Spontaneous and Artificial Activation of Neocortical Seizures

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Amzica, Florin and Mircea Steriade. Spontaneous and artificial activation of neocortical seizures. J. Neurophysiol. 82: 3123–3138, 1999. The aim of this study is to disclose the mechanisms underlying the recruitment of neocortical networks during slow-wave sleep oscillations evolving into spike-wave (SW) seizures. 1) We investigated the activation of SW seizures in a seizure-prone neocortex by means of electrical stimuli applied within the frequency range of spontaneous sleep oscillations. Stimuli were grouped in bursts of 10 Hz, similar to sleep spindles, and repeated every 2 s, to reproduce their rhythmic recurrence imposed by the slow (<1 Hz) sleep oscillation. Either cortical or thalamic stimuli, which were applied while the cortex displayed sleeplike activity, gradually induced paroxysmal responses in intracellularly recorded neocortical neurons, which were virtually identical to those of spontaneous seizures and consisted of a progressive buildup of paroxysmal depolarizing shifts (PDSs). 2) The ability of cortical networks to follow stimuli was tested at various stimulation frequencies (1–3 Hz) and quantified by calculating the entropy of the ensuing oscillation. Rhythmic PDSs were optimally induced, and the lowest entropy was generated, at a stimulation frequency around 1.5 Hz. Fast runs at 10–15 Hz, which often override PDSs, thus contributing to the polyspike-wave pattern of seizures, were induced by cortical stimuli, but were disturbed by thalamic stimuli. Spontaneous seizures generally evolved toward an accelerated discharge of PDSs. It is suggested that these accelerating trends during SW seizures act as protective mechanisms by provoking the uncoupling of cortical networks and eventually arresting the seizure.

INTRODUCTION

Rhythmic sensory stimuli reaching a hyperexcitable cerebral cortex may produce seizures. The starting hypothesis of the present study was that focal seizures can be triggered by rhythmic central stimuli delivered within the same frequency range as the spontaneous low-frequency oscillations in corticothalamic networks. As a consequence, naturally occurring synchronized sleep oscillations may develop into seizures. It was indeed shown that sleep is a favorable condition for the genesis of certain seizures, particularly those characterized by spike-wave (SW) complexes at 2–4 Hz (Kellaway 1985; Steriade 1974). During electroencephalographic (EEG)-synchronized sleep, the cortex is dominated by a slow oscillation (<1 Hz) that entrains and groups other sleep rhythms, generated in thalamus or cortex (Amzica and Steriade 1998b; Contreras and Steriade 1995; Steriade et al. 1998).

In the companion paper (Steriade and Amzica 1999) we tested the excitability of cortical networks before, during, and after SW seizures. It resulted that a seizure-prone cortex has the ability to transform incoming stimuli into paroxysmal responses and that the excitability is modulated by the ongoing synaptic activity of the network. It is therefore possible that synchronous activities in corticothalamic networks, as they occur during EEG-synchronized sleep, trigger pathological oscillations, which would spread over large cortical territories. During spontaneous seizures, neurons are subjected to the competitive influence from oscillating networks. The mechanism generating the oscillatory behavior during seizures is still unknown. However, in the class of cortically generated SW seizures, their progressive development from sleep oscillations has been proposed (Steriade and Amzica 1994; Steriade and Contreras 1995; Steriade et al. 1998).

The present study attempted to modify the oscillating pace by replacing it with a controlled source of stimulation, to measure the ability of the network to adapt to the constraint. The stimulating frequencies used in this study mimicked synchronous synaptic inputs during slow-wave sleep or seizures. The study is restricted to the behavior of cortical neurons because the priming role of the cortex in generating SW seizures has recently been emphasized (Steriade and Contreras 1998; Steriade et al. 1998).

METHODS

Animal preparation and techniques of electrophysiological recordings are described in the companion paper (Steriade and Amzica 1999). The stimulating frequencies used for this study belong to the natural frequencies occurring spontaneously in the corticothalamic network during slow-wave sleep or epileptic seizures. Thus we mainly stimulated at 0.5, 1–3, and 10 Hz. The pace of the stimulation was either constant or with progressive change. Stimuli were delivered as single shocks or in clusters (e.g., 5–8 shocks at 10 Hz, every 2 s, mimicking spindles recurring with the periodicity of the slow oscillation).

The methods for calculating the latency and the surface area below excitatory potentials have been described in the companion paper (Steriade and Amzica 1999). Some additional analytic tools were developed for this study. Because the oscillatory behavior displayed before, during, and sometimes after seizures is not perfectly periodic, we calculated instantaneous frequencies as reciprocal values of the time interval between two successive oscillatory events. In the case of intracellular recordings, we considered the paroxysmal depolarizing shifts (PDSs) or the fast runs as oscillatory events. To each of these depolarizing events we associated a time stamp corresponding to the moment where its rising slope was maximal. To determine the maximum slope point, we calculated the first derivative of the intracellular signal and detected its local maximum situated between the onset of the depolarization and the moment where the first action potential was triggered. Instantaneous frequencies were plotted as a function of real time, providing thus a dynamic measure of the rhythmicity of the ongoing phenomena.

To quantify the oscillatory behavior, we have chosen to use the

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entropy as a measure of the dispersion of instantaneous frequency values during the various epochs related to a seizure. The instant frequencies of a given period were distributed in a histogram. The coefficient $C_i$ of each bin (0.25 Hz for PDSs and 1 Hz for fast runs) was transformed into a probability $p_i$ associated to the incidence of a given frequency to occur

$$p_i = \frac{C_i}{\sum_{i=1}^{n} C_i}$$

Hence entropy was calculated according to the original formula used by Shannon (1948)

$$S = -\sum_{i=1}^{n} p_i \log p_i,$$

where log is the decimal logarithm. The minimum entropy is zero and would be achieved for processes oscillating within the frequency band of a single binwidth. The maximum entropy is equal to log $n$ and corresponds to a uniform distribution of frequencies ($p_i = 1/n$). To be able to compare the results from different cells, seizures, and animals, we normalized for each case the entropy by its maximal value (log $n$). Thus normalized entropy values ranged between 0 (highly ordered systems) and 1 (highly unordered systems).

RESULTS

First, we present the contribution of rhythmic stimulation in triggering PDSs and SW seizures. Next, we tested the ability of epileptic cortical networks to display PDSs in response to various stimulation frequencies. Finally, we studied the integration of fast runs and polyspikes within rhythmic activities generated in the corticothalamic network.

Database

A total of 98 regular-spiking neurons were recorded during SW/polyspike-wave (PSW) seizures in 22 cats. The neurons were recorded for at least 15 min (some lasted for 90 min) and had membrane potentials more negative than $-60$ mV and overshooting action potentials. In all, we recorded 267 electrically generated seizures and 149 spontaneous seizures. The typical pattern of the seizure has been previously described (Steriade et al. 1998) (see also Fig. 1). It consists of SW complexes at frequencies between 1.5 and 3 Hz and fast runs of activity (10–15 Hz). Sometimes these patterns appeared in alternation, and some other times short sequences of fast runs followed PDSs, thus constituting PSWs.

Induction of SW seizures with rhythmic stimuli

Slow-wave sleep is dominated by a slow (<1 Hz) cortical oscillation consisting of periodic depolarizing-hyperpolarizing sequences (Steriade et al. 1993b). The depolarizing epochs generate action potentials that underlie the propagation and synchronization of the slow oscillation through intracortical (Amzica and Steriade 1995a,b) and corticothalamic networks, thus triggering thalamically generated sleep spindles (Contreras and Steriade 1995; Steriade et al. 1993c). Spindles (sequences of waves at 7–14 Hz, recurring rhythmically at the frequency of the slow oscillation, mainly 0.3–0.5 Hz) often appear superimposed on the depolarizing phase of the slow oscillation. To reproduce this behavior, we delivered periodic (0.5 Hz) trains of thalamic stimuli consisting of 5 stimuli spaced by 0.1 s (Fig. 1). This procedure reliably induced SW seizures in 90 of the recorded neurons (92%). They were expressed in the gyrus where recordings were performed, and often in the neighboring gyri also. Intracellularly, these seizures started with large amplitude (20–40 mV) depolarizations with the features of PDSs, with shape and duration similar to those underlying interictal and ictal EEG spikes (Ayala et al. 1973; Johnston and Brown 1981; Matsumoto and Ajmone-Marsan 1964a,b). Fast runs at 15–20 Hz accompanied some of these PDSs.

The rhythmic stimulation of the thalamic lateral posterior nucleus produced a gradual onset of the seizure. The first train of stimuli ($a$ in Fig. 1) elicited normal synaptic responses (excitatory postsynaptic potentials (EPSPs)) at each stimulus. The second train ($b$) had similar effects, but was followed by a sustained depolarization. For the following trains of stimuli ($c$–$f$), the response to the third shock in each train became progressively larger, as demonstrated by the depolarizing surface area underlying the EPSP, and the EPSP to the fourth shock triggered a full-blown PDS crowned by increasing numbers of fast runs. This is shown by the significant increase of the surface area of depolarizing responses after the fourth and fifth stimuli. After seven trains of stimuli, the seizure became self-sustained and the stimulation was stopped. Control responses ($g$) were again elicited after the complete arrest of the seizure.

From the 90 neurons displaying SW seizures after thalamic stimulation with trains of stimuli at 10 Hz, and considering only the first seizure after impalement, the first PDS appeared in most cases during the 3rd train of stimuli (69% of the cases). In 21 neurons, the first PDS was recorded during the 4th train of stimuli (23%), while the rest of the neurons (7, representing 8% of the cases) reacted with PDSs during the 2nd or the 5th train. We selected only the first seizure after impalement to avoid the uncontrolled effect that would have been played by frequent stimulation.

Thus rhythmic pulse trains at 10 Hz repeated with the frequency of the slow oscillation (0.5 Hz) are a precipitating factor for SW seizures. We tested the behavior of the cortical network under the continuous pressure of such a stimulation pattern in 25 neurons of those where the stimulation was triggering SW seizures efficiently (Fig. 2). In 22 of them, recurrent seizures were obtained with a rhythmicity of $\sim 0.04$ Hz. The average period of such seizures was $24 \pm 3$ s (mean $\pm$ SD) and the range was between 12 and 30 s. This pattern was either not seen in the absence of the stimulation, or it occurred at a different frequency (generally slower) and less regularly.

All seizures had a stereotyped pattern (Fig. 2A, expanded period) with paroxysmal events occurring in close time-relationship with the trains of stimuli. Some PDSs appeared spontaneously (preceding a train of stimuli) suggesting that the seizure attained a self-sustained development. In all cases, the onset of the seizure was marked by the transition from normal synaptic responses to the train of stimuli (Fig. 2B, sweeps 1–4) to the generation of giant synaptic potentials toward the end of the train of stimuli (sweeps 5–7). The onset of the seizure was
accompanied by a gradual increase of the neuronal responsiveness to earlier shocks in the train (see the displacement of the PDSs latency in Fig. 2C, sweeps 8–11). Eventually, a phase was reached where PDSs were reliably evoked by the first shock in the train (sweeps 11–14). The end of the seizure was associated with a symmetrical reaction to stimulation: paroxysmal responses began to slide toward the end of the train and finally disappeared (Fig. 2D, sweeps 15–19). These results suggest that a seizure-prone cortex (e.g., a cortex that presents scars or chemical imbalance or has been stimulated repeat-
FIG. 2. Continuous stimulation mimicking sleep patterns induced recurrent seizures. A: continuous cortical stimulation (area 5) of a cortical neuron from area 7 with trains of 5 stimuli at 10 Hz delivered every 2 s induced recurrent spike-wave (SW) seizures riding over a depolarizing envelope. The middle seizure in the square is expanded below. B: detailed responses to the 1st 5 pulse trains from the seizure depicted in A. Numbers at the left of each trace indicate the ordinal of that train. Note the appearance of a paroxysmal depolarizing shift (PDS) toward the end of the 5th train of stimuli. C: each train elicited a PDS whose latency to the 1st stimulus in the train diminishes as the seizure develops. Eventually, PDSs were reliably evoked by the 1st stimulus in the train (11–14). D: arrest of the seizure was associated with delaying of the evoked PDS (15) and its eventual disappearance (16–19).
edly), which undergoes the continuous pressure of slow sleep oscillatory activities, may generate recurrent SW seizures.

**Spontaneous and evoked PDS**

Single cortical stimuli were also effective in triggering paroxysmal responses (see Figs. 4, 6, 8, 9, and 11 in Steriade and Amzica 1999). Here we deal with the ability of these responses to follow various frequencies and regard them in relation with the corresponding spontaneous oscillations. The experimental paradigm is described in Fig. 3. The spontaneous activity of neurons consisted of more or less regularly occurring PDSs. Their reflections at the EEG level were spiky waves. The instantaneous frequencies were situated between 0.5 and 1 Hz. Rhythmic cortical stimulation at a fixed frequency (1 Hz) produced faithful following by the neuron and by its environment (see rhythmic EEG spikes at 1 Hz). The evoked PDSs had the same aspect as the spontaneous ones (bottom of Fig. 3). The paroxysmal responses to stimuli occurred with an alternative pattern (inset with vertically expanded detail from the instantaneous frequency curve). This pattern consisted of instantaneous frequencies continuously alternating around the fixed stimulation frequency. It was a constant finding in all tested cells...
and is the consequence of a jittering PDS’ latency. In spite of this small variability of responses, sooner or later spontaneous activity interfered with the ability of the cortical network to follow stimuli at almost fixed latencies. This phenomenon generally coincided with the onset of a self-sustained activity. Spontaneous PDSs preceding the delivery of a stimulus provoked an acceleration and, consequently, refractory periods (Hablitz 1984; Steriade and Amzica 1999), which produced a certain disorder in the sequence of instantaneous frequencies (see right side of the inset in Fig. 3). In the case shown in Fig. 3, the stimulation was stopped and the oscillation, after a short period with faster frequencies, resumed control values (<0.8 Hz).

In our experimental conditions, under ketamine-xylazine anesthesia, spontaneous seizures had an electrographic pattern similar to that in the Lennox-Gastaut syndrome (Steriade et al. 1998), with SW complexes at relatively lower frequencies, 1–2.5 Hz (see Niedermeyer 1998b). Thus the capacity of the network to follow imposed rhythms was first tested by delivering stimuli at various frequencies (Fig. 4). The evoked PDSs optimally followed stimulation frequencies around 1.4–1.5 Hz (Fig. 4B). Responses to lower frequency stimulation (~1 Hz) often interfered with the spontaneous PDSs of the network resulting from accelerating activities (Fig. 4A). Faster stimulation rates (2 Hz and higher) were followed only for limited periods of time (Fig. 4C). They were able, however, to produce activities at submultiples of the stimulation frequency (i.e., 1 and 0.5 Hz). In other words, PDSs were triggered only every second or every fourth stimulus. This “undertuning” was usually noticed before and at the beginning of a seizure. Once self-sustained activities were promoted in the network, the stimulation at 2 Hz appeared as a superior limit at which neurons were able to respond with PDSs, and more failures were evident.

This phenomenon was associated with a certain variability. Initial attempts to test each cell with too many stimulation frequencies to obtain an optimal tuning frequency proved to be unrealistic because of the interference with spontaneous epileptic and nonepileptic activities. Besides, when more stimulation was delivered to the animal, more spontaneous (unwanted) seizures appeared, and the state of the cells degraded. Therefore only few discrete stimulation frequencies were used (0.5, 1, 1.5, 2, 2.5, and sometimes 3 Hz). We chose to quantify the ability of a seizure-prone network to follow different types of stimuli by calculating the entropy during periods with stimulation at a given frequency. The average entropy of 30 recorded cells during spontaneous SW discharges was 0.78 ± 0.36 (mean ± SD). It dropped to 0.45 ± 0.12 for 1-Hz stimulation, reached a minimum of 0.25 ± 0.07 for 1.5-Hz stimulation, and increased again to 0.8 ± 0.3 for 2-Hz stimulation frequency. These measures suggest that the cortical networks optimally follow evoked or spontaneous oscillations around 1.5 Hz.

In addition to test the responsiveness of the network with fixed stimulation frequencies, we also investigated its behavior during sequences of accelerating and decelerating stimuli (Fig. 5). Again, under these conditions neurons ceased to follow stimuli beyond 2 Hz. Once the tuning was lost, returning to lower stimulation frequencies, including those at which reliable responses were previously obtained, did not immediately produce the coupling of the network to the stimuli (n = 23). In the case illustrated in Fig. 5, two types of stimuli were delivered: single stimuli at accelerating frequencies (left part of Fig. 5) and series of stimuli at constant frequencies with acceleration between the series. In both cases, the neuron reliably followed up to 1.7 Hz and began to fail about that frequency. The latency of the PDSs remained constant throughout this period (~75 ms). No follow-up was possible above that value (1.7 Hz), although the latency of some PDSs remained in the control range during the initial period of the 2.5-Hz stimulation.

The stimulation frequency at which the neuron resumed the following was lower (1.1 Hz) than the one at which he had lost it (2 Hz). This phenomenon, suggesting a hysteresis-type behavior, was seen in a minority of cells (n = 5). The neuron had, however, no difficulty to adapt to the slowing of the stimulation frequency (right part of Fig. 5), as long as the slowing intervened in a moment when the neuron was still following the stimuli.

The evolution of the entropy confirms that there is an optimum oscillation frequency, which the cortical networks follow more readily (Fig. 6). Both acceleration beyond and slowing below that frequency is accompanied by loss of coupling and by increased entropy values, thus by increased disorder in the oscillatory behavior of the system. The panel displaying the latencies of the PDSs emphasizes that the arrest of a seizure was preceded, in all cases in which stimulation outlasted the arrest of the seizure (n = 138), by a progressive increase of the latency of the evoked PDS.

Synchronized fast (10–15 Hz) oscillations, also called fast runs, are often present during cortical SW seizures (Neckermann et al. 1998; Steriade et al. 1998). They consist of stereotyped, clocklike, and rhythmic depolarizing potentials of 2- to 15-mV amplitude. These components showed constant features for a given neuron throughout the seizure, and their amplitude decreased with the distance from the seizure focus. We asked whether this seizure pattern could be obtained with electrical stimulation, i.e., if an artificially created focus of fast runs would generate and spread like the spontaneous one. In a first instance, during dual simultaneous intracellular recordings, we delivered rhythmic (10 Hz) stimuli to a cortical region close to the one where the neurons were impaled (Fig. 7). This pattern of stimulation was applied only at a moment when the electrical activity of the brain was already displaying spontaneous paroxysmal potentials in at least one of the recorded sites. Only those cases were retained for analysis where cortical neurons showed synaptic responses to stimulation (n = 10). Synchronized activities were triggered in all cases of cortical stimulation, and the neurons were faithfully following the stimuli (Fig. 7). The neuron closer to the stimulation site displayed more ample responses than the one situated further away, and with a corresponding time lag. The latency of the area 5 neuron was 6.3 ms, whereas the latency of the area 7 neuron was 13 ms. This pattern of stimulation did not result in self-sustained oscillations with fast runs. It was, however, followed by SW seizures after the cessation of the stimulation (Fig. 7, top panel). It results that the cortex plays a reinforcing role in the
FIG. 4. Following of PDSs for various stimulation frequencies. Area 7 neuron. Each panel contains the intracellular recording, the associated depth field potential and the graph of the instantaneous frequency. In the latter, a horizontal line indicates the period and the frequency of stimulation (in area 5). A: stimulation at 1 Hz. The 1st series of stimuli was stopped when the spontaneous activity of the network interfered with the ability to follow stimuli. B: stimulation at 1.4 Hz. C: stimulation at 2 Hz. Note that some undercoupling occurs occasionally at 1 Hz (— — —).
synchronization and spreading of the fast runs during SW seizures.

Stimulation of thalamic nuclei anatomically related with the cortical recording area induced responses in which the priming epileptic event was the rebound (see Figs. 8 and 9 in Steriade and Amzica 1999). As the thalamus reflects fast runs (Steriade and Contreras 1998), we thought that, through its reentrant projections, it might modulate the fast runs generated in the cortex. This aspect was studied by stimulating the thalamic centrolateral intralaminar nucleus during fast runs (Fig. 8). The centrolateral nucleus has widespread projections to the suprasylvian association areas (Jones 1985). The stimulation frequency was tuned to the frequency of the preceding spontaneous fast runs (Fig. 8). The centrolateral nucleus has widespread projections to the suprasylvian association areas (Jones 1985). The stimulation frequency was tuned to the frequency of the preceding spontaneous fast runs (Fig. 8). The centrolateral nucleus has widespread projections to the suprasylvian association areas (Jones 1985). The stimulation frequency was tuned to the frequency of the preceding spontaneous fast runs (Fig. 8). The centrolateral nucleus has widespread projections to the suprasylvian association areas (Jones 1985). The stimulation frequency was tuned to the frequency of the preceding spontaneous fast runs (Fig. 8).

The spontaneous fast runs consisted of regular, stereotyped depolarizing potentials at ≈10 Hz (Fig. 8B1). Under control conditions, thalamic stimuli at 10 Hz elicited a low-amplitude EPSP (Fig. 8B4), occasionally followed by a larger secondary depolarizing potential (Fig. 8, B2 and B3). The latter had similar features with the spontaneous fast run complex. As shown by the instantaneous frequency diagram, and in contrast with the cortical stimulation (Fig. 7), the thalamic stimulation interfered with the spontaneous fast runs and disrupted the rhythmicity of the network for most of the time. With the exception of the period depicted in Fig. 8B3, spontaneous fast runs appeared to compete with secondary depolarizations triggered by the stimulation. It is difficult to determine which of the delayed potentials are true rebounds or occasional spontaneous fast runs. The secondary response in the first averaged trace of Fig. 8C has a longer duration (≈65 ms) and less steeper onset slopes than the average spontaneous complex (≈50 ms), because of the jittering of its onset latency.

The entropy values increased from 0.33 ± 0.03 during spontaneous fast runs to almost the double during the stimulated period by reaching 0.63 ± 0.12. We conclude that the thalamus has a rather desynchronizing and dampening contribution to the fast runs during SW seizures.

Finally, we tested the influence of tetanic stimulation (100
Hz) on the evolution of fast runs \((n = 30)\). This stimulating frequency was chosen to produce a tonic synaptic pressure on cortical neurons, by simulating the effect of an activated network or the discharge pattern crowning PDSs. The trains of stimuli at 100 Hz were applied to the cortex or the related thalamic nuclei, and lasted for 0.5–1 s. In both cases, the frequency of fast runs decreased during tetanic stimulation (Fig. 9). The neuronal membrane depolarized by 4–7 mV (average 5.2 mV, \(n = 30\)) after cortical stimulation, and by 2–5 mV (average 3.4 mV, \(n = 30\)) after thalamic stimulation. In Fig. 9, cortical stimulation at 100 Hz produced a slowing of spontaneous fast runs from an average of 13.4 to 8 Hz, which was also associated with an increase in entropy from 0.13 to 0.52 (Fig. 9A). The effect of thalamic stimulation, although basically similar, was weaker (Fig. 9B): the average frequency dropped from 15 to 11 Hz, whereas the entropy increased from 0.21 to 0.62. For the 30 recorded neurons, the average frequency decrease was 22%, accompanied by an entropy increase of 195%.

Thus we assume that tonic pressure exerted on cortical neurons during fast runs activity has the virtue of slowing and disorganizing the oscillation.

**Seizures with PSW complexes**

According to one of our previous studies (Steriade et al. 1998), 70% of the seizures contained both rhythmic SW complexes (1.5–3 Hz) and fast runs (10–15 Hz). Until now, we investigated the interaction between paroxysmal activities in corticothalamic networks and artificially created oscillatory foci. Often, a PDS is accompanied by short sequences of fast runs, the whole generating at the EEG level the PSW pattern (Fig. 10; see also Fig. 8 in the companion paper). Therefore we tested the effect of rhythmic stimulation on such seizures by focusing on PSWs.

An example of a spontaneous seizure is shown in Fig. 10A (**top panel**). Considering only the time intervals between consecutive PDSs, and calculating the respective instantaneous frequencies, the spontaneous evolution of the seizure was associated with a progressive acceleration from 1.5 to 3 Hz. Such acceleration was present in most of the spontaneous seizures (88% of the 192 observed seizures). We also calculated the instantaneous frequencies of the polyspikes components of each PDS (**bottom graph** in Fig. 10A). The values are, with the exception of the two periods with continuous fast runs, randomly distributed between 10 and 25 Hz. During the two short episodes of fast runs, instantaneous frequencies polarize around 12 Hz.
Cortical stimulation within the frequency range of spontaneous PSW activity (1–3 Hz) produced the same effect as in seizures with poor or no PSW components (data not shown). An interesting outcome resulted, however, from continuous stimulation at the frequency of the fast runs (10 Hz in Fig. 10B). The parameters of the neuronal PDSs (duration of the depolarization, duration of the hyperpolarization) adjusted such that the sustained seizure assumed a rhythmic evolution. PDSs recurred at the beginning at 2.5 Hz (every 4 stimuli) and continued at 3.3 Hz (every 3 shocks). Later on, spontaneous activity interfered with the stimulation, and the coupling was lost. In spite of this, the instant frequency values remained within a constant range (2.5–4 Hz), and no accelerating trend was detected. Polyspikes were also affected by the 10-Hz stimulation. The distribution of their instant frequencies distributed, at least during the middle part of the seizure, around two modes: 14 and 27 Hz.

This increased oscillatory organization was also confirmed by a drop in the entropy averaged over 25 such seizures: PDS oscillations went from 0.48 to 0.3 (37.5%), whereas polyspikes decreased from 0.53 to 0.48 (13.2%). The individual values were calculated over equivalent periods of time of spontaneous and stimulated seizures, and include, in the latter case, also those periods where the spontaneous activity was interfering with the stimulation. Thus the above-calculated figures are conservative.

**DISCUSSION**

We have shown that 1) periodic trains of stimuli mimicking natural sleep activities induce SW seizures, 2) the seizure-
FIG. 8. Thalamic stimulation (10 Hz) disrupts cortical fast runs. A: intracellular recording in area 7 during stimulation of the thalamic centrolateral (CL) nucleus for the period underlined by the dotted line, and instantaneous frequency graph. The stimulation frequency was adjusted to the frequency of the spontaneous fast runs (see dotted line). Note stable 10-Hz discharge rate before the onset of the stimulation and its disruption by thalamic shocks. B: details from A, displaying spontaneous fast runs (1), irregular responses to the thalamic stimulation due to interaction with spontaneous activity (2), period with good following, in which the fast runs appear as rebound responses to the thalamic stimulation (3), and effect of thalamic stimulation during polyspike-wave (PSW) components (4). C: averaged responses (n = 15) showing isolated EPSPs (bottom trace) and EPSPs followed by rebound fast runs.
prone cortical networks optimally follow oscillatory activities at 1.5 Hz, 3) the cortex favors the development of fast runs while the thalamus disrupts them, and 4) during fast runs, tetanic stimulation plays a protective role by decreasing the discharge frequency and the synchronization.

Sleep oscillations and SW seizures

All-night sleep recordings in humans have demonstrated an increase of paroxysmal discharges during light non–rapid eye movement sleep. This sleep stage is marked by the appearance of K-complexes (initial sharp component, followed by a slow component) (Roth et al. 1956) and sleep spindles. The role of K-complexes in conjunction with paroxysmal potentials was emphasized (Niedermeyer 1998a). Recently, the cellular mechanisms of the K-complex were described (Amzica and Steriade 1998a), and it has been shown that K-complexes are the electrographic expression of the slow oscillation in both humans (Amzica and Steriade 1997) and cats (Amzica and Steriade 1998a). Previous studies have shown that the cortically generated slow oscillation is relayed to the thalamus, where it triggers sequences of spindles (Contreras and Steriade 1995; Steriade et al. 1993c). The spreading and synchronization of the slow oscillation (Amzica and Steriade 1995b), as well as of epileptic discharges (Neckelmann et al. 1998), is supported by intracortical reciprocal connections (Avendaño et al. 1988). Although SW seizures of the type described in this study originate in the cortex (Maquet et al. 1995; Marcus and Watson 1998a).
FIG. 10. Spontaneous PSW seizure and its modulation by 10-Hz stimulation. A: spontaneous PSW seizure and evolution of instantaneous frequencies. Instantaneous frequencies of PDSs are measured between consecutive onsets of PDSs. The line in this graph reflects the linear fit of the values and shows an accelerating trend with the evolution of the seizure. Instantaneous frequencies for polyspikes are calculated only for depolarizing events (fast runs) overriding a PDS. B: continuous stimulation at 10 Hz in the same neuron induces a similar pattern of PSW seizure. Instantaneous frequencies are more stable and ordered both for PDSs and for polyspikes.
aptic refractoriness of cortical neurons is demonstrated by their
ported for hippocampal (Hablitz 1984) and neocortical PDSs
Marsan 1964a,b). A relative refractoriness was already re-
observation has particular implications for the results presented
occurrence of a previous PDS (evoked or spontaneous). This
action, we propose that a hyperexcitable cortex receiving peri-
are not reflex epilepsy in the classical sense, as is the case of
mediated seizures (Amzica and Steriade 1997, 1998a,b; Steriade et
complexes are present during wakefulness. Second, sleep SW
occasional stimuli may trigger SW seizures. In other terms, similarly to
seizures induced by recurrent sensory stimuli, oscillatory ac-
tively generated in the sleeping corticothalamic
network may play a similar role in triggering SW seizures. The
excitability of the cortex may be due to a series of non-
exclusive factors such as chemical imbalance, impaired inhi-
bition, hypersynchronized oscillations, and kindling. Increased
extracellular potassium concentrations are known to contribute
to epileptogenesis (Moody et al. 1974). Indeed, in baboons, seizure
onset was preceded by an increase in extracellular potassium concentration from 3.1 to 9.8 mM (Pumain et al.
Coupling of cortical neurons during epileptiform oscillations
The response of a hyperexcitable cortex to cortical and
thalamic stimuli was analyzed in the companion paper (Ste-
and 10).
Together with the graded size of the evoked PDS as a
function of the time interval from the anterior one (Steriade and
Cortical rhythms are critical for driving the network, and this
may be particularly important during sleep, when the network is
more synchronized and less responsive to external stimuli. The
ability of cortical neurons to resonate at certain frequen-
cies with extrinsic stimuli was quantified by means of the
entropy. It resulted that the optimum coupling occurred at
frequencies around 1.5 Hz, which corresponded to the highest
order in the system (Figs. 4–6). The entropy values given in
this study are relative and pertain to the conditions declared in
METHODS. They proved useful for comparing spontaneous and
triggered seizures (Fig. 10). In all cases the latter were asso-
ciated with lower entropy values (higher order). The drop in
entropy depended on the stimulation frequency, but could
reach up to 68%. Electrical stimuli to the cortex or thalamus
constitute hypersynchronous drives to the network and mimic
an epileptic focus because the responses are virtually identical
to the spontaneous PDSs (Fig. 3). Two nonexclusive reasons
could explain the fact that spontaneous seizures have higher
entropy values (less ordered behavior): 1) they develop from
multiple competing foci, or 2) they originate in a single focus
but encounter resistance from various pools of neurons.
In the present experiments, spontaneous activities interacted
with, and disrupted the, imposed inputs. In the overwhelming
majority of cases, it was an acceleration of the rhythm, eventu-
ally inducing the loss of coupling (Figs. 3 and 4). This
suggests that other pools of neurons than the ones undergoing
the stimulation became active and attempted to drive the neu-on at the time of the stimulation. Thus it appears more prob-
able that the relative increased entropy of spontaneous seizures
is due to distributed epileptogenic foci.
The origin of fast runs (10–20 Hz) is not known (see,
however, Traub and Jefferys 1994). They survive in cortical
slabs (Timofeev et al. 1998) thus being independent on tha-
lamic activities. The present data further suggest that the thal-
amus may play a rather disturbing role for this seizure com-
ponent (Fig. 8), whereas the cortex tends to organize this
oscillation (Fig. 7). Spontaneous seizures with fast runs may
create a rhythmic cortical drive on thalamocortical neurons,
which, in turn would convey the signal back to the cortex. Our data show that, when an oscillatory behavior is imposed in the thalamus by diffuse and intense stimulation at the natural frequency of the fast runs, the cortical fast runs are disturbed. This further suggests that the thalamus does not play a supportive role in the genesis of this oscillation. Although intrinsic properties, such as a persistent sodium current (Llinás 1988), may play a role in the genesis of fast runs, it is clear that cortical synaptic linkages are essential for its propagation and synchronization.

Tonic barrages of high-frequency stimuli at 100 Hz, applied either to the thalamus or to the cortex, slow down fast runs (Fig. 9). They may mimic activating inputs as well as spike bursts overriding PDs. Arousing stimuli may stop or diminish the incidence of absence seizures (Kostopoulos et al. 1987). As to their effect on fast runs, they mainly produced a steady depolarization associated with a deceleration of the discharge rate. The latter was not the mere consequence of the stimulation on intrinsic properties because it was reflected also at the population level (see EEG in Fig. 9), where synchronizing mechanisms are essential. Without leading to conclusive results for the influence of arousal on seizures, the tetanic stimulation proved that fast runs are mainly generated through synaptic coupling and that persistent stimulation reduced the order in the network, thus contributing to the arrest of the oscillation.

Concluding remarks

Stimulating at fixed frequencies increases the order (decreases the entropy) in the system. It results that there is no restricted network able to impose a given oscillatory behavior of a given frequency. Oscillatory phenomena of the types investigated in this paper are rather the outcome of distributed networks and are assisted by the intrinsic properties of individual cells, which modulate the reactivity of the whole network by allowing him to follow or to favor certain frequencies and filtering other ones. Spontaneous sleep oscillations constitute in the seizure-prone brain a precipitating factor.

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REFERENCES
