. 6). An indication that field interactions are involved in synchronizing action potentials in vitro is the fact that action potentials may arise from a transient hyperpolarization of intracellular potential (Taylor and Dudek, 1984b; Bracci et al., 1999), corresponding to a transmembrane depolarization. We obtained a similar phenomenon during synchronous firing of neurons with ripples during paroxysmal discharges in neocortical slabs (Fig. 7). Since neurons are generally close to firing threshold during paroxysmal neocortical ripples, fluctuations of transmembrane potential grouped the firing of neurons around the negative going parts of these cycles, corresponding to depolarizations in the transmembrane potential (Figs. 5 and 6).

Since neuronal firing is strongly phase-locked during their presence, neocortical ripples during paroxysmal events are probably, at least partially, field reflections of synchronous neuronal firing. We propose that they constitute an autoregenerative process: ripples’ field oscillations...
Fig. 8. Proposed mechanism for the involvement of field potentials and neocortical ripples in the initiation of seizures. The top part illustrates the position of neocortical ripples as the proposed gateway between normal activities and seizure initiation. The amplitude of neocortical ripples fluctuates during normal activity. The amplitude of ripples is linked to the intensity of neuronal activation. Weak activity or the presence of halothane leads to weak ripples, and the network remains in normal activity. Strong activity leads to strong ripples. If these ripples are still below a certain threshold, the network remains in normal activity. Neocortical ripples, upon reaching a certain threshold, can become involved in an autoregenerative process, leading to increased and synchronous neuronal firing, which in turn increases neocortical ripples (bottom part, ripples feedback loop). The increased firing and neuronal activation generates strong extracellular potentials that in turn increase neuronal activation by decreasing intracellular firing threshold through transmembrane depolarization (bottom part, field feedback loop). These two feedback loops interact and enhance each other. Increased neuronal activity also leads to decreased extracellular space, and thus increases the amplitude of field potentials (both ripples and slower components), further potentiating the explosiveness of both loops.
help generate and synchronize action potentials, and synchronous action potentials enhance ripples’ field oscillations. Modeling studies based on in vitro hippocampal data on very fast oscillations have indicated that such a phenomenon could explain the high-frequency ringing in field-potential paroxysmal events in slices (Traub et al., 1985). In another neuronal modeling study, it was suggested that action potentials in one neuron could influence the membrane potential of neighboring neurons. Although the effect observed was small, it was pointed out that it could be relevant when the affected neuron was close to firing threshold (Holt and Koch, 1999), as is the case generally during paroxysmal events. A study combining intracellular recordings and modeling has suggested that electrical coupling through both gap junctions and field effect is involved in synchronizing the activity of CA1 pyramidal neurons. In particular, spikelets in intracellular recordings were attributed to synchronous firing of adjacent neurons (Vigmond et al., 1997).

Proposed mechanisms for the initiation of focal seizures in the neocortex

Based on our results, we propose that field potentials play an active part in paroxysmal events, and particularly in their initiation. An illustration of the proposed mechanism of seizure initiation is presented in Fig. 8. Slow components of field potentials, such as EEG spikes, are most likely the result of currents through neuronal synaptic and intrinsic conductances (Matsumoto and Ajmone-Marsan, 1964; Creutzfeldt et al., 1966; Prince, 1978; Johnston and Brown, 1981; Steriade et al., 1998; Steriade and Amzica, 1999). As the field potentials build up, they result in transmembrane depolarizations in surrounding neurons. This brings more neurons to firing threshold, neuronal synaptic activation further increases, leading in turn to stronger fields. This constitutes what we call the field feedback loop. We propose that a similar loop is at play in the case of neocortical ripples (see Neocortical ripples as an autoregenerative process). The main difference is that for these very fast oscillations, the sources of the field fluctuations are the currents involved in action potentials, as described for field effects in hippocampal slices in the absence of synaptic transmission (Taylor and Dudek, 1984b). Because they result in transmembrane depolarizations, very fast field oscillations lead to more numerous and synchronous action potentials that in turn reinforce the field oscillations. Beyond a certain threshold, this reinforcing interaction between field potentials (both slow and fast components) and neuronal excitation may be responsible for the initiation and spread of paroxysmal activity. We propose that neocortical ripples are the gateway between nonparoxysmal activities and these explosive feedback loops leading to seizures. During non-paroxysmal activities, the amplitude of neocortical ripples fluctuates (Grenier et al., 2001). As long as this amplitude remains below a certain amplitude level, the network remains in a non-paroxysmal state. When these ripples go beyond this threshold, the positive-feedback loop between field potential ripples and synchronous action potentials is engaged. This leads to a fast recruitment of inactive neurons into firing. This increased activity has many consequences. (a) It brings more neurons close to threshold, and thus ready to be induced into firing by field ripples, strengthening the ripple feedback loop. (b) It also leads to more synaptic activation, and thus stronger field potentials, which in turn lower the intracellular firing threshold of neurons and facilitate their recruitment into firing, which is the field-potential feedback loop. These two regenerative processes reinforce each other, because a reduction in intracellular firing threshold facilitates further recruitment of neuronal firing through field ripples. The feedback nature of these two processes explains the transition between normal activity and seizures being accompanied by strong, very fast oscillations. Other factors probably facilitate these processes. Strong neuronal activation leads to a decrease of the extracellular space (Dietzel et al., 1980; Andrew and MacVicar, 1994). This enhances extracellular resistivity and favors the appearance of strong field potentials and electrical interactions between neurons (Faber and Korn, 1989; Jefferys, 1995; Dudek et al., 1998). Fast-spiking cells discharge at high frequencies during EEG spikes (Timofeev et al., 2002), leading to a shift to more depolarized reversal potential of chloride and, consequently, to a depolarizing influence of the GABA_A-dependent synaptic potentials.

We conclude that field potentials must be taken into account for an accurate description and understanding of neocortical paroxysmal events, along with the more frequently considered phenomena such as synaptic and intrinsic conductances, gap junction coupling and fluctuations of extracellular ionic concentrations.

Acknowledgements—This work was supported by Canadian Institutes of Health Research (grants MT-3689, MOP-36545 and MOP-37862), U.S. National Institutes of Health Research (grant RO1 NS-40522), Fonds de la recherche en santé du Québec, Human Frontier Science Program, and Savoy Foundation. Thanks to Y. Cissé for participation in some experiments and to P. Giguère and D. Drolet for technical assistance.

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(Accepted 24 January 2003)