Extracellular Calcium Fluctuations and Intracellular Potentials in the Cortex During the Slow Sleep Oscillation

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Massimini, Marcello and Florin Amzica. Extracellular calcium fluctuations and intracellular potentials in the cortex during the slow sleep oscillation. J Neurophysiol 85: 1346–1350, 2001. During slow wave sleep the main activity of cortical neurons consists of synchronous and rhythmic alternations of the membrane potential between depolarized and hyperpolarized values. The latter are long-lasting (200–600 ms) periods of silence. The mechanisms responsible for this periodical interruption of cortical network activity are unknown. Here we report a decrease of ~20% in the extracellular calcium concentration ([Ca]out) progressively taking place in the cortex between the onset and the offset of the depolarizing phase of the slow sleep oscillation. Since [Ca]out exerts a high gain modulation of synaptic transmission, we estimated the associated transmitter release probability and found a corresponding 50% drop. Thus the periods of silence occurring in the cortical network during slow wave sleep are promoted by recurrent [Ca]out depletions.

INTRODUCTION

A slow oscillation (<1 Hz) has been described in virtually all cortical neurons of anesthetized (Steriade et al. 1993b) and naturally sleeping cats (Steriade et al. 1996) and in the sleep electroencephalogram (EEG) or magnetoencephalogram of humans (Achermann and Borbély 1997; Amzica and Steriade 1997; Simon et al. 2000). This oscillation appears as the cyclic fluctuation of the neuronal membrane potential between two voltage levels: a depolarizing phase made of synaptic activity and a hyperpolarizing period characterized by the absence of network activity. The mechanisms governing the switch between these two states are still poorly understood. It has been shown that the hyperpolarizing phase is associated with disfacilitation within the cortical network (Contreras et al. 1996). As demonstrated by multiple intra- and extracellular recordings in intact and lesioned brains, the slow oscillation is cortically generated (Steriade et al. 1993c) and takes place as a synchronous network event (Amzica and Steriade 1995).

Synchronous activities in large populations of neurons are likely to produce coherent modifications of the ionic composition of the extracellular space. In particular, Ca$^{2+}$, given its relatively low resting level (1–1.3 mM), is in a critical position since small absolute changes can produce large shifts in its extracellular concentration. Experimental results (Nicholson 1980; Nicholson et al. 1977, 1978; Pumain et al. 1983; Somjen 1980) and mathematical models (Egelman and Montague 1998; Wiest et al. 2000) converge in demonstrating that a decrease in [Ca]out occurs both during physiological and pathological neuronal activations. The drop of [Ca]out is mainly due to Ca$^{2+}$ inflow at the postsynaptic level (Bollmann et al. 1998; Borst and Sakmann 1999; Heinemann and Pumain 1981; Rusakov et al. 1999). Even slight changes of [Ca]out are known to modulate, with a high gain, transmitter release and therefore the synaptic function (Bootman and Berridge 1995; Dodge and Rahamimoff 1967; Katz and Miledi 1970; Mintz et al. 1995; Qian et al. 1997).

We tested the hypothesis that, during the depolarizing phase of the slow sleep oscillation, the occurrence of a simultaneous activation in virtually all neocortical neurons induces a phasic depletion of extracellular Ca$^{2+}$. As a consequence, some degree of synaptic depression would occur in the cortex, thus favoring the onset of the hyperpolarizing phase. Further, during the ensuing silent epoch, [Ca]out resting levels would be restored together with synaptic efficacy.

METHODS

Experiments were performed on eight adult cats, under general anesthesia with ketamine-xylazine. [Ca]out and DC field potentials (FPs) were measured by means of double-barreled ion-sensitive microelectrodes (ISMs), together with intracellular membrane potentials in neocortical neurons from the suprasylvian gyrus (areas 5 and 7). In some cases the slow oscillation was disrupted by the electric stimulation of the peduncolopontine tegmental (PPT) nucleus. The ISMs were filled with the calcium ionophore I-Cocktail A (Fluka) and were calibrated, before and after recordings, in appropriate solutions with Ca$^{2+}$ concentrations between 0.2 and 6 mM; the calibration values were fitted with a logarithmic trend. Only electrodes reaching 90% of the response in <20 ms were used. The time course of the response was measured stepping the electrodes trough drops containing different Ca$^{2+}$ concentrations (0.2, 0.5, 1, 1.5, 2, 4, and 6 mM). The drops were held at close distance by silver rings, which were connected to the ground. Thus ISMs were far faster than the phenomena under investigation. Since ISM potentials could be contaminated through capacitive coupling by FPs, the latter were subtracted from the former, and the resulting signal was linearized and transposed into concentration values using the parameters extracted from the logarithmic fitting of the calibration points.

Intracellular recordings were obtained with glass microelectrodes filled with 3 M potassium acetate and DC resistance between 30 and 40 MΩ. Only stable recordings with resting membrane potentials more negative than −60 mV, overshooting action potentials and input resistances between 17 and 24 MΩ were kept for analysis. To ensure

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stability of intracellular recordings, we paralyzed the animals with gallamine triethiodide (33 mg/kg iv) and ventilated them artificially, with control of the end-tidal CO₂ concentration between 3.5 and 3.7%. Further stability was obtained by cisternal drainage, bilateral pneumothorax, hip suspension, and by filling the hole in the calvarium with a 4% agar solution. Body temperature was maintained at 37–38°C. All pressure points were infiltrated with lidocaine, and a constant state of deep anesthesia was ensured by continuously monitoring an EEG with slow waves. Additional doses of ketamine-xylazine were administered at the slightest sign of EEG activation. A high-impedance Neurodata amplifier with active bridge circuitry was used to record and inject current into the cells. The headstage amplifier for ISMs was modified with an ultra ultra low input current (<25 fA) amplifier (National Semiconductor). Signals were recorded on tape with band-pass of 0–9 kHz and digitized off-line at 10 kHz for analysis and display.

RESULTS

All recorded neurons (n = 53) and FPs displayed a spontaneous slow (<1 Hz) oscillatory pattern as previously described in detail (Steriade et al. 1993b) (see also neuron in Fig. 2A). When the double-barreled electrode was placed at the cortical surface, the field and ISM potentials had almost identical variations and, after subtraction of the field potential signal from the ISM signal, no Ca²⁺ phasic activity was recorded (Fig. 1, A and C). When the electrode was lowered in the depth of the cortex (0.5–1.5 mm), FPs displayed reversed activity with respect to the surface recording (see also Contreras and Steriade 1995), and, in spite of the similarity between ISM potentials and FPs, their subtraction disclosed phasic Ca²⁺ variations (Fig. 1, B and C). From the surface recording, it is clear that the ISM also picks up FPs. Therefore this contamination has to be present in the depth recording too, and justifies the total subtraction of FP signal from the output of the ISM. Moreover, we were concerned about the possibility that the ISM reflects a delayed FP. This was ruled out by the flat result after the superficial subtraction (Fig. 1A). The depth [Ca]out fluctuations ranged from peak values of 1.18 ± 0.03 mM (mean ± SD) down to 0.95 ± 0.05 mM around an average level of 1.04 ± 0.2 mM. The amplitude of the fluctuations ranged from 0.18 to 0.27 mM, being, on the average of all experiments, around 20% of the maximum value.

To compare the dynamics of Ca²⁺ observed during the slow oscillation with the levels occurring during an activated state, stimulating electrodes were placed in the PPT nucleus of three cats. PPT electrical stimulation (30–60 Hz) resulted in the

![FIG. 1. [Ca]out fluctuations during the slow oscillation and cortical activation. A: the double-barreled electrode was placed on the pial surface and recorded the field potential (trace 1) and the ion-sensitive microelectrode (ISM) potential (trace 2). The activity consisted of alternative positive and negative waves with an oscillating frequency of ~0.8 Hz. The former corresponded to neuronal excitation, the latter to neuronal hyperpolarization (Contreras and Steriade 1995). Note close resemblance between the field and ISM activities, which, after subtraction (trace 2 − trace 1) disclosed almost steady [Ca]out. B: in the same animal, the double-barreled electrode was lowered in the depth of the cortex (~1 mm). The field potentials were reversed with respect to the corresponding phasic activities; depth negativities were associated with neuronal depolarization, positivities with hyperpolarization (Contreras and Steriade 1995). [Ca]out, resulting from subtraction of the 2 signals, displays phasic fluctuations with amplitude of 0.2–0.25 mM. C: averaged (n = 25) cycles of the slow oscillation from the surface (at left) and depth (at right) of the cortex. For each panel, the sweeps to be averaged were extracted around (±2 s) the onset of the neuronal excitation of consecutive cycles. This stereotyped moment is marked with a vertical dotted line. D: comparison of slowly oscillating patterns with activated patterns. The electroencephalographic (EEG) activation was obtained by electrical stimulation of the pedunculopontine (PPT) nucleus with a train of 100 shocks at 50 Hz. The slow oscillation was disrupted for about 7 s and resumed thereafter. Ca²⁺ fluctuations were equally abolished, and a steady level (around 1.14 mM) was reached. This level remained between the maximum and minimum values of the slow oscillation. All signals with positivity upward.]
disruption of the slow oscillation and EEG activation as previously described (Steriade et al. 1993a). In all cases (n = 25), simultaneously with the EEG activation, the phasic fluctuations of [Ca]\textsubscript{out} were abolished and [Ca]\textsubscript{out} maintained a steady level (1.13 ± 0.05 mM) for the duration of the activation (Fig. 1D). This Ca\textsuperscript{2+} concentration was slightly below the maximum [Ca]\textsubscript{out} but higher than the average measured during the slow oscillation.

Simultaneous recordings with ISMs and intracellular pipettes linked the [Ca]\textsubscript{out} variations to the cellular activity (Fig. 2A). Calcium level reached its maximum at the end of the hyperpolarizing phase and started to decrease soon after the onset of the neuronal depolarization. The slope of calcium fall was not constant, being steeper (−0.35 to −0.46 mM/s) during the first 200–300 ms of the neuronal depolarization. The slope during the last 200–300 ms of the depolarizing phase was milder (−0.05 to −0.08 mM/s). [Ca]\textsubscript{out} reached its minimum just before the end of the depolarization of the neuron and rose almost linearly during the following hyperpolarizing phase (Fig. 2B) with a slope value in the 0.75- to 0.95-mM/s range.

**DISCUSSION**

Many factors could account for the drop of [Ca]\textsubscript{out} during the depolarizing phase of the slow oscillation with respect to the baseline of the activated periods. 1) The onset of the depolarizing phase reflects simultaneous firing of large populations of neurons (Amzica and Steriade 1995). 2) As revealed by intracellular studies in behaving cats, many neurons, firing during the depolarizing phase of the slow oscillation, become silent after EEG activation due to awakening (Steriade et al. 1999). 3) Low-threshold Ca\textsuperscript{2+} currents, expressed in some (15%) cortical neurons (Paré and Lang 1998), may be deinactivated during the hyperpolarizing phase thus contributing to a larger calcium influx into neuronal elements during the following depolarization. 4) Ca\textsuperscript{2+} might also be uptaken by glia (Pasti et al. 1997) and at the presynaptic level (Alici and Heinemann 1995; Igelmund et al. 1996). It is not possible to establish in vivo the exact balance of all these contributions. A series of studies suggests the preponderance of the postsynaptic uptake (Bollmann et al. 1998; Borst and Sakmann 1999; Heinemann and Pumain 1981; King et al. 2000; Rusakov et al. 1999).

On the other hand, overshooting extracellular calcium levels at the end of the hyperpolarizing phase with respect to the steady value measured during activated periods may result from Ca\textsuperscript{2+} extrusion by neurons (DiPolo and Beauge\textquotesingle 1983) during a period characterized by poor ongoing cortical activity. The relationship between [Ca]\textsubscript{out} and transmitter release is known from in vitro studies performed on both central and peripheral synapses. We estimated the probability of transmitter release as a power function of the percentage variation of

![Figure 2](http://jn.physiology.org/)

**FIG. 2.** Relationships between intracellular membrane potential, [Ca]\textsubscript{out} and estimated synaptic release probability. A: periodic neuronal depolarizations (lasting for ~1 s) triggering action potentials were interrupted by periods (300–500 ms) of hyperpolarization and silenced synaptic activity. The field potential displays negative deflections associated with the depolarizing phase, and positive waves during neuronal hyperpolarization. [Ca]\textsubscript{out} dropped by about 0.25 mM during the depolarizing phase reaching a minimum just before the onset of the hyperpolarization. Then, [Ca]\textsubscript{out} rose back until the beginning of the next cycle. B: 30 cycles were averaged (spikes from the neuronal signal were clipped) after being extracted around the onset of the neuronal depolarization. The vertical dotted lines tentatively indicate the boundaries of the 2 phases of the slow oscillation. C: the transmitter release probability is contained within the gray area between the estimations with α = 2.5 and α = 4 exponents. The release probability dropped to around 50% before the onset of the hyperpolarizing phase. All signals with positivity upward.
[Ca]_out with respect to the maximum concentration ([Ca]_max). The exponents of the function (α) were ranging from 2.5 (Mintz et al. 1995) to 4 (Qian et al. 1997). These two limits provide an inclusive range of release probability for each measured level of calcium concentration. During the depolarizing-hyperpolarizing sequences of the slow oscillation, the release probability was maximal at the beginning of the depolarizing phase and progressively dropped, reaching a value between 0.55 (α = 2.5) and 0.4 (α = 4) just before the beginning of the hyperpolarizing phase (Fig. 2C). The probability of synaptic release recovered almost linearly returning to the full value at the end of the hyperpolarizing phase.

A global disfacilitation in the cortical network has been shown to underlie the long-lasting hyperpolarization of the slow oscillation (Contreras et al. 1996). In particular, a progressive running down of synaptic activity during the preceding depolarizing phase is consistent with two observations. First, when slowly oscillating neurons are hyperpolarized with DC current, the last part of the depolarizing phase decreases in amplitude (see Fig. 3 in Steriade et al. 1993b), suggesting that it involves more of intrinsic (depolarization-activated) currents than synaptic ones. Second, the somatic input resistance has its lowest value at the beginning of the depolarizing phase and progressively increases for the rest of the cycle (Contreras et al. 1996), consistent with the progressive closure of synaptically driven conductances.

An active inhibition is unlikely to be a major factor inducing the long-lasting hyperpolarizations. Local circuit inhibitory cells are equally silent during the hyperpolarizing phase of the slow sleep oscillation and discharge in synchrony with pyramidal neurons (Steriade et al. 1994). Thus they undergo the same disfacilitatory action of [Ca]_out as long-axoned cells, and their inhibitory action would diminish toward the end of the depolarizing phase. Long-range active inhibition is ruled out by the existence of the slow oscillation in cortical slices (Sanchez-Vives and McCormick 2000).

Taking into account the measured calcium extracellular dynamics and their estimated reflections on synaptic function, we propose the following sequence of events as a possible scenario for the cyclic occurrence of silent periods in the cortex during the slow sleep oscillation: during the depolarizing phase, the synchronous activation of cortical neurons leads to a progressive deple tion (~20% drop) of external calcium, mainly through a calcium entry at the postsynaptic sites. At the presynaptic sites, the high sensibility of transmitter release to extracellular calcium concentration would determine a progressive decrease (~45–60% drop) of synaptic efficacy. In the network, this effect would be accumulated at each synaptic station in a cascade reaction leading to an increasing degree of functional disconnection. At the single neuron level, a reduced synaptic depolarizing pressure would reduce the contribution of persistent sodium currents (Stafstrom et al. 1985), leaving Ca^{2+}-dependent K^+ currents (Schwindt et al. 1988) to shape the offset of the depolarizing phase. During the silent period of the hyperpolarizing phase, neurons are able to restore calcium extracellular concentration. At the end of this phase, high extracellular calcium levels and full synaptic function recovery would provide favorable conditions for the onset of a new cycle.

In conclusion, our results show that [Ca]_out fluctuations are phasically associated with the depolarizing and hyperpolarizing phases of the slow sleep oscillation. They bring new insights into the oscillatory mechanisms of cortical neurons. As the slow sleep oscillation is a precursor of nocturnal spike-wave seizures (Steriade and Amzica 1994), they may be extended and included in the models of normal and paroxysmal sleep patterns.


