THE ROLE OF CHLORIDE-DEPENDENT INHIBITION AND
THE ACTIVITY OF FAST-SPIKING NEURONS DURING CORTICAL
SPIKE–WAVE ELECTROGRAPHIC SEIZURES

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Abstract—The conventional view is that the cortical paroxysmal depolarizing shift is a giant excitatory postsynaptic potential enhanced by various intrinsic neuronal currents. Other results point out, however, that synaptic inhibition remains functional in many forms of paroxysmal activities and that intense activation of GABAergic interneurons may accentuate the excitation of target pyramidal cells. To determine the role played by cortical inhibitory neurons in paroxysmal discharges, we used single and dual intracellular recordings from electrophysiologically identified neocortical neurons during spontaneously occurring and electrically induced spike–wave electrographic seizures in vivo. Conventional fast-spiking neurons (presumably local inhibitory interneurons) fired at a very high frequency during paroxysmal depolarizing shifts, which corresponded to the electroencephalogram ‘spike’ components of spike–wave complexes. The firing of fast-spiking neurons preceded the discharges of neighboring regular-spiking neurons. During electrographic seizures, the reversal potential of the GABA (type A)-mediated potentials in regular-spiking neurons was shifted to positive values by 20–30 mV. Data also show that the prolonged hyperpolarizations during the electroencephalogram ‘wave’ components of spike–wave electrographic seizures do not contain Cl−-dependent inhibitory potentials. Moreover, Cl−-dependent mechanisms were reduced or absent during the fast runs that are associated with spike–wave complexes in some paroxysms. We conclude that the strong activity of cortical inhibitory neurons during paroxysmal depolarizing shifts induces Cl−-dependent depolarizing postsynaptic potentials in target pyramidal neurons, which facilitate the development of electrographic seizures.

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Key words: electrographic seizure, spike–wave, fast runs, inhibition, fast-spiking, excitability.

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Different types of electrographic patterns are used for the diagnostic of epileptic disorders (Niedermeyer, 1999a,b). Earlier and more recent studies emphasized the leading role of neocortex in the generation of seizures with spike–wave (SW) complexes at about 3 Hz (Marcus and Watson, 1968; Steriade, 1974; Steriade and Yossif, 1974; Gloor et al., 1990; Steriade and Amzica, 1994; Kandel and Buzsáki, 1997; Neckelmann et al., 1998).

It was similarly shown that more complex seizures, consisting of SW/polyspike–wave (PSW) complexes at a lower frequency (1.5–3 Hz), often associated with fast runs (10–15 Hz), are generated intracortically (Steriade et al., 1998a; Steriade and Contreras, 1998; Timofeev et al., 1998), whereas thalamocortical neurons are steadily hyperpolarized throughout the seizure (Steriade and Contreras, 1995; Pinault et al., 1998). Depth-negative electroencephalogram (EEG) components (the EEG ‘spikes’) are associated with neuronal depolarization and firing, while depth-positive EEG components (the EEG ‘waves’) correspond to neuronal hyperpolarization and silence in all types of cortical neurons.

Epileptogenesis is associated with an increase in [K+]o (Moody et al., 1974), leading to cellular depolarization, increased excitability (Traynelis and Dingledine, 1988; McNamara, 1994), positive shift in Cl− reversal potential (Tasker and Dudek, 1991), and conversion of some regular-spiking (RS) neurons to intrinsically bursting (IB) ones (Jensen et al., 1994; Jensen and Yaari, 1997). The positive shift in Cl− reversal potential in the condition of high [K+]o might be due to the activity of the neuron-specific protein K+–Cl− (KCC2) co-transporter (DeFazio et al., 2000).

It is widely accepted that a shift in the balance between excitation and inhibition towards excitation results in the development of epileptiform activities (Dichter and Ayala, 1987; Tasker and Dudek, 1991; Galarreta and Hestrin, 1998; Nelson and Turrigiano, 1998). The easiest way to elicit acute experimental seizures consists in the blockade of inhibition (Matsumoto and Ajmone-Marsan, 1964a,b; Prince, 1978; Gutnick et al., 1982; Chagnac-Amitai and Connors, 1989a,b; Steriade et al., 1998a). The conventional point of view is that the paroxysmal depolarizing shift (PDS) consists of a giant excitatory postsynaptic potential (Johnston and Brown, 1981), enhanced by activation of voltage-regulated intrinsic currents (Wong and Prince, 1978; Westbrook...
and Lothman, 1983; Prince and Connors, 1984; Dichter and Ayala, 1987; de Curtis et al., 1999). However, other results point out that synaptic inhibition remains functional in most forms of paroxysmal activities (Higashima, 1988; Davenport et al., 1990; Traub et al., 1996; Esclapez et al., 1997; Prince and Jacobs, 1998). Thus, the working hypothesis in the present study was that GABAergic neurons may participate in paroxysmal manifestations in vivo.

**EXPERIMENTAL PROCEDURES**

Intracellular recordings from neocortical neurons were performed in 65 cats anesthetized with ketamine-xylazine (10–15 mg/kg and 2–3 mg/kg; i.m.) and 10 cats anesthetized with somnotol (35 mg/kg; i.p.). Following ketamine-xylazine anesthesia, about 30% of cats (n = 22) displayed spontaneous electrographic seizures consisting of SW/PSW complexes at 1.5–3 Hz, often associated with fast runs at about 10–15 Hz. In those cases in which cats under ketamine-xylazine anesthesia did not display spontaneous electrographic seizures, or in barbiturate-anesthetized cats, the electrographic seizures were elicited by three to four pulse-trains (10–20 stimuli at 100 Hz) applied to cortical areas, in the vicinity of the intracellular recording pipette. In three barbiturate-anesthetized cats 20 µl of a 50 mM solution of bicuculline was injected in the vicinity of intracellular recording pipettes.

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, while the inner pole was placed at 0.8–1 mm in the cortical depth.

Intracellular recordings were obtained with sharp glass micro-pipettes filled in the majority of cases with a solution of 2.5–3.0 M potassium acetate (KAc). In some experiments with dual intracellular recordings, one pipette was filled with KAc while the other pipette was filled with potassium chloride, KCl (2.0–3.0 M). Intracellular pipettes had a DC resistance of 30–80 MΩ. A high-impedance amplifier (bandpass, 10 kHz) with an active bridge circuitry was used to record and inject current into the neurons. Before the recording session, 3–5 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 bandpass of at least 0–9 kHz and digitized at 10–20 kHz for off-line computer analysis.

At the end of experiments animals were given a lethal dose of somnotol, perfused, and the position of stimulating and recording electrodes was verified on 80 µm Thionin-stained sections.

**RESULTS**

*Database and neuronal identification*

Intracellular potentials were recorded from 173 cortical neurons in pericruciate and syprrsylvian gyri during different forms of paroxysmal activities. Neocortical neurons were classified in four electrophysiological cell types, as defined in previous in vitro studies and in vivo experiments on anesthetized animals (Connors and Gutnick, 1990; Gray and McCormick, 1996; Steriade et al., 1998b). The electrophysiological identification of these neuronal types has recently been performed during natural states of vigilance in chronic experiments (Steriade et al., 2001; Timofeev et al., 2001). In the present study, we recorded activities of 101 RS neurons, 25 IB neurons, 35 fast rhythmic bursting (FRB) neurons, and 12 fast-spiking (FS) neurons. All conventional FS neurons are regarded as inhibitory (Connors and Gutnick, 1990; Gutnick and Crill, 1995; Kawaguchi, 1995; Thomson et al., 1996), whereas FRB neurons could be either excitatory or inhibitory neurons (Steriade et al., 1998b).

**Electrographic seizures are associated with decreased input resistance and intrinsic responsiveness of RS neurons**

The apparent input resistance of cortical neurons was measured by application of short-lasting hyperpolarizing current pulses (Fig. 1) or by injections of prolonged depolarizing and hyperpolarizing steady current (see below, Figs. 3–5). The dynamics of intrinsic responsiveness of neurons was estimated as changes in the number of spikes elicited by a current pulse of given intensity and duration. This is an integrative measure that roughly depends on apparent input resistance, membrane potential and activation of voltage-dependent intrinsic currents. Consecutive application of depolarizing and hyperpolarizing current pulses to the same neurons before, during and after electrographic seizures revealed that the decrease in the input resistance of cortical neurons occurs simultaneously with a decrease in intrinsic responsiveness throughout the electrographic seizures. This finding is congruent with previous (Matsumoto et al., 1969) and recent (Neckelmann et al., 2000) studies showing an increase in conductance and a decrease in responsiveness to depolarizing current pulses associated with electrographic seizures. The decreased input resistance and responsiveness were progressively recovered during postictal depression (Fig. 1). The decreased input resistance could occur because of either activation of intrinsic currents or excessive synaptic activities. Since synaptic inhibition is associated with a significant decrease in input resistance (Connors et al., 1988) and shunting of spikes, we suppose that inhibition plays a significant role in the generation of PDSs. Based on this we asked the question: Does the activity of inhibitory interneurons play a significant role in the observed phenomena?

**Inhibitory reversal potential becomes less negative during electrographic seizures**

To evaluate the reversal potential for inhibitory postsynaptic potentials (IPSPs), we applied cortical stimuli during depolarizing and hyperpolarizing current pulses before and during electrographic seizures. Before the electrographic seizures, the reversal potential for IPSPs under both ketamine-xylazine (n = 5) and barbiturate (n = 8) anesthesia was −69.7 ± 2.5 mV (mean ± S.E.M.), range −65 mV to −74 mV (see an example in Fig. 2). During the electrographic seizures, the reversal potential (measured at the same latency) was more depolarized and reached −46.7 ± 3.5 mV in barbiturate-anesthetized cats (n = 5, Fig. 2, A) and −54.2 ± 2.9 mV during ketamine-xylazine anesthesia (n = 14, not shown). The slope
of fitting line for reversal potential was identical before the electrographic seizures and during the electrographic seizures (Fig. 2, plot) suggesting similar underlying cellular process. These data suggest that during the electrographic seizure the reversal potential for IPSPs is shifted towards depolarization and, furthermore, at depolarized voltages the amplitude of IPSPs appears to be small. Barbiturates increase the time of opening of Cl⁻ channels (Twyman et al., 1989). This observation and our data show that, under barbiturate anesthesia, the depo-
larization during PDS is greater. The more Cl\(^{-}\) channels open, the larger the depolarization. As Cl\(^{-}\) channels remain open longer under barbiturate [Cl\(^{-}\)], will increase more, leading to a less negative reversal potential for GABA\(_{A}\) IPSPs.

Consistent with the idea that the PDS component of SW seizures (EEG ‘spike’) contains an inhibitory component, we were able to reverse the intracellular trace during an entire electrographic seizure by injecting strong depolarizing currents. An intracellular injection of 2.1 nA DC depolarizing current resulted in depolarization of neuron depicted in Fig. 3 to the level of -39 mV. At this voltage, Na\(^{+}\) spikes were inactivated and the entire electrographic seizures exposed a hyperpolarizing envelope. Depth-negative components of EEG that are normally associated with neuronal depolarization were hyperpolarizing during interictal PDSs (Fig. 3b), fast runs (Fig. 3c) and PDSs associated with SW complexes (Fig. 3d). A vast majority of recorded neurons revealed the presence of a long-lasting hyperpolarization associated with EEG postictal depression following electrographic seizure (see Figs. 1 and 6). In three neurons, which we were able to depolarize to the levels of spike inactivation, the current associated with postictal depression (most likely Ca\(^{2+}\)-activated K\(^{+}\) current; see Alger and Nicoll, 1980) was able to repolarize neurons to the levels of firing (Fig. 3e, see inset). Although some high-threshold intrinsic currents were activated during the PDS (Wong and Prince, 1978; de Curtis et al., 1999), the fact that PDSs were associated with hyperpolarizing potentials indicates that the membrane was clamped at a certain voltage by a strongly activated conductance. In

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**Fig. 2.** Reversal potential for IPSPs of RS neuron is positively shifted by about 20 mV during electrographic seizures. Barbiturate anesthesia. One electrical stimulus to cortex, in the vicinity of the recording micropipette, was applied during positive or negative intracellular current pulses delivered every second. Electrographic seizures were elicited by four brief pulse-trains at 100 Hz. Top panel shows an intracellular recording obtained in one experiment. Bottom left and middle panels show examples of neuronal responses to single stimuli applied before the electrographic seizure and during the early phase of electrographic seizure, respectively. Bottom right panel shows the reversal potential of neuron before the electrographic seizure (-) and during the electrographic seizure (△). Note that the slope of fitting curves is identical during electrographic seizure and before the electrographic seizure.

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**Fig. 3.** Epochs with SW complexes and fast runs during full-blown electrographic seizure, as well as interictal spikes, contain an inhibitory component. Ketamine-xylazine anesthesia. RS neuron. Interictal spikes and electrographic seizure elicited by cortical stimulation (three trains of 20 stimuli at 100 Hz, one train per second, as indicated by arrowheads in the upper panel). Prior the stimulation (a), the cortical neuron was depolarized from rest by steady injection of depolarizing current (2.1 nA). From a membrane potential of -39 mV, interictal spikes (b), as well as fast runs (c) and SW/PSW complexes (d) of electrographic seizure, were hyperpolarizing, indicating that inhibition constitutes an important part of these events. The bottom panel is an expansion of the end of electrographic seizure and of the postictal depression (e). Recovery to the resting membrane potential is illustrated in panel (f).
EEG - depth area 4

-39 mV

-68 mV

Intra-cell area 4

Fig. 3.
Fig. 4.
conditions of blocked inhibition by bicuculline, the high-threshold intrinsic currents were enhanced by injection of depolarizing current that results in an increase of the duration of PDS (see Fig. 5), thus suggesting a role of high-threshold currents in the PDS generation. Since neurons depolarized with DC current reveal hyperpolarizing envelope during the PDSs, we estimated the reversal potential for PDSs during interictal spikes and during PDSs associated with electrographic seizures. To do so, we used the mean voltage for 100–150 ms just preceding the PDS as reference voltage and the mean voltage during the PDS as voltage of PDS (Fig. 4). The reversal potential for spontaneously occurring, interictal PDSs ranged from −70 mV to −65 mV (−68.3 ± 0.6 mV, mean ± S.E.M.; n = 3; Fig. 4) and the reversal potentials for spontaneous PDSs during electrographic seizures were at mean −55.2 ± 0.8 mV (n = 8, not shown). Recordings with KCl-filled pipettes revealed much stronger depolarization, compared to simultaneous recordings with KAc-filled pipettes (see below, Fig. 8). The reversal potential for PDSs measured with KCl-filled pipettes was by 15–20 mV less negative (−38 ± 1.4 mV, n = 4) than the reversal potential for PDSs in control conditions.

In a separate group of experiments, we studied the reversal potential for PDSs recorded with KAc-filled pipettes following bicuculline (20 μl of 50 mM) injection into the cortex, near the place of intracellular recordings (Fig. 5). The estimated reversal potential of PDSs in these conditions was between −25 mV and −5 mV (mean −18.2 ± 1.8 mV, n = 9), namely, it was shifted to depolarized values by more than 25 mV as compared to the reversal potential of PDSs that occurred without bicuculline injection. The input resistance at the maximal amplitude of PDSs during interictal EEG ‘spikes’ as well as during spontaneously occurring full-blown electrographic seizures was extremely low (1–2 MΩ, n = 8) and it was higher (5–10 MΩ, n = 9) after the blockade of GABA A-mediated IPSPs by bicuculline (not shown). These data indicate the presence of an important bicuculline- and Cl−-sensitive inhibitory component during the PDS.

Role of FS neurons in electrographic seizures generation

We found at least three different aspects that specifically characterized the activity of FS neurons during electrographic seizures.

Firstly, FS neurons increased their firing rates before the electrographic seizure and especially during the first PDS (Fig. 6). The precursor-increased firing related to electrographic seizure was seen in 29 out of 36 electrographic seizures in which we recorded activities of FS neurons. The firing threshold for the first spikes within the first PDS was lower than before the electrographic seizure or during consecutive PDSs (bottom panels in Fig. 6), thus suggesting a high excitability of FS neurons in the period immediately preceding the electrographic seizures. The lowering in firing threshold may be due to field effects that accompany the electrographic seizures (Taylor and Dudek, 1984; Dudek et al., 1998; Grenier and Steriade, 2001).

Secondly, the intrinsic responsiveness of FS neurons was high during the electrographic seizures and, in particular, at the onset of electrographic seizures. In the neuron shown in Fig. 7 the mean number of spikes during spontaneous PDSs was 14.6 ± 0.8. Application of depolarizing current pulses of 1 nA to FS neurons elicited a greater number of spikes during the first PDS (from 37 to 43) within an electrographic seizure (Fig. 7; see (6) and arrowheads in plot at bottom). The same neuron between seizures responded with four to 12 spikes with current pulses of the same amplitude depending on the phase of the slow oscillation. These data indicate the highest excitability of FS neurons at the onset of electrographic seizure and also corroborate the increase in spontaneous firing rates of FS neurons prior to electrographic seizures (Fig. 6). The relatively high intrinsic responsiveness of FS neurons at the onset of electrographic seizures stood in contrast with the significantly decreased responsiveness of RS neurons during electrographic seizures (Fig. 1). The recovery of excitability of FS neurons during postictal depression usually occurred within the first few seconds (Fig. 7; see fragments (3), (4), (5) and plot). This feature also stands in contrast with the properties of RS neurons, in which the depressed excitability usually lasted for minutes (Fig. 1) or until the next electrographic seizure. Despite the fact that the excitability of FS neurons was high during electrographic seizures, it was not constant. These neurons revealed regular changes during various paroxysmal cycles, consisting of a decreased excitability during the onset of the EEG ‘wave’ of SW complexes and a dramatic increase in excitability at 100–150 ms before the next PDS. The phasic changes of excitability within the electrographic seizure were tested by application of short (100 ms) depolarizing current pulses throughout the electrographic seizure (Fig. 8). All tested FS neurons (n = 5) showed low responsiveness at the onset of the EEG ‘wave’ and increased excitability prior as well as during the PDS. As shown above, RS neurons decreased their responsiveness during the electrographic seizure (Fig. 1).

Thirdly, in contrast to RS neurons, most FS neurons...
Fig. 5. Reversal potential of PDS after intracortical bicuculline injection. Barbiturate anesthesia. Depicted period of recording was obtained 90 min after an injection of 20 µl of bicuculline 50 mM into the cortex 1.5 mm posterior to the placement of recording pipette. Intracellular recordings were obtained in current clamp mode. Upper panel illustrates a period of EEG, intracellular activities and current monitor. Steady DC intracellular current was injected in a range from −0.6 nA to +0.6 nA with an increment of 0.2 nA. Bottom left panel shows a sample of PDSs, which occurred under different levels of intracellular current injection. Traces were aligned by the first spike in PDS. Right upper panel shows estimated reversal potential. Voltage values were taken at time instances indicated in left panel. Right bottom panel shows that the duration of PDS depends on the membrane potential.
Fig. 6. Activity patterns of FS neurons during spontaneously occurring electrographic seizure with PSW complexes. Ketamine-xylazine anesthesia. Top inset shows identification of FS neuron by response to depolarizing current pulse. The cat displayed numerous electrographic seizures and the FS neuron was silent during the postictal depression. Four to 6 s before the next electrographic seizure, the neuron started firing. Maximal firing frequency (800 Hz) was reached at the onset of the first PDS. The onset of electrographic seizure is expanded below (horizontal bar and arrow; further expanded at right). Small arrows in the right panel indicate firing threshold. The firing threshold remained decreased during the onset of following paroxysmal cycles (bottom panels).
Fig. 7. Excitability of FS neuron increases prior to electrographic seizures and remains high during electrographic seizures. Ketamine-xylazine anesthesia. Spontaneous electrographic seizures. Excitability was estimated by application of intracellular current pulses (1.0 nA, 200 ms). Upper panel shows simultaneous recording of surface-EEG, DC depth-EEG recorded within 200 μm from the neuron, and intracellular activity of FS neuron during two electrographic seizures and the postictal depression between them. Fragments indicated by numbers are expanded in the middle panel. Current pulses were applied during: (1) EEG ‘wave’; (2) EEG ‘spike’; (3), (4), (5) postictal depression; and (6) first EEG ‘spike’ of the second electrographic seizure. Note a progressive increase in the number of spikes during postictal depression although the membrane potential remained unchanged. At bottom a plot showing neuronal responsiveness, expressed as a number of spikes elicited by the current pulse. Filled squares underlie the fragment shown in upper panel.
Fig. 8. Alternation in excitability of FS neuron during electrographic seizures. Ketamine-xylazine anesthesia. Fragment of spontaneous electrographic seizure. Excitability was tested using short (100 ms) depolarizing current pulses. The intensity of current pulse (1.5 nA) was adjusted to not elicit a spike during the early phases of EEG ‘wave’ (depth-positivity) of SW complexes. Upper panel shows a fragment of EEG and intracellular recording. Left column shows intracellular responses to current pulses during different phases of paroxysmal cycles. Middle right, spike threshold for current pulse is more positive than that for the first spike in PDS. Bottom right, plot showing changes of responsiveness of neuron as a function of the two components (‘wave’ and ‘spike’) in the paroxysmal cycle. Gray square indicates number of spikes and time of their occurrence during spontaneous paroxysmal cycles. Horizontal dotted line indicates the mean firing rates during PDS.
did not display complete spike inactivation during the PDS (Figs. 7–9, 11; but see Fig. 6 in which spike inactivation in FS neuron occurred). Since RS neurons fired only one to two spikes during PDSs, they cannot maintain synaptic depolarization of postsynaptic neurons. Thus, the postsynaptic effect of FS neurons firing during PDSs becomes an important synaptic factor contributing to the maintenance of the membrane potential in target structures.

Fast runs (10 Hz) do not contain a significant inhibitory component

We recorded 12 FS neurons during 27 electrographic seizures that contained fast runs longer than 2 s. In 24 electrographic seizures, FS neurons did not fire at all or presented only one to two spikes in some cycles (Fig. 9). By contrast, most RS neurons fired on almost every cycle during fast runs (Fig. 10) or were depolarized to the level of spike inactivation (see fig. 5 in Steriade et al., 1998a). The behavior of FS neurons during fast runs is likely ascribable to asynchronous synaptic bombardment. It is quite common for fast runs to reveal either slight changes in the frequency of oscillation in neighboring sites of recordings or a phase shift of up to 180° (Timofeev et al., 1998). These features are reflected in FS neurons as a high degree of asynchronous synaptic events that might be modulated at a given frequency of fast runs or the double of this frequency (Fig. 9). The asynchronous synaptic input is probably not able to depolarize FS neurons to their firing threshold (see Discussion). Even in cases where FS neurons discharged several spikes during each cycle of fast runs, the firing ratio FS/RS was lowest during fast runs (see Fig. 11).

Effects of intracellular Cl⁻ on PDS

We evaluated the role of Cl⁻-dependent potentials
during SW components and fast runs in simultaneously recorded pairs of closely located neurons (lateral distance, < 0.5 mm). One neuron was recorded with KAc-filled pipette, while the other neuron was recorded with KCl- and DIDS-filled (n = 8) pipettes to increase the effects of intracellular Cl⁻ by blocking the Cl⁻ pump (Lang and Paré, 1997). The results show a significantly increased depolarization during PDSs of SW complexes in all neurons recorded with Cl⁻-containing pipette and a small difference between the membrane potential of the two neurons during fast runs (Fig. 10). We calculated the membrane potential during 100 ms preceding the PDS and during 100 ms within the PDS in recorded pairs of neurons. The mean hyperpolarization achieved by neurons recorded with KAc and KCl in the pipette during the ‘wave’ did not differ (−71.3 ± 2.5 mV and −67.0 ± 2.0 mV correspondingly, P = 0.17), indicating that hyperpolarizing potentials between PDSs were not generated by Cl⁻-dependent IPSPs. The mean depolarization during the PDS was significantly different (−56.3 ± 1.8 mV and −34.9 ± 2.7 mV correspondingly, P < 0.001). These data
Fig. 11. Leading role of FS neuron during PDSs of SW complexes, and of RS neuron during fast runs. Ketamine-xylazine anesthesia. Middle panel shows a fragment of a electrographic seizure with simultaneous EEG and dual intracellular recordings from FS and RS neurons. Fragments of fast runs and a SW complex, indicated by horizontal bars and arrows, are expanded in top panel. Top left inset shows a comparison of action potentials fired by the two neurons before the electrographic seizure onset. Bottom plots show the time relation of firing between the two neurons and the firing ratio (FS/RS) during the electrographic seizure. Upward lines in the bottom panel correspond to the fragment of electrographic seizure that is expanded in the middle panel.
support the idea that Cl−-dependent mechanisms are implicated in the generation of PDSs belonging to SW complexes, but to much lesser extent in the generation of fast runs.

Temporal relations between the activities of FS and RS neurons during electrographic seizures

Since the activity of FS neurons dominates that of RS neurons during PDSs, and their relative role significantly decreases during fast runs, we studied the temporal relations of cell firing in five simultaneously recorded cell pairs, in which one neuron was FS and the other RS. In all these cell couples, recorded with lateral distance <0.2 mm, the first spike of FS neuron preceded by 2–10 ms the first spike of RS neuron during the PDSs of SW/PSW complexes, whereas the first spike of RS neuron preceded by 2–10 ms the spike of FS neuron during fast runs (Fig. 11).

DISCUSSION

The present results show that depolarizing components during cortical SW complexes contain an important inhibitory component that depends on the firing of conventional FS neurons prior to and during the PDSs, and that Cl−-dependent depolarizing potentials are imposed upon target RS neurons. During electrographic seizures, the reversal potential of IPSPs is shifted to depolarized values, which results in the depolarization of postsynaptic neurons.

Mechanisms of depolarization through activity of GABAergic neurons during PDSs

Repetitive firing of inhibitory neurons during PDSs produces a prolonged opening of Cl− channels. Due to the postsynaptic uptake of Cl−, the [Cl−] increases. This causes a decrease in the amplitude and even a change in the polarity of IPSPs that become depolarizing (Thompson and Gahwiler, 1989). Prolonged high-frequency stimulation (Kaila et al., 1997; Taira et al., 1997) or spontaneous high-frequency firing of inhibitory interneurons (present study) may induce a rapid GABAA-mediated bicarbonate-dependent increase in the [K+]o. An increase in [K+]o in mature neocortical pyramidal neurons would result in further increase in [Cl−] (DeFazio et al., 2000). These factors seem sufficient to mediate the Cl−-dependent postsynaptic depolarization during electrographic seizures.

It is widely accepted that inhibitory interneurons in the neocortex play a coordinating role in different forms of activity (Lytton and Sejnowski, 1991; Whittington et al., 1995; Somogyi et al., 1998), which is maintained via chemical and electrical synapses (Galarreta and Hestrin, 1999; Gibson et al., 1999). Previous studies on hippocampal slices treated with 4-aminoypyridine or with high [K+] (Michelson and Wong, 1991) and recent data on neocortical slices indicate that the reversal potential for IPSPs in cortical FS neurons is significantly more depolarized than the reversal potential for IPSPs in RS neurons (Martina et al., 2001). The IPSPs directly depolarize FS neurons to the firing threshold. These results suggest that a spike occurring in a small number of FS neurons will produce an excitation in other FS neurons with very short delays. In conditions of high [K+]o, the IPSPs generated by FS neurons will depolarize and actually excite all target neurons. This is confirmed by our data showing that, in local cortical networks, the FS neurons fire before the RS neurons (see Fig. 11). Prolonged firing of FS neurons significantly decreases the input resistance of postsynaptic cells and induces a shunting inhibition, which prevents postsynaptic neurons from firing (Eccles, 1964; Borg-Graham et al., 1998; Hirsch et al., 1998). During the PDS, this would be reflected as a reduction in spikes’ amplitude or their abolition, which is the case in the majority of neocortical RS neurons.

Cortical networks during fast runs

Our data show that the paroxysmal fast runs, which are usually associated with tonic components of epileptic seizures (Niedermeyer, 1999b), do not contain a significant inhibitory component. This conclusion is based on two sets of data: FS neurons significantly reduce their discharges, up to their abolition; and the FS/RS ratio of spikes per cycle and intracellular recordings with Cl−-filled pipettes indicate that the Cl−-dependent synaptic component during the fast runs is significantly reduced as compared to SW complexes. We suggest that the reason for such a low firing rate of FS neurons is the low level of synchrony during the fast runs. The local synchrony during the fast runs varies from one epoch to another as is reflected in the different amplitude of field potentials. However, the long-scale synchrony between different sites of recordings is rather low as it has slightly different frequencies and/or phase shifts throughout an individual fast run epoch (Timofeev et al., 1998). FS neurons reveal linear voltage-current and frequency-current relations, thus indicating their ability to perform faithful conversion of input to output (Connors and Gutnick, 1990). We suggest that the leak currents and high level of synaptic activity almost exclusively contribute to the maintenance of the membrane potential and firing rates of FS neurons. This stands in contrast to other types of cortical neurons in which synaptic potentials at ‘normal’ levels of the membrane potential in vivo (around ~62 mV) are significantly amplified by intrinsic currents (Timofeev et al., 2000a,b,c, 2001). Thus, asynchronous synaptic activities, which reach FS neurons during the fast runs, do not depolarize these cells enough to reach firing threshold.

The cellular mechanism that generates the cortical fast runs is unclear. One of the possible mechanisms could be the intrinsic propensity of IB neurons to generate bursting activities at frequencies up to 16 Hz (Nuñez et al., 1993). Indeed, fast runs are associated with maximal depolarization of glial cells (Amzica and Steriade, 2000), indicating that [K+]o is at its highest level (Futamachi and Pedley, 1976; Pedley et al., 1976). In the conditions of high [K+]o, some RS neurons trans-
form their firing pattern to that displayed by IB neurons (Jensen et al., 1994; Jensen and Yaari, 1997). Thus, relatively numerous bursting neurons firing at their intrinsic frequency and even weak synaptic synchronization would be sufficient to generate fast runs at about 10–15 Hz.

The role of FS neurons in SW activities at 1.5–3 Hz

We propose that the activity of FS neurons has a leading role in the cortically generated seizures with SW/PSW complexes at 1.5–3 Hz. Neuronal responsiveness is accounted for by synaptic and intrinsic excitability, which depend on the parameters of network activity (Abbott et al., 1997; Desar et al., 1999a,b; Galarreta and Hestrin, 1999, 2000). Thalamocortical volleys excite FS neurons. Indeed, a strong inhibitory component was revealed in cortical RS neurons, recorded intracellularly with Cl⁻-filled pipettes, during thalamically generated spindle (Contreras et al., 1987). Depending on the activity in input systems, the excitability of inhibitory interneurons might be significantly enhanced (Gupta et al., 2000). Most electrographic seizures described in the present study started from prolonged trains of spikes of FS neurons. The high frequency firing causes a rapid Cl⁻ accumulation in neurons (Bracci et al., 2001) and leads to local increase in [K⁺], that will be followed by a depolarizing shift in Cl⁻ reversal potential. Thus, continuous firing of FS neurons would result in the depolarization of postsynaptic cells. Does the depolarizing action of GABAergic neurons actually excite or inhibit postsynaptic neurons? We think that both these scenarios are valid. The depolarization of a neuron brings the membrane potential close to the firing threshold enhancing probability of firing. On the other hand, the high GABA_A-dependent conductance should shunt the membrane, thus preventing postsynaptic neurons from firing. Intracellular recordings with Cl⁻-filled pipettes produce depolarizing IPSPs that are crowned by sodium spikes (Contreras et al., 1997). Thus, depolarizing IPSPs could still be excitatory and IPSP-related shunting does not abolish firing of postsynaptic neurons, although some studies performed on bicuculline-treated slices have suggested that even depolarizing IPSPs inhibit postsynaptic neurons (Wells et al., 2000). Since the shunting effect of reversed IPSPs still persists, the role of these potentials would be to control the level of depolarization in postsynaptic neurons.

We envisage the following mechanism for the generation of paroxysmal oscillatory activity at 1.5–3 Hz. Any excitation in the cortical network reaches a subset of neurons. The RS neurons reveal a low input resistance during the electrographic seizures (Fig. 1; see also (Neckelmann et al., 2000) and, thus, the initial excitation does not depolarize neurons to firing threshold. An increased intrinsic excitability of FS neurons prior to PDSs (Figs. 7, 8) facilitates the firing of FS neurons. These neurons fire before RS neurons during SW complexes (Fig. 11) and induce powerful depolarizing potentials in postsynaptic neurons. During the ascending phase of the PDS, the shunting effect of inhibition is not strong enough to prevent neurons from firing and all neurons involved in the PDS display at least one spike. Thereafter, the shunting effect of inhibition and the depolarizing block truncate the firing of the majority of RS neurons. Strong depolarization during the PDS supports abundant entry of Na⁺ and Ca²⁺ into neurons. An increased intracellular concentration of Na⁺ and Ca²⁺ activates Na⁺- and Ca²⁺-activated K⁺ currents (Schwindt et al., 1989, 1992), which hyperpolarize neurons during the ‘wave’ component of SW complexes (I. Timofeev, F. Grenier and M. Steriade, in preparation). Recent experiments suggest that approximately 20% of cortical neurons display an I_H-like current, which in conditions of high [K⁺], would be strong enough to bring some neurons to firing threshold (Timofeev et al., 2002). These neurons would trigger the next paroxysmal cycle.

Overall, our data suggest that the massive activity of inhibitory neurons in vivo leads to paradoxical excitation in postsynaptic networks. The paroxysmal feature of this excitation contrasts to the normal conditions of waking cats, in which IPSPs control the level of membrane potential and the precise timing of firing in postsynaptic neurons (Timofeev et al., 2001).

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