Membrane Capacitance of Cortical Neurons and Glia During Sleep Oscillations and Spike-Wave Seizures

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Amzica, Florin and Dag Neckelmann. Membrane capacitance of cortical neurons and glia during sleep oscillations and spike-wave seizures. J. Neurophysiol. 82: 2731–2746, 1999. Dual intracellular recordings in vivo were used to disclose relationships between cortical neurons and glia during spontaneous slow (<1 Hz) sleep oscillations and spike-wave (SW) seizures in cat. Glial cells displayed a slow membrane potential oscillation (<1 Hz), in close synchrony with cortical neurons. In glia, each cycle of this oscillation was made of a round depolarizing potential of 1.5–3 mV. The depolarizing slope corresponded to a steady depolarization and sustained synaptic activity in neurons (duration, 0.5–0.8 s). The repolarization of the glial membrane (duration, 0.5–0.8 s) coincided with neuronal hyperpolarization, associated with dissipation, and suppressed synaptic activity in cortical networks. SW seizures in glial cells displayed phasic events, synchronized with neuronal paroxysmal potentials, superimposed on a plateau of depolarization, that lasted for the duration of the seizure. Measurements of the neuronal membrane capacitance during slow oscillating patterns showed small fluctuations around the resting values in relation to the phases of the slow oscillation. In contrast, the glial capacitance displayed a small-amplitude oscillation of 1–2 Hz, independent of phasic sleep and seizure activity. Additionally, in both cell types, SW seizures were associated with a modulatory, slower oscillation (~0.2 Hz) and a persistent increase of capacitance, developing in parallel with the progression of the seizure. These capacitance variations were dependent on the severity of the seizure and the distance between the presumed seizure focus and the recording site. We suggest that the capacitance variations may reflect changes in the membrane surface area (swelling) and/or of the interglial communication via gap junctions, which may affect the synchronization and propagation of paroxysmal activities.

INTRODUCTION

The cortical network is made of neuronal and glial cells. The membrane of each element undergoes the influence of synaptic and/or ionic currents. The ensuing changes are reflected by corresponding variations of the conductance and/or capacitance of the membrane. Glial cells initially were considered to accomplish only connective and trophic functions for neurons. Growing evidence, however, attributes them with important tasks in the electrical dialog with neurons (Nedergaard 1994; Parpura et al. 1994). Recent reports bring into attention reciprocal neurotransmitter-based exchanges between neurons and glia. On one hand, glial membranes possess receptors for a variety of transmitters (Nicholson 1995). On the other hand, glia release neurotransmitters (Levi and Gallo 1995). Glial cells possess receptors for most of the neurotransmitters involved in behavioral state control, such as glutamate [non-N-methyl-D-aspartate (non-NMDA)] (Sontheimer et al. 1988; Steinhaüser and Gallo 1996), GABA (GABA_A) (Bormann and Kettenmann 1988; MacVicar et al. 1989; Rosier et al. 1993), acetylcholine (Tse et al. 1992), and norepinephrine (McCarthy et al. 1995). Glutamate, an ubiquitous transmitter in the CNS, depolarizes glia through receptors similar to the ones of the neurons (Bowman and Kimelberg 1984; Kettenmann and Schachner 1985) and triggers slow Ca^{2+}-dependent oscillations in cortical and hippocampal astrocytes (Pasti et al. 1995, 1997). Calcium waves may travel through gap junctions between astrocytes and may unidirectionally cross glia-neuron gap junctions (Nedergaard 1994).

During the state of resting sleep, neurons have been shown to display a slow (<1 Hz) oscillatory activity generated within the cortex (Steriade et al. 1993a,b). This activity consists of periods of neuronal depolarization associated with intense neuronal activity alternating with periods of neuronal hyperpolarization and cessation of cellular firing. Although this slow oscillation first was described under various anesthetics, its relevance to sleep has been demonstrated (Achermann and Borbély 1997; Amzica and Steriade 1998; Steriade et al. 1996). The behavior of glial cells during the slow oscillatory sleep pattern is not known.

Sleep enhances the propensity of the brain to generate spike-wave (SW) seizures (Kellaway 1985; Steriade 1974). SW seizures gradually develop from the slow (<1 Hz) sleep oscillation (Steriade and Amzica 1994; Steriade et al. 1998). The neuronal activity during SW seizures is similar to that observed during slow sleep oscillations, only the synchrony of the neuronal pool is higher and the depolarizations and hyperpolarizations are more ample.

In simple preparations, glial cells are reliable potassium detectors (Kuffler et al. 1966; Nicholls and Kuffler 1964). Neuronal excitatory activity is associated with the opening of transmitter- and voltage-gated channels resulting in Na^+ influx and K^+ efflux. The latter may induce increases of the extracellular K^+ by several mM (Somjen 1979), which in turn will depolarize neighboring glial cells due to their high K^+ conductances. Janigro et al. (1997) have shown that, in hippocampal slices, astrocytic inwardly rectifying K^+ channels play a crucial role in regulating the extracellular K^+ concentration. A major part of the K^+ uptake occurs via passive Donnan-like redistribution of K^+ and Cl^- (Ballanyi et al. 1987; Kettenmann 1987; Walz 1989). The accumulation of K^+ in the extracellular space is associated with two phenomena: water influx in glial cells leading to swelling (Ballanyi et al. 1990); increased propensity to seizure onset leading to regenerative events un-
underlying the seizure (Fetziger and Ranck 1970; McBain et al. 1993; Zuckermann and Glaser 1968). It becomes therefore of particular interest to understand the cellular mechanisms of neuron-glial interactions as they evolve from physiological slow sleep oscillations to patho logical paroxysms.

Our experimental approach is based on double intracellular recordings in vivo, which enables the recording of simultaneous neuronal and glial activities as they develop in intact cortical networks. This is especially useful in the case of glial ("silent") cells, which lack overt signaling that can be recorded extracellularly.

In this paper we also investigate the membrane conductance and capacitance of cortical neurons and glia during normal (slow sleep oscillation) and paroxysmal (SW seizures) electrical patterns. Changes in the membrane capacitance may betray variations in the somatic membrane surface, in a similar way exocytosis is detected using capacitance measurements (Moser and Neher 1997). Hippocampal slices display activity-dependent swelling (Andrew and MacVicar 1994) and transient shrinkage of the extracellular space occurs during periods of increased extracellular potassium ([K\(^{+}\)]\(_{o}\)) (Dietzgel et al. 1980), leaving open the issue of whether neurons or glia are responsible for this phenomenon. Evidence presented here points to the glial cells and suggests mechanisms through which they may influence neuronal behavior. It also will be shown that several types of capacitance changes occur during normal and paroxysmal oscillations. This is the first report of such capacitance variations in vivo, i.e., under physiological conditions, where the cortical network is preserved totally.

**METHODS**

**Animal preparation and recordings**

Twenty adult cats were anesthetized with a mixture of ketamine and xylazine (10–15 and 2–3 mg/kg im, respectively), intubated, paralyzed with gallamine triethiodide, and artificially ventilated (20–30/ min). All pressure and incision points were infiltrated with lidocaine. To avoid any pain, the electroencephalogram (EEG) and the heart rate were monitored continuously and supplementary doses of anesthetic were readministered at the slightest activation of the EEG or acceleration of the pulse rate. The end-tidal CO\(_2\) concentration was maintained at 3.7% (±0.2). Surgery consisted of craniotomy exposing mainly the suprasylvian gyrus. Cisternal drainage, hip suspension and pneumothorax were performed to improve the mechanical stability of the brain. To further reduce eventual pulsations, after installing the recording electrodes, the hole in the calvarium was filled with a solution of 4% agar.

Intracellular recordings were performed with glass micropipettes (tip diameter <0.5 μm) filled with potassium acetate (3 M, impedance 30–40 MΩ) and, on some occasions, with neurobiotin (2%, Vector Laboratories). Staining of cells with neurobiotin was performed by applying depolarizing pulses (150 ms at 3.3 Hz) of 1–2 nA for 5–15 min. Signals were passed through a high-impedance amplifier with active bridge circuitry, and recorded on tape (band-pass: DC to 9 kHz). Field potentials (AC traces in figures) were recorded with tungsten macroelectrodes (0.5–1 MΩ impedance), inserted in the depth of the suprasylvian cortex (~1 mm), amplified and band-pass filtered (0.3–1,000 Hz). For off-line computer analysis, signals were passed through an analogue-digital converter at a sampling rate of 20 kHz. SW seizures occurred spontaneously (22% of the seizures), or were triggered by electric stimulation (15% of the seizures), or were induced by inserting the needle of a syringe, filled with 10 μl of a 0.2 mM solution of bicuculline in saline, into the rostral part of the suprasylvian gyrus (cortical area 5); very small amounts of bicuculline (0.02–0.05 μl) leaked slowly into the cortex. Intracellular recordings were performed at some distance (>10 mm) from the bicuculline focus to avoid the direct action of the drug on the cellular membrane.

At the end of the experiments where neurobiotin was used, the deeply anesthetized cats were perfused transcardially with saline followed by 10% paraformaldehyde. The brain was removed and stored in formalin with 30% sucrose for 2–3 days, then it was sectioned at 50 μm, and processed with the avidin-biotin standard kit (ABC standard kit, Vector Laboratories): the sections were incubated overnight at room temperature in the avidin-biotin-horseradish peroxidase complex solution at a dilution of 1:200 and 0.5% Triton X-100. After rinsing, the sections were reacted with 3,3′-diaminobenzidine tetrahydrochloride (0.05%), H\(_2\)O\(_2\) (0.003%), incubated in a solution of glial fibrillary acidic protein (GFAP) at a dilution of 1:800, mounted on gel-dipped slides and cover-slipped.

At the end of all other experiments, the cats were given a lethal dose of intravenous pentobarbital sodium.

**Analysis**

Conductance and capacitance of the cellular membrane were derived from the charging curve of hyperpolarizing pulses applied intracellularly. Constant care was taken to keep the bridge balanced throughout the period with hyperpolarizing current pulses, regardless of the ongoing spontaneous synaptic activity. Only recordings with a balanced bridge were considered for analysis. Time constants were estimated with a double-exponential fit using the Levenberg-Marquardt algorithm (IGOR software, WaveMetrics). In the case of neurons, the double-exponential fit is required by the nonisopotential intracellular voltage distribution (Rall 1959). Isopotentiality also may be compromised in glial cells due to their coupling through gap junctions. Indeed, synaptic neuronal activity may modulate interglial intercellular communication (Marrero and Orkand 1996), and a recent model (McKhann et al. 1997) points toward transient changes of the coupling ratio between glia. Thus the charging curve was fitted with a function of the type:

\[
\frac{k_0 + k_1 \exp(-t/\tau_1) + k_2 \exp(-t/\tau_2)}{1 + \exp(-t/\tau_0)}
\]

where the two exponentials were always arranged such that \(\tau_1\) was always the largest and \(\tau_2\) the smallest coefficient. Thus \(\tau_1\) becomes the main time constant of the charging curve. The length of the charging curve (\(T_{10\%}\) in Fig. 1A) is 10 times the expected main time constant (10\(\tau_1\)). The fit produces the five parameters \((k_0, k_1, k_2, \tau_1, \tau_2)\) and the respective standard deviations.

The duration of the injected current (\(I\)) was ≥60% longer than 10 times the expected main time constant (10\(\tau_1\)), to obtain a reliable plateau to be used as \(V_{\infty}(\infty)\). Thus single glial impalements required pulses of ≥10 ms, whereas double-glia-neuron recordings or single-neuron impalements were imposed with pulses of ≥50 ms. In each cell, we applied a series of depolarizing and hyperpolarizing pulses (Fig. 1B) to find the current limit at which active conductances were still not triggered. In some cases, depolarizing pulses were associated with rectifying components (not shown). Hence we decided to use hyperpolarizing pulses. As shown in Fig. 1B, hyperpolarizing pulses of up to ~2 nA were devoid of overt active responses. In the sequences of pulses depicted in Fig. 1B, we also estimated the dependence of the time constants on the intensity of the injected current. The main time constant (\(\tau_1\)) was extremely stable (variation <3%), whereas the second time constant (\(\tau_2\)) presented larger variations (~25%), but these were unrelated with the intensity of the pulse. Thus for the testing of the dynamic evolution of the capacitance and conductance, we generally applied current pulses of 1 nA.

Pulses were delivered at a frequency of 5–10 Hz, and in some cases they were delivered with variable frequencies to verify whether some of the oscillations reported here could have been artifacts of the
stimulation rate. Several parameters were extracted from each pulse (Fig. 1A). The average membrane potential before the onset of the hyperpolarizing pulse \( V_{\text{dep}} \) was calculated over a period \( t_{\text{dep}} \) representing five main time constants \( t_{\text{dep}} = 5 \tau_1 \). Similarly, \( V_{\text{m}}(\infty) \), or \( V_{\text{hyp}} \) is the average of the membrane potential toward the end of the hyperpolarization calculated over an identical time span \( t_{\text{hyp}} = 5 \tau_1 \). The input conductance of the cell \( Y_N \) is

\[
Y_N = \frac{I}{\Delta V} = \frac{I}{V_{\text{dep}} - V_{\text{hyp}}}
\]

Estimation of time constants was performed according to Lux and Pollen (1966) data obtained from neurons. Because \( \tau_N = R_m C_m \), with \( R_m = V Y_N \), \( R_m \) being the input resistance, the calculation of the capacitance is straightforward \( C_m = \tau_1 Y_N \).

In some of the figures, the hyperpolarizing pulses were removed artificially from the intracellular trace to reconstruct the membrane potential. This was achieved by displacing the charging curve from the bottom of the hyperpolarizing pulse toward the level of the resting membrane potential and by canceling the eventual transients. These traces were used only for descriptive, graphic purposes, never for calculating time constants.

**RESULTS**

**Database and cellular identification**

From the total of intracellularly recorded cells, we retained for analysis only the best recordings, i.e., 58 neurons and 53 glia, of which 29 were double intracellular impalements. The criteria for this choice were, in the case of neurons: stable...
resting membrane potentials more negative than \(-60\) mV \((-71 \pm 2.8\) mV; mean \pm SD) for \(\geq 15\) min and overshooting action potentials. Although a wide range of glial resting membrane potentials has been described recently (McKhann et al. 1997), we retained for analysis only glial recordings starting with a sudden drop of membrane potential from 0 mV to resting membrane potentials more negative than \(-70\) mV \((-85 \pm 3.4\) mV; see Fig. 4). Membrane potentials had to remain stable for \(\geq 15\) min and never needed the application of steady hyperpolarizing currents. In no circumstance were action potentials triggered spontaneously after impalement or by depolarizing pulses. At the end of the glial recording, the micropipette was withdrawn from the cell and field potentials were recorded. These displayed a reversed polarity for the main components recorded intracellularly. During SW seizures, glial recordings displayed intracellularly steady depolarizations of 10–30 mV, and extracellularly corresponding negative shifts of 3–6 mV (see Fig. 4). These values were in accordance with previous in vivo intraglial recordings (Grossman and Hampton 1968; Sypert and Ward 1971). Moreover, values of the resting time constants calculated in this paper are in agreement with those of previous studies (e.g., Trachtenberg and Pollen 1970).

All glial and neuronal elements were recorded within the cortex, at \(<1.5\) mm from the surface, as read from the stepping device carrying the microelectrode (the precision of this estimate was better than 85% when compared with the precise location of stained cells). Cellular staining with neurobiotin and reactivity to GFAP (Fig. 2B) show that most of the glial cells (88%) were protoplasmatic astrocytes (Fig. 2, C and D), whereas neurons were generally pyramidal shaped (Fig. 2A). Only a few dye-coupled cells (4 couples) were disclosed. Of these, only two couples contained glial cells, but they failed to react to GFAP immunocytochemistry and are therefore not shown. However, the low level of dye-coupling observed here may be explained by fact that the use of neurobiotin generally leads to false negative estimation of the dye-coupling (Moser 1998). In the case of neurons, dye-coupling diminishes dramatically with age (Connors et al. 1983).

**Slow sleep and paroxysmal activities in neuron-glia networks**

During natural sleep and under anesthesia, corticothalamic neuronal networks oscillate at a frequency \(<1\) Hz (Steriade et al. 1993a). The membrane of cortical neurons displayed a continuous variation between depolarized and hyperpolarized values (Fig. 3). The depolarization is made of synaptic potentials (excitatory and inhibitory) and is associated with negative depth field potentials. The hyperpolarization is marked by the abolition of synaptic potentials and is associated in depth field recordings with positive waves. All glial cells reflected this slow oscillation (Fig. 3). Their membrane underwent periodic depolarizing-repolarizing sequences in close temporal relationship with activities recorded in simultaneously impaled neurons nearby. The onset of the depolarizing phase of the slow oscillation induced a round-like depolarization of glial cells. Toward the end of the depolarizing phase and with the onset of the hyperpolarizing phase, the glial membrane began to repolarize with a decaying slope to reach more or less the initial

**FIG. 2.** Neurons and glia recorded during slow oscillatory activities and spike-wave seizures. A: pyramidal neuron filled with neurobiotin in layer V. B: glial fibrillary acidic protein (GFAP)-reactive astrocytes. C and D: double-labeled (neurobiotin and GFAP) astrocytes. All calibration bars are 10 \(\mu m\).
potential. The amplitude of the glial depolarization ranged between 1.5 and 3 mV and was proportional to the neuronal depolarization during an oscillatory cycle.

Figure 4 depicts the general pattern of cortical SW seizures as recorded with dual impalements and field potentials. The latter shows the evolution of seizures from a slowly oscillating sleep-like pattern to a few rhythmic EEG spikes. The rhythm accelerated (1–2 Hz), produced a short epoch of fast runs (≈10 Hz), and continued with recurrent polyspike waves at ≈1.3 Hz. A first seizure was recorded with a pipette inside a neuron and with another pipette in the extracellular space (Fig. 4A). A few seconds later and only few micrometers below, the second pipette impaled a glial cell (see the brisk drop of potential), and a new seizure occurred (Fig. 4B). This glia corresponds to the one depicted in Fig. 2C.

The neuronal rhythmic (1–2 Hz) depolarizing potentials evolved from the depolarizing sequences of the slow oscillation that turned into paroxysmal depolarizing shifts (PDSs; Fig. 4B1), accelerated their rhythm from <1 to 1–2 Hz and generated the SW pattern. Seizures often displayed periods of continuous fast runs (≈10 Hz; Fig. 4B2) or bursts of fast runs tailing the recurrent PDSs (Fig. 4B3), thus contributing to the polyspike shape of the EEG. All these neuronal phasic events were reflected in glial potentials. The glial PDS grew ampler than any cycle of the slow oscillation. As the seizure reached its most paroxysmal expression, fast neuronal depolarizing events were reflected partially reversed by the glial cell (neuronal fast excitatory events were associated with sharp negativities in glia). This is not a voltage-dependent phenomenon because it does not happen at the same membrane potential at the beginning of the seizure. Rather it might be caused by the glial swelling (see following text) that brings neuronal and glial membranes closer. Thus some intraglial potentials may reflect direct extraneuronal transients. However, slower depolarizing neuronal potentials continued to be associated with depolarizing glial waves. Therefore the glial potential during SW seizures reflects the superimposition of intraglial currents and extraglial field potentials.

The macroscopic glial membrane potential was characterized by a steady depolarization for the whole duration of the seizure (Fig. 4B). When recorded with DC electrodes, the extracellular expression of the sustained excitation appeared as a persistent negative shift (Fig. 4A).

**Capacitance variations during slow (<1 Hz) sleep activities**

Previously most of the studies monitoring the cellular capacitance were conducted to disclose secretory mechanisms, where modified capacitance values are connected to exocytotic or endocytotic processes (Moser and Neher 1997; Neher and Marty 1982). Our study extrapolates the principle of the capacitance technique to in vivo cortical neuronal and glial somatic recordings. The membrane may be viewed as a battery of capacitors (Holmes et al. 1992; Rall 1959). Three intrinsic membrane factors may affect the capacitance: the composition of the membrane lipid bilayer (dielectric constant), the surface, and the thickness of the membrane. The first two parameters induce proportional effects while the third has a reciprocal relationship with the capacitance. Although it appears as a limitation, we consider that the dielectric constant is invariable over the time scale of our experiments. It is reasonable to believe that the chemical and physical structure of the cellular membrane is not modified during seizures lasting for no more than a few minutes, especially because the electrical behavior...
returns to its preseizure values at the end of the seizure. This assumption, however, may be questioned over longer time spans.

The average time constants of neurons ($\tau_1$ and $\tau_2$) calculated during slowly oscillating activities, were respectively $4.02 \pm 0.23$ ms and $1.06 \pm 0.78$ ms (all results are provided as means ± SD). The first exponential coefficient ($k_1$) was $122.6 \pm 24.3$ and the second exponential coefficient ($k_2$) was $47.8 \pm 15.6$. The errors of the time constants due to the fitting procedure were $33.4 \pm 12.1\%$ and $175.3 \pm 2\%$, respectively. All these values were calculated for 30 neurons for an average period of 60 s/cell. The errors were quite high due to the superimposition of synaptic activities over the charging curves. This is why capacitance measurements were considered reliable only in fewer neurons ($n = 5$), in which errors for at least the main time constants were below 10% and could not affect the oscillatory evolution of their values (Fig. 5). The detail in Fig. 5B depicts a few hyperpolarizing pulses delivered during a cycle of the slow oscillation together with the fitting curves, the two time constants and the standard deviation errors of the fits. It is shown that the main time constant ($\tau_1$) was diminished during the depolarizing phase of the slow oscillation and increased during the hyperpolarization. The second time constant ($\tau_2$) displayed irregular variations that could not be related to any activity in the network. The behavior of the first time constant ($\tau_1$) produced a capacitance oscillating in a reciprocal manner to oscillations of the membrane potential of the neuron (Fig. 5C). The neuronal trace in this panel was reconstructed from the original trace (see METHODS) and is depicted to outline the inverse relation of the capacitance with the neuronal activity. The average neuronal capacitance was $170$ pF (range from 100 to 800 pF). The amplitude of the capacitance variation during slow oscillations was proportional to the amplitude of the slow oscillation and ranged from 50 to 150 pF.

The glial capacitance, measured as in the case of neurons, during periods with slow oscillations, expressed a periodic variation of $1–2$ Hz ($n = 53$; Fig. 6). The main time constant ($\tau_1$) was $0.148 \pm 0.02$ ms and had a coefficient $k_1 = 134.81 \pm 25.57$ (fitting error $3.67 \pm 0.6\%$). The second time constant ($\tau_2$) was $2.14 \pm 0.24$ ms with $k_2 = 25.12 \pm 3.57$ and the fitting error of $13.57 \pm 3.2\%$. Both time constants oscillated at roughly the same frequency (see autocorrelograms in Fig. 6D). The average of the glial capacitance during slow oscillations was $13.45$ pF (range 10–25 pF) and its variation (amplitude of 0.5–3 pF) was independent of synaptic activities and the slow ($\leq 1$ Hz) oscillation of the network (Fig. 6A). Figure 6C also presents a comparison between the capacitance evolution derived from fitting the charging curves with a double exponential (top) and a single exponential (bottom). Although both traces show basically the same oscillation, as proven by their cross-correlation (CROSS in Fig. 6D), some differences were evident: the average conductance value was higher ($\sim 30\%$)
with single than with double-exponential fit and the oscillatory variations were less pronounced and regular with the simple exponential. Adding to this the fact that the fitting error was higher with single exponentials (19 ± 3.4%, \( n = 23 \)), we decided to use only the double-exponential fit.

We propose to call the capacitance oscillation displayed by glia the pulsatory capacitance variation (PCV). The term “pulsatory” is suggested by the stereotyped pattern of the capacitance trace. The PCV had an average frequency of 1.52 ± 0.4 Hz (in 53 cells) as derived from the maximum peak of the power spectrum of the capacitance curves. These microswellings were not induced by eventual heart pulsations (the heart rate was monitored during experiments). They were not time-related to the oscillatory activity of the network (see also following text). On the contrary, none of the tested neurons displayed PCV, their only capacitance changes being related to the ongoing network activity (slow oscillation).

Glial capacitance variations during SW seizures

SW seizures in glial cells were associated with increased membrane conductance and capacitance. These variations were dependent on the distance between the presumed seizure focus (site of bicuculline infusion or of electrical stimulation) and the location of the impaled cell. They also depended on the severity of the seizure.

In the case of epileptic foci induced in cortical area 5 and glial cells recorded 10–15 mm away from the focus in area 7, the onset of the seizure was marked by a progressive build-up of the persistent glial depolarization, in contrast with the relative fast onset showed by the focal DC field potential in area 5 (Fig. 7A). In spite of this, the glial cell responded with depolarizing waves from the first PDS, suggesting that the propagation of the seizure was quite fast (>2 m/s). This estimated propagation velocity resulted from a time lag of <5 ms between the onset of corresponding PDSs in area 5 (close to the focus) and area 7 and knowing that the distance between the two electrodes was of ~10 mm. Here too, as was often the case for epileptic foci induced by intracortical infusion of bicuculline, recurrent SW seizures occurred every minute or so, separated by silent periods with postictal depression (PID). The membrane conductance followed a similar time-course (Fig. 7B) but was even more delayed from the onset of the seizure (>25 s). Once the conductance began to increase, it followed an accelerated course to finally mark a 27% increase. Out of 53 glial cells, 27 were situated at some distance (>5 mm) from the presumed epileptic focus, and all of them had increased conductances during the seizure with an average of 32 ± 7.5%.

The membrane capacitances of distantly located glial cells followed a similar time-course as the conductance and started to increase ~25 s after the onset of the seizure (Fig. 7C). Several components could be distinguished: 1) the PCV was
still very obvious throughout the recording (thin line in Fig. 7C; see also narrow peak at 0.88 Hz in the power spectrum of Fig. 7D). The PCV was not affected by the various membrane potential activities displayed during the seizure (1.5 Hz of the SW seizure, 10 Hz of the fast runs, and PID after the end of the seizure). It was not seen in the $t_2$ curve (not shown).

A slow modulation of $0.2 \text{ Hz}$, hereafter called modulatory capacitance variation (MCV), appeared with the development of the seizure (e.g., the few higher peaks marked with open arrowheads in Fig. 7C, and the corresponding peak in the power spectrum of Fig. 7D). The MCV appeared only during seizures, independently of the distance from the focus, in 48 of the 53 tested glial cells (90%). The amplitude of the MCV reached a maximum during the most paroxysmal segment of the seizure. The average capacitance increase due to the MCV compared with the control value was of $92 \pm 11\%$. Peak values from various seizures ranged between 66 and 400%. And finally, 3) there was a slow trend reflecting a progressive capacitance increase of the glial cells with the progression of the seizure (thick line in Fig. 7C). This glial capacitance component was obtained as a polynomial fit of fourth order of the original curve.

Capacitance variations of glial cells during SW seizures depended on the severity of the seizures. The severity was considered as a function of the duration of the seizure, the presence of fast runs ($\approx 10 \text{ Hz}$), and the amount of depolarization displayed by intracellular recordings. In the same intraglial recording, two seizures of different severity induced different modifications at the level of the membrane conductance and capacitance (Fig. 8A). The first seizure was the most severe one. The conductance increased almost twofold and the capacitance increase was $50\%$ higher compared with the second seizure. The overall dependence of glial capacitance on two parameters defining the severity of SW seizures is presented in Fig. 8B.

SW seizures also were elicited with electrical stimulation of the cortex (Fig. 9). Here again, more severe seizures (Fig. 9, 2) induced increased capacitance modifications. During the first sei-
Fig. 7. Small-amplitude oscillations of the glial membrane capacitance during SW seizures and their modulation by a slower (~0.2 Hz) oscillation. A: SW seizure induced by intracortical diffusion of bicuculline in area 5. Glia was impaled in area 7 and shows a much slower progression of the sustained depolarizing shift, and an earlier onset of the repolarization, than the one occurring close to the epileptic focus. Only the reconstructed trace is depicted here; however, conductance and capacitance values are calculated from the real recording trace (pulses delivered at 1 Hz). B: conductance of the glia begins to increase only 25–30 s after the onset of the seizure. C: individual capacitance values (thin line oscillating at ~0.9 Hz) were fitted with a polynomial curve (thick line) to emphasize the slow persistent increase toward the end of the seizure. Note the appearance of modulatory peaks at ~0.2 Hz in the capacitance trace (open arrowheads) during the most paroxysmal phase of the seizure. Dotted horizontal lines tentatively mark resting values for conductance and capacitance. D: power spectra of the capacitance trace to disclose the oscillating frequencies. Note the 0.88 Hz peak (black arrowhead) corresponding to the pulsatory capacitance variation (PCV) and the lower peak at 0.23 Hz (open arrowhead) for the modulatory capacitance variation (MCV).

 seizure (Fig. 9, I), the capacitance displayed small-amplitude PCVs and MCVs, especially during the seizure. If a more severe seizure was triggered (Fig. 9, 2), capacitances were drastically increased and followed the time course of the seizure. A sustained swelling can be inferred from the persistent increase in capacitance. The onset of this increase was delayed from the onset of the seizure and coincided with the reaching of a depolarizing plateau in the intraglial recording. This could explain why, in the case of the seizure depicted in the left panel without such a depolarizing plateau, the capacitance modifications were not so dramatic.
Neuronal capacitance variations during SW seizures

During SW seizures, neurons displayed increased conductances of their membrane. This could contribute to the inactivation of somatic sodium spikes during the steady depolarization (Fig. 10). In contrast to glia, changes in neuronal capacitance during seizures rarely were seen (only in 3 of 58 neurons, i.e., 5%, Fig. 10). In none of the cases could we find PCVs. This does not mean that they do not exist but that synaptic activity and slower time constants often make the extraction of precise values for capacitance more difficult.

FIG. 8. Relationship between glial swelling and the severity of the seizures. A: intraglial recording in cortical area 5 during 2 SW seizures. First seizure is more severe than the 2nd. Conductance curve is a smoothed traces of the individual values. In the capacitance trace, points linked through dotted lines represent individual values, the thick line represents the smoothed trace to emphasize the slow trends present during the seizure. Amount of swelling depends on the duration of the seizure and on the amount of excitation expressed by the intracellular potential. B: relative capacitance increase as a function of the distance from the presumed seizure focus (left) and of the seizure duration (right). Relative capacitance increase was calculated as the ratio between the maximum capacitance increase during a given seizure, and the value of the capacitance before the onset of that seizure. Left: black squares represent individual values, while open squares are averaged values for each group. Lines depict the general tendency of the relative capacitance increase.
these cases interpolations between neighboring pulses free of synaptic interferences were considered. However, the absence of PCV during periods free of synaptic activity, such as the hyperpolarizing phase of the slow oscillation or the PID, suggests that neurons do not possess this kind of capacitance variations.

The two cells illustrated in Fig. 10 were recorded at very short distance (<0.5 mm) and presumably belonged to a network homogeneously affected by the SW seizure. The neuronal capacitance displayed irregular variations in the range of the MCV (0.3–0.4 Hz) and a very discrete steady increase during the seizure (see --- in the neuron's graph).

It is clear from the fitting curve of the capacitance values that the surface area of the neuron and of the glia had different overall evolutions. The first increase appeared in the neuron and was paralleled by a corresponding decrease in the glia. Glial capacitance increase coincided with the middle of the seizure. The correlative analysis of time constants from the recording presented in Fig. 10 is depicted in Fig. 11. The two time constants of the neuron (top left) followed similar evolutions regardless of whether individual values (gray curve) or overall tendencies (black curve) were considered. Even better correlations were disclosed between the two time constants of the glia (top left). Cross-correlations between neuronal and glial time constants (bottom panels in Fig. 11) show mostly negative central peaks suggestive of a complementary interaction between the volume variations of the neurons and glia (see black traces from the curves that depict the general tendency during the seizure in Fig. 10). The positive superimposed peak present in the cross-correlation of the first time constant (τ1, bottom left, gray curve in Fig. 11) is probably due to the epoch where both neuronal and glial time constants increase simultaneously during the middle of the seizure.

**DISCUSSION**

This paper describes patterns of slow (<1 Hz) sleep oscillations in cortical neuron-glia networks and their development into paroxysmal patterns of SW type. Three types of capacitance variations, which are proposed to reflect volume and interglial communication modifications, accompany these activities in glial cells. These may impose regulatory processes on the ionic currents flowing through the cellular membranes and the extracellular space. Neurons display only small-amplitude capacitance variations time-locked with synaptic activities. All cortical cells increased their conductance during SW seizures, in accordance with previous reports (Hablitz 1987; Oakley et al. 1972).

**Patterns of neuro-glial interactions**

Although the role of glial cells in uptaking [K+]o released by neurons has been known for a long time (Kuffler et al. 1966; Nicholls and Kuffler 1964), the neuron-glia interaction always has been studied through indirect methods. To our knowledge, Figs. 3 and 4 are the first to show the simultaneous behavior of neuronal and glial membrane potentials in vivo. Such dual neuron-glial impalements are particularly useful because SW seizures preferentially take place during sleep (Kellaway 1985; Steriade 1974) and extracellular K+ accumulation is a major factor in triggering SW seizures (Fritzinger and Ranck 1970; Janigro et al. 1997; Zuckermann and Glaser 1968). Figure 3 suggests a tight relationship between neuronal and glial activities during physiological sleep oscillation. The resting concentration of [K+]o is ~3 mM (Futamachi et al. 1974). Although the variations of [K+]o were not measured in our experiments, we may extrapolate them from other studies.
We made the assumption that, for the voltage range recorded in this study, the relationship between the glial membrane potential and the \([K^+]_o\) is linear (Walz et al. 1984). The \([K^+]_o\) elevation during SW seizures of the type we report here was 9–10 mM (Moody et al. 1974; Sypert and Ward 1974). This would correspond to an average sustained depolarization of glial cells of 20 mV (range 10–30 mV, as a function of distance from the presumed focus), seen in the present study. Equally, the 1.5- to 3-mV rhythmic depolarizing potentials recorded in glia during the slow oscillation would bring the concentration of the \([K^+]_o\) somewhere between 3.75 and 4.5 mM. This is within the range where, as shown in the cerebellum, neurons increase their excitability but close to the limit (5 mM) where their excitability starts to diminish (Kocsis et al. 1983). This reasoning suggests that the pacing of the slow \((1 \text{ Hz})\) sleep oscillation may result from the critical balance between periods with enhanced neuronal excitability (depolarized phase) and with diminished excitability (hyperpolarized phase). Glial cells could play an important role by setting the \([K^+]_o\) concentration.

Impaired uptake of \(K^+\) could result in regenerative processes underlying SW seizure (Fetziger and Ranck 1970; Janigro et al. 1997). During these periods, as suggested by reduced time lags between corresponding phasic events, the synchrony between cortical neurons (Steriade and Amzica 1994) and between neurons and glia (present data) is increased.

Three types of capacitance variations

The capacitance variations reported in this study (Figs. 5–10) were measured by means of hyperpolarizing pulses. The underlying principle already has served to detect exocytosis in chromaffin cells (Moser and Neher 1997). Here it was used only after ensuring that the hyperpolarizing pulses did not trigger any visible active conductances susceptible to affect the charging curve (Fig. 1B). Some differences in the behavior of glia between in vivo and in vitro preparations are discussed elsewhere (Somjen 1995).

These capacitance variations tend to point toward swelling and, in the case of glial cells, to modulation of their coupling through gap junctions. The ability of glial cells to swell has been acknowledged repeatedly in cultures and in slice preparations (for a review, see Kimelberg 1995). However, the actual evolution of glial volume in situ during slow sleep
oscillations and during seizures, in close time relation to electrical patterns, has not been shown before.

Increases of capacitance may be due to an increased surface area of the cellular membrane or to its thinning. However, the capacitance increases cannot be exclusively accounted for by surface enlargement. Knowing that a surface increase with a factor of \( k \) would be translated into a volume increase of about \( k^{2/3} \), it would suggest that for a sustained mean capacitance increase of 55% during SW seizures (values from Fig. 8B, corresponding to cells located at 2–5 mm from the focus), the glial volume would increase by 94%. It is known that the extracellular space has a limited volume representing \( 6 \pm 20\% \) of the total brain volume (Nicholson 1995). Increases of the glial volume beyond that limit, although possible during severe edema, would induce displacement of the cells and loss of the impalements. Because this was not the case, it appears that an enlarged surface has to be accompanied by thinning of the membrane and possibly by other phenomena (see following text). Thinning of the membrane also could be a consequence of increased surface if this were provoked by increased tension and not by added substance, as is the case during exocytosis. Limiting the volume increase to the whole available 20% of extracellular space, by an inverse reasoning, would correspond to a maximum surface enlargement of 13%. Thus to attain the same 55% capacitance increase, a thinning of the membrane by 73% would be required.

Our set of cortical glial cells display a basic oscillatory capacitance variation (PCV) of the membrane during physiological sleep oscillations (Fig. 6), suggesting a continuous volume regulation at a frequency of 1–2 Hz. These pulsatile movements are independent of heart rate or respiration. They are not affected by the electrical activity of the network, and they survive during most of the SW seizure and the PID following the seizure. Only during severe paroxysms, was the PCV abolished (see Fig. 9, right). This effect may be due to the fact that the local extracellular space has reached a critical shrinkage, where cellular membranes begin to press against each other and prevent PCV.

During SW seizures, the membrane capacitance displayed a slower variation of \(-0.2\) Hz (Figs. 7–10). The amplitude of the MCV increased with the development of the seizure (Fig. 7) and could outlast the arrest of the seizure. In the cases of recurrent seizures where the seizure-free periods were short enough (<20 s), the MCV was present throughout the recording, with a small-amplitude reduction before the onset of a new ictal episode (Fig. 9). It may be suggested that the MCV corresponds to a supplementary protection of the extracellular space against accumulation of \( K^+ \) via at least two possible mechanisms.

First, a series of swelling-dependent conductances have been found in glial cells. Astrocytes and other cell types counteract volume increases by an efflux of \( Cl^- \) and other anions (Crépel et al. 1998; Pasantes-Morales et al. 1994). Besides the receptor- and voltage-gated currents, which are active during paroxysmal activities, stretch-activated-currents may play an important role in equilibrating the membrane potential of the glial
cells. If the MCV corresponds to variations of the cellular surface, it would result that the stretch-activated channels are periodically opened to allow ion efflux accompanied by water expulsion. This also could explain why the membrane potential during seizures saturates at a given level, regardless of the severity of the seizure (Moody et al. 1974).

Second, the periodic (every 5 s) swellings of glia would produce equivalent periodic squeezing pressure on the intracellular ionic content and would help to evacuate the excess to more distant locations through the network of gap junctions. This process may be assisted by the fact that the steady depolarization observed during SW seizures (Figs. 4 and 7–10) would induce intracellular alkalization, which results in increased permeability of gap junctions (see Ransom 1995). It appears that the phasic potentials superimposed on the steady depolarization are not related to the MCV, probably because they are not ample enough to produce an observable modulation of the gap junction opening.

In parallel with the development of the MCV, there was also a sustained increase in the membrane capacitance of glial cells (Figs. 7–10), which generally followed the course of the SW seizure. The intensity of this phenomenon decreased with the distance from the presumed seizure focus, suggesting its implication in the evacuation of K⁺ and eventually of other ions (Fig. 8B).

It should be equally noted that the increased capacitance also might result from modified gap junction properties. It is well established that glial cells, especially astrocytes, extensively communicate through gap junctions (Binnmöller and Müller 1992; Gutnick et al. 1981; for review, see Dermietzel and Spray 1993). It is possible that the modification of intraglial voltages or ionic concentrations during certain activities (especially during seizures) affect gap junction communication, for example, to increase the global capacitance recorded by the somatic electrode, thus creating the illusion of a swollen membrane. This hypothesis is supported by the fact that dye coupling of astroglia is significantly upregulated by membrane depolarization, both by increases in the extracellular K⁺ concentration and directly by ionophores (Enkvist and McCarthy 1994). The increased values for the second time constant reported here during SW seizures (e.g., Fig. 11) may suggest a modulation of remote glial membranes, possibly by the opening of gap junctions and creating the illusion of increased membrane surface area. At this time, however, our experimental approach is not able to discern between the contribution of surface, thickness, and gap junction communication to the reported capacitance variations.

With minor exceptions (<5%), cortical neurons involved in slow (<1 Hz) sleep oscillations or in SW seizures were less inclined to swell. This finding does not imply that neurons are deprived of volume regulatory mechanisms of the PCV type. In fact, the ability of molluscan neurons to swell has been reported as a result of hypo-osmotic insult (Herring et al. 1998; Wan et al. 1995). Additionally, exocytotic processes are certainly accompanied by increased membrane surface, as demonstrated in a series of preparations with secretory cells (Moser and Neher 1997; Zimmerberg and Whitaker 1985). It is feasible that such processes also may take place in the cortical neurons of the cat, but because they occur at remote sites from the soma, they would not be detected by the technique used in this study. It is also true that synaptic activities might interfere with the somatic estimation of the capacitance in neurons. Indeed, when recording the response of the somatic membrane to the hyperpolarized pulse, the electrode equally picks up the synaptic potentials generated in and conducted through the dendritic arbor. Thus the charging curve undergoes the superimposition of synaptic potentials.

However, besides the small-amplitude capacitance variations related to the slow (<1 Hz) sleep oscillation (Fig. 5), sustained swelling rarely was detected in neurons during SW seizures (see, however, Fig. 10). Several mutually nonexclusive explanations may be envisaged: the ionic channels work efficiently and do not produce intracellular electrolytic accumulations; the glial swelling generates a pressure on neurons and keeps their volume unchanged; the incidence of gap junctions between neurons diminishes dramatically with age (Connors et al. 1983); and the SW seizures we induced were neither strong enough nor sufficiently generalized to induce more serious swelling effects in neurons. As such we are brought to suggest that glial cells have a specific role in assuming the initial volume regulatory function in the cortex. This also would protect the neuronal membrane from mechanical tension and would better preserve the fine tuning of the channel-mediated ionic exchange.

**Functional implications of glial swelling**

SW seizures are associated with substantial increases in synchrony but, at the same time, with impaired ability to generate action potentials evoked by intrasomatic depolarizing pulses (Steriade et al. 1998). Several lines of evidence have suggested that signal transmission and synchronization may be achieved through extrasynaptic mechanisms, of which ephaptic interactions are of particular interest for the present discussion (Dudek et al. 1986; Hochman et al. 1995; Rosen and Andrew 1990; Taylor and Dudek 1982; Yam et al. 1986). Transmembrane potential measurements have shown that during paroxysmal events, the signal reaches amplitudes in the order of 10 mV (see Fig. 11 in Dudek et al. 1986). It is therefore possible that during SW seizures, when the extracellular space shrinks (Dietz et al. 1980), the glial swelling (present data) would draw near the cellular membranes, thus facilitating the intercellular ephaptic transmission. Further spreading of the signal also may occur through the gap junctions of the glial syncytium. This process also may be amplified by the swelling-induced release of amino acids by astrocytes (Kimelberg et al. 1990), as well as direct glia-to-neuron signaling (Parpura et al. 1994).

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