Intracellular Study of Excitability in the Seizure-Prone Neocortex In Vivo

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Steriade, Mircela and Florin Amzica. Intracellular study of excitability in the seizure-prone neocortex in vivo. J. Neurophysiol. 82: 3108–3122, 1999. The excitability of neocortical neurons from cat association areas 5–7 was investigated during spontaneously occurring seizures with spike-wave (SW) complexes at 2–3 Hz. We tested the antidromic and orthodromic responsiveness of neocortical neurons during the “spike” and “wave” components of SW complexes, and we placed emphasis on the dynamics of excitability changes from sleeplike patterns to seizures. At the resting membrane potential, an overwhelming majority of neurons displayed seizures over a depolarizing envelope. Cortical as well as thalamic stimuli triggered isolated paroxysmal depolarizing shifts (PDSs) that eventually developed into SW seizures. PDSs could also be elicited by cortical or thalamic volleys during the wave-related hyperpolarization of neurons, but not during the spike-related depolarization. The latencies of evoked excitatory postsynaptic potentials (EPSPs) progressively decreased, and their slope and depolarization surface increased, from the control period preceding the seizure to the climax of paroxysm. Before the occurrence of full-blown seizures, thalamic stimuli evoked PDSs arising from the postinhibitory rebound excitation, whereas cortical stimuli triggered PDSs immediately after the early EPSP. These data shed light on the differential excitability of cortical neurons during the spike and wave components of SW seizures, and on the differential effects of cortical and thalamic volleys leading to such paroxysms. We conclude that the wave-related hyperpolarization does not represent GABA-mediated inhibitory postsynaptic potentials (IPSPs), and we suggest that it is a mixture of disfacilitation and Ca2+-dependent K+ currents, similar to the prolonged hyperpolarization of the slow sleep oscillation.

INTRODUCTION

Repetitive sensory volleys or synchronous stimuli applied to central pathways can trigger epileptic seizures in susceptible animals and humans. Initially focal seizures can be elicited by different types of stimuli, such as stroboscopic flash-lights, repeated sounds, cerebral scars, and electrical stimuli to cortex or thalamus, all acting on a hyperexcitable cortex. Studies on focal epilepsy have recently been performed in brain slices maintained in vitro, and, because of the high incidence of temporal seizures in humans, the investigations have mainly focused on ionic mechanisms and network operations in the hippocampus, entorhinal and piriform cortices (De Curtis et al. 1998; Pelletier and Carlen 1996; Traub et al. 1993, 1996; Wheal et al. 1998). The intrinsic cellular properties, synaptic mechanisms, and spread of seizure activity have also been studied in neocortical slices, generally after application of GABAA receptor antagonists, 4-aminopyridine, or other epileptogenic drugs (Aram et al. 1991; Barkai et al. 1995; Chagnac-Amitai and Connors 1989; Chervin et al. 1988; Gutnick et al. 1982; Sutor et al. 1994).

In this and the companion paper (Amzica and Steriade 1999) in vivo studies, we asked whether the two main components of spike-and-wave (SW) seizures, associated with opposite features of membrane polarization, are characterized by a differential excitability in neocortical neurons, whether precursor changes in the responsiveness of neocortical neurons can be detected before gross electrographic signs of seizures, and how do central stimuli modulate the timing of such paroxysmal episodes. In particular, as the “wave”-related hyperpolarization of cortical neurons was repeatedly regarded as produced by GABA-mediated inhibitory postsynaptic potentials (IPSPs) (Destexhe 1998; Giaretta et al. 1987; Pollen 1964), we tested the responsiveness of cortical neurons to antidromic and orthodromic volleys during both (“spike” and “wave”) components of SW complexes. We used a model of seizures consisting of SW or polyspike-wave (PSW) complexes at 2–3 Hz, often associated with fast runs at 10–20 Hz (Steriade et al. 1998a). Such seizures appear spontaneously, develop without discontinuity from the slow oscillation (<1 Hz) which characterizes the state of anesthesia (Steriade et al. 1993b) as well as natural slow-wave sleep (Acherman and Borbély 1997; Amzica and Steriade 1997) in animals and humans, and can also be triggered by electrical stimulation of the neocortex or thalamus. These paroxysms originate in the neocortex as their appearance in the thalamus lags by a few seconds the initiation in neocortex (Neckelmann et al. 1998; Steriade and Amzica 1994), and they can occlude after ipsilateral thalamectomy (Steriade and Contreras 1998). The neocortical origin of continuous SW discharges, and the absence of parallel metabolic alterations in the thalamus, was also shown by means of positron emission tomography (PET) studies in children (Maquet et al. 1995). The relatively high incidence of spontaneous SW/PSW seizures in our animal experiments, in the absence of GABA_A receptors antagonists or other convulsive substances and without deliberate electrical stimulation, may be explained by the widespread corticothalamic coherence of low-frequency oscillations during natural sleep, the hypersynchronizing action of ketamine-xylazine anesthesia in acute experiments, the large numbers of stimulating/recordings electrodes, and the repeated stimuli used for neuronal identification (Steriade et al. 1998a).

It is indeed known that cortical SW seizures preferentially occur in the state of drowsiness and resting sleep in behaving monkeys (Steriade 1974) and in humans (Kellaway 1985; Maquet et al. 1995). Rhythmic or sustained electrical stimuli are constantly used to produce models of complex seizures in
limbic and thalamocortical systems (Goddard 1967; Rafiq et al. 1993, 1995; Shouse and Ryan 1984; Steriade and Yossif 1974).

We investigated the dynamics of neocortical responsiveness during the transition from sleep patterns to seizure with emphasis on the differences between the spike and wave components of SW/PSW complexes, and we hypothesized that precursor signs in neuronal excitability will be observed in advance of full-blown seizures at the electroencephalographic (EEG) level.

METHODS

Experiments were conducted on 32 adult cats of either sex, anesthesitized with ketamine and xylazine (10–15 mg/kg and 2–3 mg/kg im, respectively). In addition, all tissues to be excised and pressure points were repeatedly infiltrated with lidocaine (2%). When the EEG displayed sleeplike patterns, a muscle relaxant (gallamine triethiodide) was administered, the animal was placed in the stereotaxic apparatus and artificially ventilated with the control of end-tidal CO₂ between 3.5 and 3.8%. The heart beat was kept constant (90–110/min) and the body temperature maintained at 37–39°C. To compensate for fluid loss, 20–30 ml iv saline was administered during the experiment. The depth of anesthesia was continuously monitored by EEG recording, and additional doses of the general anesthetic were given at the slightest tendency toward an activated EEG (fast and low-amplitude waves). The stability of intracellular recordings was ensured by hip suspension, drainage of cisterna magna, bilateral pneumothorax, and covering the hole made in the skull with a warm solution of agar (4% in 1% saline). At the end of experiments, the animals were given an intravenous lethal dose of pentobarbital sodium.

Recordings and stimulation

Field potential and intracellular activities were simultaneously recorded from suprasylvian association areas 5 and 7. 1) For field potentials we used coaxial electrodes, with the ring placed at the cortical surface and the tip inserted in deep cortical layers (0.8–1 mm). Small adjustments of macroelectrodes were made so that the reversal of depth-to-surface signals typical for the slow oscillation is obtained (Contreras and Steriade 1995). In all figures, we illustrate monopolar recordings from the cortical depth (the indifferent electrode was placed on neck muscles), with the relative positivity up, as for intracellular recordings. 2) Intracellular activity was recorded by means of glass micropipettes filled with 3 M potassium acetate (DC resistance, 25–50 MΩ). A high-impedance amplifier with active bridge circuitry was used to record the membrane potential (Vₘ) and inject current into the neurons. Intracellular signals were recorded, together with field potential activity, on an eight-channel tape with a band-pass of 0–9 kHz. 3) Stimulation was applied in the vicinity of the recording micropipette [within the same cortical area, or in the adjacent suprasylvian area (i.e., area 7 when recording was made from area 5)] or to appropriate dorsal thalamic nuclei [lateral posterior (LP) or rostral intralaminar central lateral (CL) that have reciprocal projections to and from areas 5 and 7; (see Jones 1985)]. We used bipolar stimulation through coaxial electrodes, similar to those used for recording the field potential activity from cortex.

Analyses

1) For the calculation of the latency of synaptically evoked responses, maximal and minimal values of the presimulus period were calculated over a time span of at least twice the maximum expected latency. These limits represent a voltage range within which the Vₘ may vary spontaneously even after the delivery of the stimulus. The first point crossing the maximal threshold in the depolarizing sense was considered as the latency of the excitatory component of the response. The point where the excitatory potential was crossing again the upper threshold was considered to mark the end of the excitation and served to the calculation of the duration of excitatory postsynaptic potentials (EPSPs). 2) The amount of excitation produced by a stimulus was quantified by the surface area lying below the excitatory response and above the threshold. The surface depolarization area was calculated as the integral of the response over its duration.

RESULTS

Database and neuronal identification

We analyzed data from 166 regular-spiking neurons (mainly slow-adapting, but some fast-adapting) that were recorded during SW/PSW seizures which occurred 1 as episodic paroxysms, developing without apparent discontinuity from the sleeplike patterns of the slow oscillation (e.g., Figs. 1 and 10); or 2 as one of the numerous spontaneous seizures that appeared during the same experiment and were separated from preceding and succeeding paroxysms by long periods of postictal depression (e.g., Figs. 7 and 8). No epileptogenic substances were used in the present experiments.

All 166 neurons were recorded for at least 15–20 min (but up to 90 min), had Vₘ’s more negative than −60 mV and overshooting action potentials. Antidromically evoked spikes were differentiated from orthodromically evoked ones by take-off directly from the baseline, fixed latency, and collision with spontaneous action potentials at proper time intervals.

General pattern of spontaneously occurring seizures

At the intracellular level and at a resting Vₘ most numerous seizures (158 of 166 neurons) consisted of SW/PSW complexes, generally at 2–3 Hz, associated or not with fast runs at 10–20 Hz, developing over a depolarizing envelope that lasted as long as the seizure (generally 10–20 s) and accompanied by a reduction in the amplitude of action potentials (Fig. 1). The onset of EEG seizures was considered at the moment where the acceleration of the slow oscillation (<1 Hz) crossed 1 Hz, frequency known to mark the upper limit of the sleeplike oscillation under ketamine-xylazine anesthesia; this time was also associated with increased amplitude of EEG waves (see rightward arrows in Fig. 1, A and B). The sustained, prolonged neuronal depolarization started a few seconds before the seizure was visible at the macroscopic EEG level (Figs. 1 and 2). As a rule, such seizures started with isolated paroxysmal depolarizing shifts (PDSs; Fig. 1A1) that increased their incidence and eventually became rhythmic SW or PSW complexes at 2–3 Hz. Although the generalized SW seizures are known to not be associated with postictal depression at the EEG level, intracellular recordings showed a short period of hyperpolarization (3–5 s) after cessation of seizure (Fig. 1A).

Synaptic and antidromic responses to cortical stimuli

Orthodromic responses, tested during control (pre- and postseizure) and seizure epochs consistently showed a progressively decreased latency, increased amplitude, and faster slope at the EPSP onset, during the transition from the slow oscillation (or isolated, interictal PDSs) to SW/PSW paroxysms (n = 52). In Fig. 2, the cortically evoked EPSPs...
decreased their latency from 4–4.5 ms to 2–3 ms, and the duration of the depolarization increased roughly by 60% (from ~40 ms to ~65 ms) during the 10 s approaching the seizure (compare traces a to f during the preseizure epoch). Stimulation with the same parameters were applied throughout seizures, and contrasting results were obtained during the two main components of SW paroxysms, as follows. 1) During the hyperpolarization following each PDSs and related to the wave component of EEG SW complexes, cortical stimuli reliably elicited PDSs that started at ~3 ms, quite similarly to the EPSP latency before seizure. 2) When falling during the PDSs associated with the spike component of SW complexes, the same stimuli were completely ineffective in eliciting an overt response (Fig. 2C2).

The comparison between antidromic and orthodromic responses in the same cortical neuron provided evidence for the dependency of both responses on the $V_m$ and network activity. The unusually long latency of antidromic response (13 ms) in the neuron illustrated in Fig. 3 is attributable to the low-intensity stimulation of a thin axonal collateral. 1) The anti-
A dromic response was elicited during the wave component of SW complexes only on steady depolarization, bringing the $V_m$ to $-65 \text{ mV}$ (Fig. 3B1), but failed at the hyperpolarized level of the resting $V_m$ ($-80 \text{ mV}$). During the spike component, antidromic action potentials were only elicited during the declining, repolarizing phase of PDSs (Fig. 3C1), but were absent if stimuli fell during the middle of PDSs. This was not due to $V_m$ because at the same $V_m$ ($-50 \text{ mV}$), but during a different
FIG. 3. Comparison between antidromic and orthodromic responses during the spike and wave components of seizures. Intracellular recording of area 7 neuron activated antidromically and orthodromically from the posterior part of area 5. A: antidromic response to low (L), intermediate, and higher (H) intensities (to obtain antidromic responses, the neuron was steadily depolarized by injecting 1 nA through the recording pipette). Note take off from the baseline. Note also the depolarizing plateau potential whose amplitude and duration was a function of stimulus strength; at H intensity, single action potentials were occasionally triggered at a latency of 30–40 ms. B: during the “wave” of SW seizure, cortical stimulus (intensity H) elicited an antidromic spike followed, after 30–40 ms, by a PDS when the V_m was depolarized (−65 mV); the same stimulus elicited only a PDS at the resting V_m (−80 mV). C: during the “spike,” the antidromic response survived, but only toward the end of PDSs, when the V_m tended to hyperpolarize. See also text. Averages (n = 5) of cortically evoked early responses during wave, under steady depolarization (1) and at rest (2), are depicted at bottom left. Averages at different periods of the spike component (full depolarization, 2; and declining period, 1) are depicted at bottom right.
FIG. 4. Dynamic evolution of cortically evoked synaptic responses during the wave component of the SW seizure and of antidromic spikes during given times of the spike component. Intracellular and depth-EEG recordings from area 7. This neuron responded before the seizure (see D) with an antidromic spike (latency 1 ms) followed by an EPSP (onset latency 1.5–1.7 ms). A: occurrence of seizure, with initial PDS triggered by cortical stimuli to area 5, delivered every 1 s. Open circles below the intracellular trace indicate orthodromic responses, and filled circles indicate antidromic activation of the neuron. Places without circles indicate that stimuli fell during the PDSs and no clear response could be elicited (see Fig. 3C2). Antidromic responses were elicited only during the depolarizing envelope of the seizure, and their incidence decreased as the seizure faded. B: each of the 5 panels depicts 4 superimposed sweeps with orthodromic responses; in all panels, the ordinal numbers of sweeps are indicated at left (and correspond to those indicated below the intracellular trace in A); the dotted horizontal line prolongs the $V_m$ before the stimulus; arrow indicates $-64$ mV; and the vertical line after the stimulus (marked by an empty circle) indicates the take-off time of the synaptic response. Before the full-blown seizure, each stimulus elicited a PDS (1st 4 stimuli). C: antidromic responses at different $V_m$ s during the spike component. D: superimposition of typical antidromic responses during the depolarizing envelope of the seizure, and of synaptic responses before and during the seizure as well as during postictal depression (PID).
phase, the antidromic spike was elicited (Fig. 3C1). Collision with orthodromic spikes is also precluded because stimuli were delivered during an epoch with no spontaneous discharges (Fig. 3C2). 2) Synaptically elicited PDSs were present during the wave, after the antidromic spike (Fig. 3B1) or in isolation, at a more hyperpolarized $V_m$ (Fig. 3B2). At the hyperpolarized level ($-80\,\text{mV}$), when the antidromic response failed, the onset of PDSs constantly revealed small-amplitude EPSPs that, by summation, gave rise to the giant synaptic potential crowned by high-frequency spike bursts (Fig. 3B2). An embryonic PDS could follow the antidromic response toward the end of the spike component, when the $V_m$ repolarized (Fig. 3C1), but was absent when the stimulus was delivered during the PDSs (Fig. 3C2).

The analysis of dynamic responsiveness throughout SW seizures in neurons displaying both antidromic and synaptic responses to cortical stimuli ($n = 14$) is illustrated in Fig. 4 with a neuron from area 7 that was antidromically as well as synthetically driven by stimuli applied to area 5. This is a typical example of the reciprocal relations between the two association areas in cat, demonstrated morphologically (Grüner et al. 1974) and electrophysiologically (Amzica and Steriade 1995).

As shown in Fig. 4A, the first four cortical stimuli triggered PDSs that opened the scene for a prolonged ($\sim 60\,\text{s}$) seizure. As in the preceding figure, synaptic activation elicited PDSs only during the wave component of SW complexes, whereas antidromic invasion was effective during certain periods of the spike component. 1) The latency of the EPSP during preseizure epochs was $1.5-1.7\,\text{ms}$ (Fig. 4Ba) and decreased to $1.0-1.4\,\text{ms}$ from the beginning of the seizure to its climax (Fig. 4, Bb and Bc), to control values after the postictal depression (Fig. 4, Bd and Be). This progression was accompanied by a similar evolution of the slope of EPSP onsets, which became steeper toward the middle of the seizure, was sluggish during postictal depression, and returned later to control values. In a sample of eight neurons, the EPSPs latency dropped from $1.8 \pm 0.1\,(\text{SD})\,\text{ms}$ to $1.3 \pm 0.1\,\text{ms}$ (28%), and the slope increased by 74% (the slope was measured as the tangent of the segment between the take-off of the depolarization and the initiation of the spike). 2) The antidromic response appeared during the spike component but only at $V_m$'s more negative than $-45\,\text{mV}$, at a time when the PDSs repolarized toward the wave (Fig. 4C). The absence of antidromic response at more positive $V_m$ was not due to collision with spontaneous action potentials.

The duration and overall spread of synaptically evoked PDSs depended on the state of network activity. The largest PDSs were triggered at some time distance ($>500\,\text{ms}$) from previous PDSs (Fig. 5A). When preceding, spontaneously occurring PDSs ended at time intervals of $\sim 100\,\text{ms}$ before the tested PDSs, they produced a shortening of $\sim 50-70\,$% in the evoked PDSs (Fig. 5B). Further reduction in the evoked PDSs was observed when they were shorter at time intervals before spontaneously occurring paroxysmal events (Fig. 5C). Figure 6 quantifies the relation between the size of PDSs and the time interval from the preceding PDS, as shown in Fig. 5. It shows that the depolarizing surface of the PDS was most sensitive to short time lags and that it saturated for longer time intervals. It also emphasizes that PDSs are not all-or-none events in the classical sense, but rather display a graded size. The time constant of the best exponential fits was $152 \pm 12\,\text{ms}$ (mean $\pm$ SD) for spontaneous seizures (Fig. 6A) and $409 \pm 54\,\text{ms}$ for seizures elicited by electrical stimulation (Fig. 6B). This time constant represents the time lag (between the offset of a PDS and the onset of the next one) after which the excitatory surface area increases by 63%.

**Synaptic responses to thalamic stimuli**

Similarly to the induction of seizures by cortical stimuli (see Fig. 4), thalamic volleys triggered isolated PDSs that eventually developed into seizures with SW/PSW complexes and fast runs (Figs. 7A and 8A; $n = 38$). After a series of thalamic volleys, the evoked EPSPs in related cortical neurons became gradually ampler (Fig. 7B1), and the postinhibitory rebound excitation developed into PDSs (Fig. 7B2). Occasionally, however, the PDSs developed directly from the early EPSPs, the large hyperpolarizations that usually follow PDSs were suppressed, and the PDSs were followed by fast runs at $10-20\,\text{Hz}$ over a depolarizing plateau (Fig. 7B3). Eventually, the seizure became self-sustained and, depending on the “time-distance” from the previous PDSs (see Figs. 5 and 6), thalamic stimuli evoked longer or shorter PDSs, followed by sequences of fast runs (Fig. 7B4).
We analyzed the evolution, throughout thalamically evoked seizures, the latencies and surface areas of EPSPs' and postinhibitory rebound excitations \((n = 16)\). The results matched those obtained by the analysis of cortically elicited EPSPs during the preseizure epoch and during the wave component of SW complexes (see Fig. 4). A typical example of the shortened latency and increased surface area of EPSPs is shown in Fig. 8. Throughout the seizure, the EPSPs' latencies decreased from \(\approx 5\) ms (during the control, preseizure epoch) to \(3.7\) ms. The depolarization surface area of EPSPs increased only slowly during the first part of the seizure (Fig. 8, inset between arrows in \(B\)) but, starting with the time when the early EPSPs merged with the rebound depolarizations to constitute giant PDSs (see trace 33 in \(D\)), the surface area of EPSPs displayed a huge increase (part corresponding to stimulus 33 in \(B\)). A similar increase was observed in the surface area of the postinhibitory rebound (\(C\)). In fact, the surface of EPSPs almost tripled (it increased by 273\%), and the depolarization surface of the postinhibitory rebound increased \(\approx 17\) times (1,724\%).

The basic difference between the mechanisms underlying seizures generated by cortical and thalamic stimuli, i.e., the occurrence of isolated PDSs from the early EPSPs in the former case and from the postinhibitory rebound in the latter, is illustrated in Fig. 9 for the same neuron tested with both types of stimuli. The early excitation elicited by local cortical (area 7) stimulation was slightly more synchronous and occurred at a shorter latency than that evoked by thalamic (LP) stimulation: four action potentials at a latency of 1 ms in the former case, three action potentials at 1.6 ms in the latter. However, this slight difference was associated with a dramatic change in the evoked PDS that appeared immediately after the early, cortically evoked excitation, whereas it was generated after the thalamically evoked postinhibitory rebound, at a latency of \(\approx 230\) ms (see DISCUSSION). It should be emphasized that this contrasting aspect only took place during isolated PDSs, but not with full-blown seizures when the thalamically evoked PDSs merged with the early EPSPs in cortical neurons (see again traces 11 and 33 in Fig. 8D).

**Responses to depolarizing current pulses**

The cell’s excitability was further tested with depolarizing current pulses. This procedure provided a comparison of neuronal excitability to impulses reaching the dendritic arbor with the readiness of the soma to fire action potentials in response to direct somatic depolarizations (the recording pipette is generally located in the soma). We injected direct depolarizing pulses during control periods (slow sleep oscillation) and SW seizures. As known, SW seizures may develop without discontinuity from the slow oscillation, and the spikes and waves of
FIG. 7. Thalamically evoked synaptic responses in cortical neuron during a SW seizure. Depth-EEG and intracellular recordings from cortical area 5. A: stimuli were delivered to the thalamic lateral posterior (LP) nucleus at a frequency of 1 Hz before and during a SW seizure. The 4 underlined periods correspond to a control (preseizure) epoch (1), the appearance of isolated PDSs (2), an epoch with fast runs at ~10 Hz (3), and a period with sustained PSW complexes at ~3 Hz (4). B: details of the stimuli marked with triangles in A. In C, superimposed details of the early responses from B1 and B4, respectively, are expanded.
FIG. 8. Progression of thalamically evoked synaptic responses in cortical neuron during a SW seizure. Depth-EEG and intracellular recordings from area 5. A: rhythmic stimuli at 1 Hz were applied to the thalamic rostral intralaminar central lateral (CL) nucleus. Different responses, before (No. 5 and 10) and during the SW seizure (No. 11 and 33), as indicated by triangles below the intracellular trace, are expanded at bottom right, in D. B: the latency of the early EPSP is plotted as a function of the time of seizure (same time course as in A). Triangles depict real values, and the dotted line is a linear fit of real values. Panel below plots the evolution of the depolarization surface area. The period between arrows is redrawn in the inset by expanding the ordinate. At the end of this period, the appearance of PDSs produced a merging of the early EPSP and postinhibitory rebound excitation, resulting in a huge increase of depolarization area (middle of the panel, after right arrow; see response 33 in D). The last period corresponds to the self-sustained seizure activity where the size of the evoked response varied according to the “time-distance” between the stimulus and the preceding PDS. Note that the EPSPs’ latencies decreased from ~5 ms (during the control, preseizure epoch; 1st 8–9 stimuli in B) to 3.7 ms (during the seizure). C: latency and surface area of the postinhibitory rebound excitation, only for the period where this component was present in isolation (see sweeps 10 and 11 in D), before merging with the early EPSP (as in sweep 33 of D). B and C are time-aligned with A.
SW complexes are an exaggeration of the depolarizing and hyperpolarizing components of the slow oscillation (see Fig. 4 in Steriade et al. 1998a). On one hand, the hyperpolarizing phase of the slow oscillation was compared with the wave phase of the SW seizure, and, on the other, the depolarizing phase of the slow oscillation was compared with the spike component of the SW seizure.

The results from SW seizures developing over a depolarizing envelope are depicted in Fig. 10. 1) The intensity of the depolarizing pulses was adjusted in relation with the $V_m$ during the hyperpolarizing phase of the slow oscillation, to generate a few action potentials (Fig. 10, Slow oscillation 1). During the seizure, the $V_m$ during the wave component was more depolarized (due to the depolarizing envelope), whereas the deflection induced by the same current pulse was reduced when compared with the control conditions (Fig. 10, Seizure 1). The reduction was of in the range of 60–70% in 12 tested neurons. No average value can be provided because there were variations within the same seizure as a function of its development.

2) Depolarizing pulses applied during the excitatory phase of the slow oscillation produced smaller deflections (Fig. 10, Slow oscillation 2) when compared with their equivalents during the hyperpolarizing phase. The following results are, however, to be considered with caution because the amplitude of the depolarization was limited by the firing of the somatic action potentials. In the cases where an equivalent number of action potentials was triggered as in the control situation, there was a reduction of the depolarizing deflection by 20–30%. The changes from the depolarizing phase of the slow oscillation to the spike component were much more drastic. Figure 10, Seizure 2, displays a rather conservative case in which a decreased voltage deflection of $\sim25\%$ was seen. Generally, during the PDS depolarization reaching a $V_m$ of $\sim40$ mV, intrasomatic current pulses were unable to produce any voltage deflections, and no additional action potentials were triggered.

**DISCUSSION**

The main results of this investigation are 1) the differential excitability of neocortical neurons during the wave and spike
components of the SW/PSW complexes, i.e., the possibility of triggering PDSs during the hyperpolarizations associated with waves, but the ineffectiveness of incoming signals to elicit such paroxysmal discharges during the spikes; 2) the precursor signs announcing the occurrence of full-blown seizures, consisting of progressively increased amplitude, slope, and duration of the evoked EPSPs, before any paroxysmal sign was visible at the gross EEG level; and 3) the difference between the effects of local cortical and thalamic stimulation, the former triggering PDSs immediately after the early EPSPs, whereas the latter producing paroxysmal discharges as a consequence of the postinhibitory rebound excitation.

**Constituent elements of SW seizures**

At the resting $V_m$, the overwhelming majority of SW/PSW seizures developed over a depolarizing envelope (see Fig. 1). Intra- and extracellular recordings in anesthetized (Pollen
neurons, due to the synaptic engagement of GABAergic phasic IPSPs during the paroxysmal discharges of cortical spindles (Avoli and Gloor 1982). Moreover, more than a decrease in cortical excitability, produced by topical application from thalamically generated spindle waves, a selective reduction in susceptibility to seizures in which the paroxysms were regarded as developing (Golshani and Jones 1999). Even in those models of SW seizures after thalamectomy (Steriade and Contreras 1998). Also, in intact-brain animals was demonstrated by their presence intracellularly stained and showing aspiny dendrites and locally arborizing axons) discharge at very high rates (500–600 Hz) during the spike component of SW seizures (Steriade et al. 1998a).

The absence of stimulus-evoked PDSs during the spike-related giant depolarization (20–25 mV) is explained by shunting IPSPs and a large increase in conductance during this component. However, PDSs could easily be evoked during the wave, because PDSs comprise numerous IPSPs underlying GABA_A IPSPs (Destexhe and Sejnowski 1995); 3) the wave-related hyperpolarization is obliterated in recordings with Cs^+-filled pipettes (unpublished data); 4) measurements of R_{in} using short hyperpolarizing pulses, reveal that, during PDSs, the R_{in} is many times lower than during the wave-related hyperpolarization, because PDSs comprise numerous IPSPs (Steriade et al. 1998b); and R_{in} decreases by only 20–30% during the wave-related hyperpolarization, compared with the hyperpolarizing phase of the slow sleep oscillation (Neckelmann et al. 1999), much less than what would be expected if this component of SW complexes were a GABA-mediated IPSP. In sum, we propose that the wave-related hyperpolarization is a mixture of Ca^{2+}-dependent K^+ currents (Schwindt et al. 1988, 1992) and disfacilitation, quite similar to the mechanism of the prolonged hyperpolarization of the slow sleep oscillation (Contreras et al. 1996; Steriade et al. 1993a).

Responsiveness of cortical neurons during the spike and the wave, dynamics of excitability changes, and differential effects of cortical and thalamic stimuli

The neocortical origin of PDSs during SW seizures in intact-brain animals was demonstrated by their presence after thalamectomy (Steriade and Contreras 1998). Also, in developing thalamocortical slices in vitro, PDSs are present in cortex isolated from thalamus, and connections between deep cortical layers are sufficient for their synchronization (Golshani and Jones 1999). Even in those models of SW seizures in which the paroxysms were regarded as developing from thalamically generated spindle waves, a selective decrease in cortical excitability, produced by topical application of KCl to cortex, caused the SW discharges to revert to spindles (Avoli and Gloor 1982). Moreover, more than half of thalamocortical neurons are tonically hyperpolarized throughout such cortically generated seizures and exhibit phasic IPSPs during the paroxysmal discharges of cortical neurons, due to the synaptic engagement of GABAergic thalamic reticular neurons, but do not display postinhibitory rebound spike bursts (Lytton et al. 1997; Steriade and Contreras 1995). The unexpected finding of hyperpolarization in thalamocortical neurons during SW seizures and the precursor events in cortical neurons have been corroborated by data from experiments on animals with genetic absence epilepsy (Pinault et al. 1998; Seidenberger et al. 1998).

1) The PDSs that form the spikes can be produced by systemic or local application of substances blocking inhibitory processes, such as penicillin (Prince and Farrell 1969) or bicuculline (Steriade and Contreras 1998), but neuronal events similar to those produced by bicuculline are generated spontaneously or after electrical stimulation (Steriade et al. 1998a). The spike patterns that were described during the penicillin model of generalized SW seizures (Giaaretta et al. 1987; Gloor et al. 1977) were probably less ample than the PDSs in the present experiments. The depolarization underlying the PDS is a large synaptic potential in CA1-CA3 hippocampal neurons (Johnston and Brown 1981, 1984) and in neocortex (Ayala et al. 1973). This view is supported by small, repetitive EPSPs that build up the onset of PDSs during seizures (see present Figs. 3 and 5). In addition to EPSPs, the spike component of SW seizures also contains an important inhibitory component. Indeed, recording with Cl^-filled pipettes revealed depolarizing shifts by 10–30 mV during the spike component, and conventional fast-spiking inhibitory interneurons (some of them intracellularly stained and showing aspiny dendrites and locally arborizing axons) discharge at very high rates (500–600 Hz) during the spike component of SW seizures (Steriade et al. 1998b).

In view of data resulting from the extracellular measures of [K^+]_o (Dichter et al. 1972; Lux and Neher 1973), it can be suggested that the giant depolarization recorded intracellularly during the PDS also reflects the increase in [K^+]_o, which produces a positive shift of the Nernst equilibrium potential. Some intrinsic properties of neocortical neurons, activated by the synaptic depolarization, may be a supplementary factor for the generation of this epileptic event. Therefore the synaptic origin and the altered intrinsic membrane properties of “epileptic neurons” (Gutnick et al. 1982; Prince 1967) are not incompatible notions. Interestingly, a peculiar class of neocortical cells, that fire rhythmic, fast, high-frequency spike bursts in response to depolarizing current pulses, have been recently disclosed to play an important role in the induction of SW/PSW seizures at the level of association areas 7 and 5 (see Fig. 15 in Steriade et al. 1998a).

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2) The mechanisms of the hyperpolarization during the wave component are debated. It was initially assumed that this is an inhibitory phase (Pollen 1964), presumably elicited by synaptic activation of local GABAergic interneurons. In keeping with this idea, some suggested that the first part of the hyperpolarization during the wave is constituted by a GABA_A receptor-mediated IPSP (Giaaretta et al. 1987), whereas a biophysical modeling study predicted the genesis of this component by a slow, GABA_B receptor-mediated inhibition due to a K^+ current (Destexhe 1998). Our data show that 1) with Cl^-filled pipettes, the wave-related hyperpolarization is not significantly altered (Steriade et al. 1998b); 2) with pipettes filled with QX-314, the wave-related hyperpolarization is also unaffected (I. Timofeev, F. Grenier, and M. Steriade, unpublished data); QX-314 promotes Na^+ conductance inactivation, but also blocks GABA_B IPSPs (Nathan et al. 1990) and G-proteins (Andrade 1991) that are required to activate K^+ channels underlying GABA_B IPSPs (Destexhe and Sejnowski 1995); 3) the wave-related hyperpolarization is obliterated in recordings with Cs^+-filled pipettes (unpublished data); 4) measurements of R_{in} using short hyperpolarizing pulses, reveal that, during PDSs, the R_{in} is many times lower than during the wave-related hyperpolarization, because PDSs comprise numerous IPSPs (Steriade et al. 1998b); and R_{in} decreases by only 20–30% during the wave-related hyperpolarization, compared with the hyperpolarizing phase of the slow sleep oscillation (Neckelmann et al. 1999), much less than what would be expected if this component of SW complexes were a GABA-mediated IPSP. In sum, we propose that the wave-related hyperpolarization is a mixture of Ca^{2+}-dependent K^+ currents (Schwindt et al. 1988, 1992) and disfacilitation, quite similar to the mechanism of the prolonged hyperpolarization of the slow sleep oscillation (Contreras et al. 1996; Steriade et al. 1993a).

Responsiveness of cortical neurons during the spike and the wave, dynamics of excitability changes, and differential effects of cortical and thalamic stimuli

The absence of stimulus-evoked PDSs during the spike-related giant depolarization (20–25 mV) is explained by shunting IPSPs and a large increase in conductance during this component. However, PDSs could easily be evoked during the wave, thus corroborating the only moderate decrease in R_{in} during the hyperpolarizing component of SW seizures and further disproving the prevalent GABAergic origin of this phase. The preserved excitability during the wave-related hyperpolarization of SW seizures is in line with the increased EPSP of neocortical neurons in response to cortical stimulation during the hyperpolarization of the slow rhythm, as compared with the depolarizing phase of this sleep oscillation (Timofeev et al. 1996). In fact, these two (normal and pathological) events, sleep and seizures, are related, and the relations between intracellular events...
and field potentials are virtually identical in both cases (Steriade et al. 1998a). The induction of PDSs during the wave-related hyperpolarization is ascribable to a network phenomenon in which repetitive EPSPs are summated and eventually reach the threshold of the giant depolarizing, epileptic event (see Figs. 3B2 and 5). This process is similar to the increased frequency of EPSPs, just before the occurrence of PDSs, in CA3 neurons from hippocampal slices (see Fig. 1 in Chambérlin et al. 1990). The shape and duration of PDSs depend on the history of the network as well as on synthetically activated intrinsic conductances, because such evoked paroxysmal events are largely diminished when they follow by short time distances spontaneously occurring PDSs (Figs. 5 and 6). This relative refractoriness may constitute a self-protective mechanism and could explain the fact that PDSs’ frequencies do not generally exceed 4 Hz during SW seizures. As the seizure accelerates (and this is the common signature of the presently described SW paroxysms that start with isolated PDSs, continue with low-frequency SW complexes at 1–2 Hz, and eventually reach higher frequency SW complexes), the increased rate of excitatory drives from the network is counteracted by the rather long refractory period of PDSs. Shorter refractory phases may be present in the genetic absence of epilepsy and other types of rat seizures in which SW complexes appear at higher frequencies, 7–9 Hz (Danobe et al. 1998; Kandel and Buzsáki 1997). In slices treated with bicuculline, PDSs often fail to occur at a frequency 0.1 Hz or higher (Hwa and Avoli 1991). The network origin of PDSs is further indicated by the progressive increase in amplitude and decrease in latency of cortically evoked EPSPs that precede by a few seconds the appearance of seizure at the global EEG level (see Figs. 2, 4, 7, and 8). Although these seizures are initiated and generated within the cortex even in the absence of thalamic, once entrained in paroxysms thalamocortical neurons may contribute to the development of seizures. However, whereas cortically evoked PDSs originate immediately after the early excitation, thalamic stimulation produces isolated PDSs only after the postinhibitory rebound excitation. This is probably due to the fact that, whereas the initial thalamic stimulus set into action a restricted pool of thalamic neurons, the postinhibitory rebound was more widely distributed (due to thalamic reticular projections) and contained many more action potentials (see trace 11 in Figs. 8D and 9). The role of the thalamic postinhibitory rebound in promoting an increased cortical excitability was demonstrated in dual intracellular recordings (Grenier et al. 1998). This justifies the assumption that, even in processes initiated and generated in given structures, i.e., the neocortex in the present case, the intact connectivity with related subsystems contributes to the full development of various events.

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