Contribution of Intrinsic Neuronal Factors in the Generation of Cortically Driven Electrographic Seizures

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Timofeev, Igor, François Grenier, and Mircea Steriade. Contribution of intrinsic neuronal factors in the generation of cortically driven electrographic seizures. J Neurophysiol 92: 1133–1143, 2004. First published January 28, 2004; 10.1152/jn.00523.2003. Some electrographic seizures are generated intracortically. The cellular and ionic bases of cortically generated spontaneous seizures are not fully understood. Here we investigated spontaneously occurring seizures consisting of spike-wave complexes intermingled with fast runs in ketamine-xylazine anesthetized cats, using dual intracellular recordings in which one pipette contained a control solution and another pipette contained blockers of K⁺, Na⁺, or Ca²⁺ currents. We show that closely located neocortical neurons display virtually identical fluctuations of the membrane potential during electrographic seizures, thus directly demonstrating a high degree of focal synchrony during paroxysmal activity. In addition to synaptic drives, the persistent Na⁺ current [I_{Na_{persistent}}] and probably the high-threshold Ca²⁺ current contributed to the generation of paroxysmal depolarizing shifts (PDSs) during cortically driven seizures. Ca²⁺-activated K⁺ current [I_{K(Ca)}] took also part in the control of the amplitude and duration of PDSs. The hyperpolarizing components of seizures largely depended on Cs⁺-sensitive K⁺ currents. I_{K(Ca)} played a significant role, while not exclusive, role in the mediation of hyperpolarizing potentials related to EEG “waves” during spike-wave seizures. We conclude that intrinsic cellular factors have significant role in the generation of depolarizing and hyperpolarizing components of seizures.

INTRODUCTION

There are tens of distinct types of epileptic seizures (Commission on Classification and Terminology of the International League Against Epilepsy 1989; Niedermeyer 1999a,b), and different neuronal substrates contribute to the generation of various electrographic paroxysms. Studies on experimental animals show that some electrical seizures are generated intracortically, even in the absence of the thalamus (Steriade and Contreras 1998). These seizures are characterized by spike-wave (SW) or spike-wave/polyspike-wave (SW/PSW) complexes of 1.5–3 Hz, intermingled with episodes of fast runs at ~10–20 Hz (Neckelmann et al. 1998; Steriade and Contreras 1998; Steriade et al. 1996; Timofeev et al. 1998). Generally, these seizures evolve from the cortically generated slow oscillation (Steriade and Contreras 1995; Steriade et al. 1998) that might be shaped by the thalamus (Hughes et al. 2002). The electrographic pattern of these seizures as well as their occurrence during slow-wave sleep resemble the clinical Lennox-Gastaut syndrome of humans (Halasz 1991; Kotagal 1995; Niedermeyer 1999a,b).

The treatment of these seizures is usually based on the use of antiepileptic drugs that limit sustained repetitive firing via use-dependent blockade of voltage-gated Na⁺ channels, facilitate the inhibitory action of GABA, combine the preceding actions (Brodie and French 2000; Brodie and Kwan 2001), or act simultaneously on persistent Na⁺ current, Ca²⁺-activated K⁺ current and possibly low-threshold Ca²⁺ current (Crusini and Lerescu 2002). However, despite antiepileptic drug treatment, more than one-third of patients continue to display seizures (Kwan and Brodie 2000). This suggests that other abnormalities than impaired inhibition and enhanced activity of Na⁺ currents may be involved in the generation of such paroxysmal activities.

The cellular mechanisms underlying the generation of electrical seizures were explored since the 1960s (Matsumoto and Ajmone-Marsan 1964a,b). Despite significant efforts, we still lack data to account for all neuronal mechanisms that are involved in paroxysmal activities. Initial studies suggested that the paroxysmal depolarizing shift (PDS) consists of a giant excitatory postsynaptic potential (EPSP) (Johnston and Brown 1981) enhanced by activation of voltage-regulated intrinsic currents (de Curtis et al. 1999; Dichter and Ayala 1987; Prince and Connors 1984; Westbrook and Lothman 1983; Wong and Prince 1978). These and other similar studies were performed in the presence of GABA_A receptor antagonists. Some experimental data point out that synaptic inhibition remains functional in many forms of paroxysmal activities (Davenport et al. 1990; Esclapez et al. 1997; Higashima 1988; Prince and Jacobs 1998; Timofeev et al. 2002b; Traub et al. 1996), and more recent studies suggest that GABAergic activities in the epileptic foci depolarize postsynaptic neurons (Cohen et al. 2002; Timofeev et al. 2002b).

In conjunction with our recent studies (Bazhenov et al. 2004; Timofeev et al. 2002a,b), we address here the question: what ionic conductances mediate the depolarizing and hyperpolarizing components of electrographic seizures in which inhibition was not impaired via application of GABA blockers? We provide evidence that, besides synaptic drives, the voltage-gated Na⁺ and probably the high-threshold Ca²⁺ conductances contribute to the generation of the depolarizing components of seizures, while Ca²⁺-activated K⁺ [I_{K(Ca)}] and other K⁺ conductances mediate the hyperpolarizing components of cortically generated electrographic seizures. These data may create a new avenue for the development of antiepileptic drugs.

METHODS

Intracellular recordings from neocortical neurons were performed in 92 cats anesthetized with ketamine-xylazine (10–15 and 2 and 3 mg/kg). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The electroencephalogram (EEG) was monitored continuously during the experiments to maintain a sufficient level of anesthesia. Additional doses of anesthetic were given at the slightest tendency toward an activated EEG pattern. In addition, all pressure points and tissues to be incised were infiltrated with lidocaine (0.5%). Gallamine triethiodide (20 mg/kg) was given intravenously, and cats were artificially ventilated to an end-tidal CO₂ of 3.5–3.8%. The heartbeat was monitored and kept constant (acceptable range, 90–110 beats/min). Body temperature was maintained at 37–39°C. Glucose saline (5% glucose, 10 ml ip) was given every 3–4 h during the experiments, which lasted for 8–14 h. The stability of intracellular recordings was ensured by cisternal drainage, bilateral pneumothorax, hip suspension and filling the hole made in the skull with a solution of agar-agar (4%). All experimental procedures were performed according to national guidelines and were approved by the committee for animal care of Laval University.

Field potential recordings and stimulation were obtained by using bipolar coaxial macroelectrodes inserted into the cortex. The outer pole of the electrode was placed at the cortical surface or 0.1 mm deeper, whereas the inner pole was placed at 0.8–1 mm in the cortical depth. After ketamine-xylazine anesthesia, ~30% of cats (n = 28) displayed spontaneously occurring electrographic seizures consisting of SW/PSW complexes at 1.5–3 Hz, often associated with fast runs at ~10–15 Hz. The reason accounting for a relatively high incidence of spontaneous seizures, in addition to the propensity to paroxysms under ketamine anesthesia, are discussed elsewhere (Steriade et al. 1999b).

Intracellular recordings from cortical neurons were obtained with sharp glass micropipettes having resistance of 30–80 MΩ. We aimed to obtain dual intracellular recordings from pairs of closely located neurons (lateral distance <0.5 mm; see Table 1). In all cases, one of the electrodes was filled with a solution of 2.5–3.0 M K⁺ acetate (KAc), while another electrode was filled either with KAc, or 2.0 M Cs⁺ acetate, or KAc containing 50 mM of 1,2-bis(2-aminophenoxy)-N,N,N',N'-tetraacetic acid (BAPTA), or 50 mM of N-(2,6-dimethyl-phenylcarbamoylmethyl)-triethylammonium bromide (QX-314). All drugs were purchased from Sigma. In some experiments, 2% of neurobiotine (Vector, Canada) was added to pipettes. A high-impedance amplifier (band-pass, 10 kHz) with an active bridge circuitry was used to record and inject current into the neurons. Before the recording session, 3- to 5-nA current pulses were passed through all the pipettes to test the correctness of balance of each pipette at a wide range of injected currents. The signals were recorded on a tape with band-pass of ±0–9 kHz and digitized at 10–20 kHz for off-line computer analysis. In the latest experiments data were digitalized on-line and recorded on Vision (Nicolet, WI) at 20 kHz.

At the end of all experiments animals were given a lethal dose of pentobarbital sodium (50 mg/kg iv), perfused, and processed for revealing intracellularly stained neurons (see Fig. 1).

### Table 1. Number of neurons recorded with different substances in the pipette

<table>
<thead>
<tr>
<th>Substance</th>
<th>Total Number of Neurons</th>
<th>Number of Dual Recordings in Which 1 Neuron Was Recorded with KAc (2.5 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAc (2.5 M)</td>
<td>270</td>
<td>20</td>
</tr>
<tr>
<td>CsAc (2.5 M)</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>KAc (2.5 M) + BAPTA (50 mM)</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>KAc (2.5 M) + QX 314 (50 mM)</td>
<td>30</td>
<td>12</td>
</tr>
</tbody>
</table>

**Results**

**Database**

We aimed to obtain dual simultaneous intracellular recordings from pairs of closely located neurons (lateral distance: <0.5 mm) and the actual distance measured from intracellularly stained pairs of neurons (n = 15) was <0.3 mm (Fig. 1). We recorded intracellular activities from 391 cortical neurons located at depths between 300 and 1,800 μm (micromanipulator reading). In paired recordings, the depth difference between two neurons did not exceed 200 μm. Of the neurons tested, 270 were recorded with pipettes filled with KAc (2.5 M). Other neurons were recorded with pipettes containing different substances (Table 1). All neurons were formally identified by electrophysiological criteria as regular-spiking (RS), intrinsically bursting (IB), fast-rhythmic-bursting (FRB), and fast-spiking (FS) (Connors and Gutnick 1990; Gray and McCormick 1996; Steriade et al. 1998).

**Control experiments**

Dual intracellular recordings during seizures from pairs of neurons located in different cortical areas disclosed synchronous behavior at a large scale, but close examination showed that there were significant time delays in the onset of PDSs and differences in amplitudes (Neckelmann et al. 1998). At variance, recordings from closely located (~<0.5 mm) neurons indicated that the excursion of intracellular traces of simultaneously recorded neurons during paroxysmal activities was almost identical (no current injection was needed to line up the traces) and, at any given moment of time, the difference in voltage of neurons that belonged to the same electrophysiological class did not usually exceed 3 mV (Fig. 1). The exception to this rule concerned only action potentials, spike-related prepotentials, and spike afterhyperpolarizations (AHPs). Those components contributed to the histogram bins of 5–6 mV (Fig. 1). During periods outside the seizures, the membrane potential in the active periods was similar too, but the difference in the membrane potential during the silent phases of oscillations, which correspond to depth-positive EEG waves, could reach ±10 mV (not shown). Because most of paired recordings were obtained from RS neurons that represent the majority of neocortical neurons, the analysis of actions exerted by intracellular drugs on different components of intracellular activities during seizures was mainly performed on pairs of RS neurons.

**Hyperpolarizing components of seizures**

Two types of seizure-related hyperpolarizing potentials were considered in the present study: relatively short-lasing hyperpolarizations (200–300 ms), which occurred between two consecutive PDSs, and long-lasting hyperpolarizations at the end of seizures. The input resistance of neurons during both these components was lower than during the preseizure epoch (Neckelmann et al. 2000; Timofeev et al. 2002b), suggesting the strong activation of some currents during the seizure-related hyperpolarizations as well as during the postictal depression. These hyperpolarizations were not Cl⁻ dependent inhibitory postsynaptic potentials (IPSPs) because they were not affected by intracellular Cl⁻ infusion and were not asso-
associated with a simultaneous firing of FS neurons (Timofeev et al. 2002b). Thus we hypothesized that K⁺ currents mediated these hyperpolarizing potentials.

To test this hypothesis, we performed intracellular recordings with Cs⁺-containing pipettes (2 M), which blocks intracellularly most K⁺ currents (Hille 2001). Intracellular recordings with Cs⁺-containing pipettes during seizures had a profound influence on intracellular activities. One to 2 min after impalement, during the pause between successive electrographic seizures, the neuron revealed depolarizing plateau potentials (Fig. 2, green); however, during the seizure, these large depolarizing potentials were truncated by PDSs. Two to 3 min after beginning of intracellular recordings with Cs⁺-containing pipettes, during the postictal depression, the membrane potential of neurons was depolarized and remained in the range between −10 and −30 mV. Under these conditions, the intracellular manifestation of seizure appeared to be reversed, that is, the PDSs were hyperpolarizing (Fig. 2, blue; see arrow). The membrane potential during PDSs reached the same value or only a few millivolts more depolarized values than the membrane potential of the same neuron during PDSs immediately after impalement. EEG “waves” of spike-wave complexes, which are normally associated with hyperpolarizing potentials, were accompanied by depolarizing events, or the membrane potential did not change significantly between consecutive PDSs. This pattern did not change over tens of minutes of recordings.

During both hyperpolarizations associated with EEG “waves” and postictal depression, the apparent input resistance of neurons recorded with CsAc-filled pipettes was three times higher than the input resistance of neurons recorded with KAc-filled pipettes (P < 0.01; Fig. 3). However, the input resistance during EEG “spikes” was similar in both conditions (CsAc- and KAc-filled pipettes). These data suggest that the hyperpolarizing potentials associated with seizures were almost exclusively mediated by Cs⁺-sensitive K⁺ currents and the relative role of these currents was small during the PDS.

The exact type(s) of K⁺ currents contributing to seizure-related hyperpolarizing potentials should be further elucidated. The PDSs are associated with strong depolarization of cortical neurons that could lead to Ca²⁺ and Na⁺ influx, which in turn could activate Ca²⁺- and Na⁺-activated K⁺ currents. We

FIG. 1. Similarity of intracellular traces in 2 closely located and simultaneously recorded neurons. Top: a fragment of spontaneous electrographic seizure. Top traces are field potentials from the surface and from the depth of neocortex; 2 bottom traces are intracellular activities of 2 different neurons. Fragments indicated by horizontal bars are expanded below at 2 different magnifications. Histogram shows the distribution of differences in the voltage in 2 neurons. To obtain this histogram, the intracellular trace of 1 neuron was subtracted from intracellular trace of another neuron and resulting points were plotted as histogram. Microphotographic inset shows the lateral distance between 2 simultaneously recorded neurons. Two neurons were collected from 2 consecutive sections of 80 μm each.
hypothesized that at least one of those currents could contribute to the generation of seizure-related hyperpolarizing potentials. To study the role of Ca\(^{2+}\)-activated K\(^{+}\) current \(I_{\text{K(Ca)}}\) in the EEG wave components of seizures, we filled up pipettes with BAPTA (50 mM), an intracellular Ca\(^{2+}\) chelator. The major effect of BAPTA on paroxysmal intracellular activities was a decrease in the amplitude of EEG wave-related hyperpolarizing potentials by 7–11 mV \([9.3 \pm 0.2 (SE) \text{ mV}]\) and a slight \(1–2 \text{ mV}\) increase in the maximal depolarization achieved during the PDSs (Fig. 4) as was estimated from histograms of membrane potential distribution. This effect was fully reached within the first 3–5 min of recording and did not change for the duration of the recording (maximum 60 min). Long-lasting recordings with BAPTA also decreased by a few millivolts the spike AHP. Similarly to other studies (Lang and Paré 1997), this effect was saturated after 30–40 min of stable recordings \((n = 7, \text{ not shown})\). These data indicate that \(I_{\text{K(Ca)}}\) contributes to the generation of hyperpolarizing potentials during seizures. The fact that the time course of effects exerted by intracellular BAPTA on spike AHP and on EEG wave-related hyperpolarizations was different might suggest, but not demonstrate directly, that the subtypes of Ca\(^{2+}\)-dependent K\(^{+}\) channels, which mediate these two potentials, were either different (Sah and Louise Faber 2002; Schwindt et al. 1988) or located in different compartments of the neuron.

Because intracellularly applied BAPTA depolarized neuron during hyperpolarizing phases of seizures, it could affect some voltage-dependent currents that were implicated in the generation of PDS. To study the effects of BAPTA on the generation of PDSs, we performed dual intracellular recordings in which the neurons impaled with BAPTA-containing pipette were injected with steady hyperpolarizing current to maintain the level of hyperpolarization that was observed in the neighboring neurons recorded with KAc-filled pipettes during the EEG wave-related hyperpolarizations. These recordings revealed that \(I_{\text{K(Ca)}}\) controls both the amplitude and the duration of PDSs (Fig. 5). The maximal amplitude of PDSs in neurons recorded with BAPTA-filled pipettes was in average by 10 mV more depolarized than the PDSs’ amplitudes in neurons in which \(I_{\text{K(Ca)}}\) was not affected by intracellular drugs. The duration of PDSs measured at half-width was 88.0 \(\pm 4.2 \text{ ms}\) in control neurons and significantly increased \((109.3 \pm 4.8 \text{ ms})\) in the neurons recorded with BAPTA. The paired comparison obtained from dual recordings yields a highly significant difference \((P < 0.001)\). The apparent input resistance during EEG waves in neurons in which \(I_{\text{K(Ca)}}\) was blocked by BAPTA was by 50–100% higher than the input resistance of simultaneously recorded control neurons (Fig. 6), but it was never as high as the input resistance of neurons recorded with Cs\(^{+}\)-filled pipettes. The differences in input resistance in recordings with BAPTA and without BAPTA in the pipettes were not voltage dependent (compare Fig. 6, A with B). These data suggest that \(I_{\text{K(Ca)}}\) significantly contributes to the generation of hyperpolarizing phases of seizures but also indicate that other K\(^{+}\) currents were implicated in the generation of these hyperpolarizing components.

**Depolarizing components of seizures**

The depolarizing components of seizures under scrutiny are represented by PDSs during SW/PSW complexes and the steady depolarization of cortical neurons during the fast runs. Because neurons achieve a significant depolarization during both these components, often higher than the firing threshold, we hypothesized that in addition to synaptic drive (Johnston and Brown 1981) some high-threshold voltage activated currents may contribute to the generation of PDS. In a previous study, we have shown that PDSs increase their duration on
in intracellular injection of steady depolarizing current (Timofeev et al. 2002b). This suggests that high-threshold Ca\(^{2+}\)/H\(^{1+}\) currents and the persistent sodium Na\(^{+}\)/H\(^{1+}\) current \(I_{\text{Na}(p)}\) could contribute to those depolarizations because these currents are activated at depolarized voltages. In vivo intracellular tools are rather limited, and we were not able to use intracellular blockers of Ca\(^{2+}\)/H\(^{1+}\) currents. However, our data shown in Figs. 4–6, in which BAPTA (a Ca\(^{2+}\)/H\(^{1+}\) chelator) was used, indirectly suggest the contribution of Ca\(^{2+}\)/H\(^{1+}\) currents in the generation of PDSs. Indeed, \(I_{K(Ca)}\) significantly contributes to neuronal hyperpolarization during the EEG wave component, thus suggesting that substantial amounts of Ca\(^{2+}\) enter the neurons during the PDSs and thus Ca\(^{2+}\) currents may be implicated in the generation of PDSs.

To study whether or not \(I_{\text{Na}(p)}\) contributes to the generation of PDSs, we performed intracellular recordings with pipettes filled with 50 mM of QX-314, an intracellular blocker of voltage-dependent Na\(^{+}\)/H\(^{1+}\) currents. These recordings revealed that voltage-dependent Na\(^{+}\) currents significantly contribute to the generation of both PDSs (Fig. 7) and fast runs at \(~10\) Hz (Fig. 8). The earliest effects of intracellularly diffused QX-314 were seen in 1–2 min after impalement and consisted of small reduction in the maximal depolarization achieved by the neuron during the PDS. At that time, the fast spikes generated by the transient Na\(^{+}\) current (the amplitude, rise time, and firing threshold) were not yet affected by QX-314. The maximal effect of QX-314 was achieved after 5–8 min from the beginning of recording and consisted of complete or partial abolition of Na\(^{+}\) spikes and significant reduction of PDSs’ amplitudes. The effects of QX-314 on intracellular potential were voltage dependent. At the beginning of a seizure, the maximal depolarization reached by control neurons (no QX-314 added) was from \(~50\) to \(~60\) mV. The mean difference in membrane potential between control neurons and simultaneously recorded neurons loaded with Cs\(^{+}\)-filled pipettes \((n = 20)\) during postictal depression, EEG “waves” and EEG “spikes.”

FIG. 3. Intracellular blockage of K\(^{+}\) currents with Cs\(^{+}\) increases the input resistance of cortical neurons during electrographic seizures. Top: a fragment of field potential and intracellular recording during repetitive seizures. In the depicted period, a small \((-0.1\) nA) steady current was injected into recorded neuron to prevent overt depolarization similar to that seen in Fig. 2. Hyperpolarizing current pulses \((-0.5\) nA, 200 ms) were applied throughout recording to estimate the apparent input resistance of neurons. Middle: the plot showing values of individual measurements of input resistance during seizures and postictal depression. At the bottom left, expanded trace of seizure onset depicted in the upper panel. Bottom right, a comparison of input resistance between control neurons (pipettes filled with KAc \((n = 87)\) and neurons recorded with Cs\(^{+}\)-filled pipettes \((n = 20)\) during postictal depression, EEG “waves” and EEG “spikes.”
EEG depth-negativity was taken as zero (Fig. 8). These data showed that not only the amplitude, but also the slope, of PDSs was slower in neurons recorded with pipettes filled with QX-314. This strongly suggests that $I_{Na(p)}$ significantly contributes in boosting the amplitude, slope, and the maintenance of depolarization reached during the PDS.

**DISCUSSION**

The major findings of this study are as follows. 1) Closely located neocortical neurons display virtually identical fluctuations of the membrane potential during electrographic seizures, directly demonstrating a high degree of focal synchrony during paroxysmal activities. 2) In addition to synaptic drives, $I_{Na(p)}$ contributes to the generation of PDSs during cortically generated seizures. 3) $I_{K(Ca)}$ takes part in the control of the amplitude and duration of PDSs. And 4) hyperpolarizing components of seizures almost exclusively depend on Cs$^+$-sensitive K$^+$ currents. $I_{K(Ca)}$ plays a significant, while not exclusive, role in the mediation of EEG wave-related hyperpolarizing potentials during electrographic seizures.

**Some methodological issues**

Each seizure is unique, and each neuron is different as it has a given number of synapses, located at various compartments, and a unique set of intrinsic currents. The relative contributions of all synaptic and intrinsic currents experienced by cortical neurons during an electrical seizure at a given neocortical site are different. We stained some of the recorded neurons, and we were able to reveal during subsequent morphological analysis that the lateral distance between these neurons was $<0.3$ mm (see Fig. 1). Given such close distance between recorded neurons, similar to studies in other forebrain structures (Stern et al. 1998), the large-scale synchrony in fluctuations of the membrane potential was similar but on a smaller scale, and during normal brain oscillations the membrane potential of neurons was different. However, during seizures, the membrane potential of closely located recorded neurons was virtually identical (Fig. 1). Thus to evaluate the role of possible currents contributing to the generation of different components of seizures, we used simultaneous dual intracellular recordings in which one of the pipettes was filled with a substance to block some currents. This method allowed us to compare the effects of different intracellular drugs with control recordings during the same seizure. Most of the drugs used in the present study had a fast action and, after stabilization of recording, the drug already affected intracellular activities. In those instances in which the quality of recordings was good even immediately after impalement, we were able to study the activity of the same neuron at the beginning of recording as well as several minutes later compared with the activity of a nearby, simultaneously recorded neuron (Fig. 5). In such conditions, the intracellular activities of neurons recorded with pipettes filled with KAc and with pipettes containing other solutions were very similar at the beginning of recordings, and differences between recordings with two different pipettes (as described in RESULTS) became apparent after several minutes. Thus we are confident that the reported results represent the effects of intracellularly applied drugs on neuronal activities.

Another issue concerns the specificity and the spatial extent of the intracellular drugs. The intracellular Cs$^+$ used in this study blocks most of K$^+$ conductances (Hille 2001). As such, the major outward currents were turned off; the neuron became depolarized, and in this state, the high-threshold currents were nonspecifically activated. QX-314 is usually used to block the voltage-gated Na$^+$ currents and G-protein-dependent K$^+$ currents (Andrade 1991; Andreasen and Hablitz 1993; Benardo et al. 1982; Wilson and Kawaguchi 1996), and it also partially

![EEG depth-negativity](image-url)

**FIG. 4.** Ca$^{2+}$-activated K$^+$ current contributes to the generation of hyperpolarizing potentials during paroxysmal EEG waves. Field potential and intracellular traces in 2 experimental conditions. The maximum of field depth negativity was taken as zero point. The amplitude of hyperpolarizing potentials associated with EEG waves decreased.
blocks high-threshold Ca\(^{2+}\) currents (Talbot and Sayer 1996). However, in our experiments, the QX-314 did not affect the hyperpolarizing phases of the seizures, suggesting that most of the observed effects were initiated by changes in voltage-gated Na\(^{+}\) conductances. The block of \(I_{\text{Na(p)}}\) in the perisomatic region significantly decreased the amplitude of PDSs. We cannot exclude that blockage of Na\(^{+}\) conductances did not affect high-threshold Ca\(^{2+}\) currents because after blockage of Na\(^{+}\) depolarizing influences, the membrane was not sufficiently depolarized to drive Ca\(^{2+}\) currents.

All in all, our data suggest which conductances contribute to the generation of different components of seizures but cannot indicate the extent of the action. To compensate for this incompleteness of measurements, we used a computational modeling approach that is fully described in the companion paper (Bazhenov et al. 2004).

Sequence of events leading to the seizure generation, maintenance, and termination

Different seizures have different origins. It has been shown that the slow sleep oscillation and cortically generated seizures share many similarities as, during both slow oscillation and seizures, the field EEG potentials reveal periodic positive and negative waves and cortical neurons are depolarized during the EEG depth-negativity and hyperpolarized during the EEG depth-positivity (Steriade et al. 1998b). Due to spontaneous variations in the degree of membrane polarization, the depolarizing component of the cortical slow oscillation may induce higher than usual neuronal firing (see Fig. 1 in Bazhenov et al. 2004). The increased firing rapidly increases the local [K\(^{+}\)]\(_{o}\), which creates conditions for neuronal swelling leading to tighter synchronization between neurons (Andrew and MacVicar 1994; Dietzel et al. 1980; Grenier et al. 2003a). Consequently, in the focus of seizure generation many cortical neurons start to fire simultaneously at frequencies ~100 Hz, generating the initial PDS (Grenier et al. 2003b). Another possible mechanism of seizure initiation might be based on advanced firing of IB neurons. In in vitro models of epileptiform activities, those bursts preceded the onset of field paroxysmal discharges (Chagnac-Amitai and Connors 1989; Jensen and Yaari 1997; Sanabria et al. 2001). Although this possibility exists, we cannot support it with our data because the firing of
none of >100 IB neurons recorded in the present and our previous in vivo studies (not shown) did not anticipate the field potential paroxysmal spikes and the relative number of IB neurons is very low (4%) in behaving animals (Steriade et al. 2001), whereas many of them display spontaneous seizures.

What are the main elements that contribute to PDS generation? In the majority of neurons, the initial depolarization during PDS is driven by EPSPs arising in neighboring or more distant cortical neurons. Studies in bicuculline-treated slices suggested that the PDS is a giant EPSP (Johnston and Brown 1981). More recent data suggest, however, that synaptic inhibition remains functional in most forms of paroxysmal activities (Davenport et al. 1990; Esclapez et al. 1997; Higashima 1988; Prince and Jacobs 1998; Traub et al. 1996) and significantly contribute to PDS generation because of the depolarizing driving force in conditions of high \([K^+]_o\) (Timofeev et al. 2002b). So far, the enhancement of PDS by activation of voltage-regulated intrinsic currents was only shown in vitro (de Curtis et al. 1999; Richter and Ayala 1987; Prince and Connors 1984; Westbrook and Lothman 1983; Wong and Prince 1978).

In the present study, the effects exerted by QX-314 support the idea that \(I_{Na}(p)\) (Crill 1996; Stafstrom et al. 1982) contributes to the generation of PDSs in vivo (Figs. 7 and 8). The fact that intracellularly infused BAPTA affected both de- and hyperpolarizing components of seizure indicates that significant quantities of \(Ca^{2+}\) entered the neurons during the PDS. An important contribution of \(N\)-methyl-D-aspartate (NMDA)-dependent \(Ca^{2+}\) contribution is unlikely because our experiments were done under ketamine-xylazine anesthesia, and ketamine at anesthetic doses blocks NMDA-dependent synaptic events (MacDonald et al. 1991). Our data do not allow the exact estimation of the ratio of different depolarizing factors because

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**Fig. 6.** Intracellular blockage of \(Ca^{2+}\)-activated \(K^+\) current with BAPTA increases the input resistance during the EEG wave. A: 1 pair of neurons. Top: depth-EEG, simultaneous dual intracellular recording from a pair of neurons and current monitor. Intra-cell 1 was recorded with pipette filled with potassium acetate (2.5 M), Intra-cell 2 was recorded with pipette filled with potassium acetate (2.5 M) and BAPTA (50 mM). During recording, 0.5-nA current pulses were applied to estimate apparent input resistance. A fragment indicated by horizontal bar is expanded in A, bottom left. Right: averaged responses \((n = 20)\) to current pulses applied during EEG wave. B: 2nd pair of neurons. A slight negative current (0.2 nA) was applied to intra-cell 1 to maintain a similar membrane potential during the hyperpolarizing phase of activity in both neurons. During recording, 0.25-nA current pulses were applied to estimate apparent input resistance. Right: averaged responses \((n = 10)\) to current pulses applied during EEG wave. In both examples the neuron recorded with BAPTA-containing pipette revealed higher input resistance.
synaptic depolarizing influences activate intrinsic Na\(^+\) and Ca\(^{2+}\) conductances that boost each other. However, given the fact that \(I_{\text{Na(p)}}\) is activated at voltages that are by 10 mV lower that the firing threshold of neurons (Crill 1996), we expect that the initial intrinsic drive depends on this ionic current. An interaction of somatic firing with dendritic depolarization (Stuart and Hausser 2001) may further boost paroxysmal depolarization.

The seizure’s hyperpolarizations and/or postictal depression (related to the EEG waves) were strongly dependent on Cs\(^+\)-sensitive K\(^+\) currents. Within 1–2 min after impalement, all RS neurons revealed depolarizing potentials during paroxysmal activities and were depolarized by about −20 mV during the pause between successive epileptiform events (Fig. 2), thus pointing to a dominant role of K\(^+\) currents in the generation of seizure-related hyperpolarizing potentials. However, such a strong depolarization was probably achieved by activation of Ca\(^{2+}\) currents, in the absence of the majority of hyperpolarizing K\(^+\) currents, rather than by the simple absence of hyperpolarizing influences. This conclusion is also based on the fact that, during seizures, when the time between consecutive PDSs was short and there was no depolarizing drive to generate depolarizing plateau potentials, the membrane potential remained unchanged (Fig. 2). Intracellular BAPTA significantly decreased hyperpolarizing potentials during the seizure (Fig. 4), but its effect was much less strong, compared with that of intracellularly infused Cs\(^+\), suggesting that other K\(^+\) currents contributed to the generation of hyperpolarizing potentials. One of the most likely candidates is \(I_{\text{K(Na)}}\) (Schwindt et al. 1989). Indeed, a significant amount of Na\(^+\) enters cortical neurons during the PDSs and may thus activate this current.

The putative role of GABA\(_{A}\)-receptor-mediated IPSPs in the generation of seizure-related hyperpolarizations, previously proposed in modeling studies (Destexhe 1998), is unlikely because long-lasting recordings with pipettes containing QX-314, a potent intracellular blocker of GABA\(_{B}\) IPSPs (Nathan et al. 1990) did not affect the generation of seizure-related hyperpolarizing potentials (Figs. 7 and 8). Thus we conclude that \(I_{\text{K(Ca)}}\) and a “leak” current are major contributors for the generation of seizure-related hyperpolarizing potentials. When a significant hyperpolarization occurred, in conditions of increased [K\(^+\)], the \(I_{\text{h}}\) depolarized a part of cortical neurons to firing threshold, which led to the generation of the next paroxysmal cycle (Chen et al. 2001; Timofeev et al. 2002a).

During the seizure, the long-range synchronization between remote sites of recordings increases (Steriade and Amzica 1994; Topolnik et al. 2003), leading to increased maximal depolarization, which is achieved by neurons that are involved in PDSs toward the end of the seizure (Steriade et al. 1998a). The increase in the depolarization is associated with increased intracellular concentrations of Na\(^+\) and Ca\(^{2+}\), which in turn activate \(I_{\text{K(Ca)}}\) and \(I_{\text{K(Na)}}\) currents, resulting in an increase in the maximal hyperpolarization achieved by neurons (Steriade et al. 1998a). When this hyperpolarizing potential overwhelms the depolarization induced by \(I_{\text{h}}\), the seizure is terminated.

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FIG. 8. Persistent sodium current boosts cellular depolarization during both runs of fast spikes and PDSs. Top: depth-EEG and simultaneous dual intracellular recording from a pair of neurons. Intracellular 1 was recorded with pipette filled with potassium acetate (2.5 M), Intracellular 2 was recorded with pipette filled with potassium acetate (2.5 M) and QX-314 (50 mM). Bottom left: an expanded period shows the effects of QX-314 on intracellular activities during PDSs and fast runs. Bottom right: WTA (n = 15) of 2 intracellular traces. The 1st EEG maximum during EEG “spike” was taken as reference time. Note that intracellular QX-314 decreased not only the maximal amplitude of PDS but also the maximal slope of depolarization.


G R A N T S

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