Spike-Wave Complexes and Fast Components of Cortically Generated Seizures. IV. Paroxysmal Fast Runs in Cortical and Thalamic Neurons

IGOR TIMOFEEV, FRANÇOIS GRENIER, AND MIRCEA STERIADE

Laboratoire de Neurophysiologie, Faculté de Médecine, Université Laval, Quebec, Quebec G1K 7P4, Canada

Timofeev, Igor, François Grenier, and Mircea Steriade. Spike-wave complexes and fast components of cortically generated seizures. J. Neurophysiol. 80: 1495–1513, 1998. In the preceding papers of this series, we have analyzed the cellular patterns and synchronization of neocortical seizures occurring spontaneously or induced by electrical stimulation or cortical infusion of bicuculline under a variety of experimental conditions, including natural states of vigilance in behaving animals and acute preparations under different anesthetics. The seizures consisted of two distinct components: spike-wave (SW) or polyspike-wave (PSW) at 2–3 Hz and fast runs at 10–15 Hz. Because the thalamus is an input source and target of cortical neurons, we investigated here the seizure behavior of thalamic reticular (RE) and thalamocortical (TC) neurons, two major cellular classes that have often been implicated in the generation of paroxysmal episodes. We performed single and dual simultaneous intracellular recordings, in conjunction with multisite field potential and extracellular unit recordings, from neocortical areas and RE and/or dorsal thalamic nuclei under ketamine-xylazine and barbiturate anesthesia. Both components of seizures were analyzed, but emphasis was placed on the fast runs because of their recent investigation at the cellular level. 1) The fast runs occurred at slightly different frequencies and, therefore, were asynchronous in various cortical neuronal pools. Consequently, dorsal thalamic nuclei, although receiving convergent inputs from different neocortical areas involved in seizure, did not express strongly synchronized fast runs. 2) Both RE and TC cells were hyperpolarized during seizure episodes with SW/PSW complexes and relatively depolarized during the fast runs. As known, hyperpolarization of thalamic neurons deactivates a low-threshold conductance that generates high-frequency spike bursts. Accordingly, RE neurons discharged prolonged high-frequency spike bursts in close time relation with the spiky component of cortical SW/PSW complexes, whereas they fired single action potentials, spike doublets, or triplets during the fast runs. In TC cells, the cortical fast runs were reflected as excitatory postsynaptic potentials appearing after short latencies that were compatible with monosynaptic activation through corticothalamic pathways. 3) The above data suggested the cortical origin of these seizures. To further test this hypothesis, we performed experiments on completely isolated cortical slabs from suprasylvian areas 5 or 7 and demonstrated that electrical stimulation within the slab induces seizures with fast components and SW/PSW complexes, virtually identical to those elicited in intact-brain animals. The conclusion of all papers in this series is that complex seizure patterns, resembling those described at the electroencephalogram level in different forms of clinical seizures with SW/PSW complexes and, particularly, in the Lennox-Gastaut syndrome of humans, are generated in neocortex. Thalamic neurons reflect cortical events as a function of membrane potential in RE/TC cells and degree of synchronization in cortical neuronal networks.

INTRODUCTION

The preceding papers in this series have demonstrated the cortical origin of seizures including spike-wave (SW) or polyspike-wave (PSW) complexes at 2–4 Hz and fast runs at 10–15 Hz. In those studies, we have shown the emergence, without discontinuity, of such seizures from the cortically generated slow sleep oscillation, the similar relations between field potentials and intracellular activities during the slow oscillation and seizures (Steriade et al. 1998a), and the intracortical synchronization processes of seizures (Neckelmann et al. 1998). Although these data, and particularly the presence of the bicuculline-induced seizures in athalamic animals (Steriade and Contreras 1998), led to the assumption that intrinsic cortical mechanisms may support SW/PSW seizures, the possible participation of the thalamus should be investigated. Indeed, the cortical origin of the slow oscillation was demonstrated by studies in thalamectomized and decorticated animals (Steriade et al. 1993c; Timofeev and Steriade 1996), but the rebound spike-bursts of thalamocortical (TC) neurons that follow their prolonged periods of hyperpolarizations during the slow oscillation (Contreras and Steriade 1995) may play an additional, ancillary role in synchronizing this sleep oscillation by assisting cortical networks in triggering the depolarizing phase of the oscillation. Mutatis mutandis, this might also be the case in seizures, at least for almost one-half of TC neurons, because ~60% of them remain steadily hyperpolarized and inactive during cortical SW seizures (Steriade and Contreras 1995). Nonetheless, the remaining TC cells may display rebound spike bursts at appropriate levels of membrane potential ($V_m$) and, thus, play a role in coherent corticothalamic SW seizure activity.

As to the other component of cortical seizures, the fast runs, they are usually associated with tonic seizures (Niedermeyer 1993). Early investigations of penicillin-induced seizures indicated that neocortical neurons display rhythmic oscillations at depolarized $V_m$ levels, but there was no direct evidence that their frequency depends on a rhythmic synaptic bombardment (Matsumoto and Ajmone-Marsan 1964). Subsequent studies of SW seizures induced by parenteral administration of penicillin reported depolarization of cell somata to the point of spike inactivation, and ascribed these phenomena to PSPs from network activity (Fisher and Prince 1977). In fact, the high-frequency bursts fired by fast-rhythmic-bursting (FRB) cortical neurons (Steriade et al. 1998b) during the fast runs of spontaneous or electrically elicited seizures (Steriade et al. 1998a) are likely due to both the intrinsic properties of FRB neurons and synaptic drives from related cortical and thalamic neurons. The possible role of thalamic reticular (RE) and TC neurons in the genesis and synchronization of paroxysmal fast runs has not yet been explored.
In this paper, we investigate the intracellular patterns of thalamic neurons during spontaneously occurring and electrically induced seizures. Emphasis is placed on the different $V_m$ levels that are associated with SW/PSW complexes and fast runs as well as the corticothalamic synaptic drives during the two major components of seizures. Because data indicate the cortical origin of seizures, we also attempted to demonstrate the presence of these seizures in isolated cortical slabs.

**METHODS**

**Preparation**

Experiments were conducted on 37 cats anesthetized with ketamine-xylazine (10–15 and 2–3 mg/kg im) or pentobarbital sodium (35 mg/kg ip). We monitored the stable state of deep anesthesia by continuously recording the electroencephalogram (EEG). We monitored the heart rate and the control of end-tidal CO$_2$ during artificial ventilation after administration of gallamine triethiodide, compensated fluid loss during experiments, and ensured stability of intracellular recordings by using the same procedures as described for acutely prepared animals in a companion paper (Steriade et al. 1998a).

In a further 22 experiments, neuronally isolated slabs were prepared from cat suprasylvian areas 5 or 7. After opening the bone, a small perforation was made in the dura above a part of the pia that did not contain large blood vessels. A homemade crescent knife was inserted along its curve into the cortex until the tip of the knife appeared under the pia, 10 mm frontally. Then the knife was turned by 90° in both right and left directions. The pia remained intact, with the exception of the place where the knife was inserted. Bleeding was not observed. The slab was ~10 mm long, ~6 mm wide, and 4–5 mm deep. The completeness of neuronal transection was verified on 80-μm thionine-stained sections (see Fig. 12).

**Recordings and stimulation**

Field potential recordings and stimulation were obtained through bipolar coaxial macroelectrodes inserted in cortical precuneate area 4 and suprasylvian areas 5, 7, and 21. The ring of the macroelectrodes was placed at the cortical surface or 0.1 mm deeper and the tip at 0.8–1 mm in the cortical depth. For thalamic recording and stimulation, we used an oblique approach to insert electrodes within the ventral lateral (VL), intralaminar central lateral (CL), and lateral posterior (LP) nuclei. Field potentials and extracellular unit discharges were also obtained by using tungsten microelectrodes (resistance 4–8 MΩ) inserted into different thalamic and neocortical regions. In all monopolar recordings, the indifferent electrode was placed on neck muscles. For extra- and intracellular recordings from thalamic VL and RE nuclei, the surface of cortex that corresponds to the anterior half of the marginal and suprasylvian gyri was cauterized with silver nitrate. The cortex and white matter were removed by suction until the head of the caudate nucleus was exposed. Micropipettes were then lowered through the head of the caudate nucleus to reach the rostral pole and rostromedial sector of the RE nucleus and the VL nucleus. Other micropipettes were inserted in the precuneate area 4, in the vicinity of at least one EEG electrode.

Intracellular recordings were performed with glass micropipettes filled with a solution of 2.5–3 M potassium acetate (DC resistance 30–80 MΩ). A high-impedance amplifier with active bridge circuitry was used to record from, and inject current into, neurons. The signals were recorded on an eight-channel tape with band-pass of 0–9 kHz and digitized at 10–20 kHz for off-line computer analysis.

Seizures occurred spontaneously or were elicited by repetitive stimulation of various thalamic nuclei (10 Hz) or cortical areas (10 and 100 Hz). Stimuli were delivered with durations from 0.05 to 0.2 ms and intensities from 0.05 to 0.3 mA.

At the end of experiments, the animals were given a lethal dose of pentobarbital and perfused for histological confirmation of location of electrodes and completeness of slab isolation.

**RESULTS**

First, we present data on the fast runs of cortically generated seizures, showing that these components occur with slightly different frequencies in different cortical foci and that out-of-phase fast paroxysmal oscillations arising in various neuronal pools may eventually coalesce. This aspect is relevant for the synchronization of fast runs within corticothalamic networks. Next, we show differential types of activity in RE and TC cells during the fast runs and SW/PSW complexes of cortical seizures, related to different membrane potential ($V_m$) of thalamic neurons, and present evidence about relations between cortical and thalamic oscillatory patterns. Last, we demonstrate the cortical origin of fast runs by showing their presence, in conjunction with that of SW/PSW complexes, in isolated cortical slabs.

**Database and neuronal identification**

Intracellular recordings, with stable $V_m$ more negative than −55 mV and overshooting action potentials, were obtained from 224 cortical neurons, 95 RE neurons, and 188 TC neurons.

1) Out of 150 cortical neurons in which depolarizing current pulses were injected intracellularly, 62% were regular-spiking (RS) cells, 14% intrinsically bursting (IB) cells, and 22% were FRB cells. We did not analyze fast-spiking (FS) neurons that are supposed to represent local-circuit GABAergic neurons (reviewed in Gutnick and Mody 1995) because of their small number in our present sample (2%), the heterogeneous characteristics of these neurons (Kawaguchi 1993; Thomson et al. 1996), and because two major features of the recently described FRB neurons have conventionally been attributed to only FS neurons. Indeed, FRB neurons fire brief spikes (0.25–0.4 ms at half-amplitude), and, if the strength of the depolarizing pulse slightly exceeds the level where it produces high-frequency (300–600 Hz) spike bursts recurring rhythmically at 20–40 Hz, they display very fast tonic firing without frequency adaptation (Steriade et al. 1998b). Despite the fact that the action potential of FRB neurons is very brief and might thus suggest their local inhibitory action, FRB neurons from layers V–VI, with morphologically identified pyramidal shape, have been antidromically activated from different thalamic nuclei (Steriade 1997; Steriade et al. 1998b) and are thus excitatory in nature.

2) RE neurons, recorded from the rostral pole and periventricular rostromedial sector of the RE nucleus, were recognized from their typical high-frequency bursts with long duration and accelerating-decelerating patterns (Domich et al. 1986; Steriade et al. 1986). These patterns, observed with both extra- and intracellular recordings (see Figs. 5–7) are different from the short spike bursts of TC cells that display a progressive lengthening in interspike intervals.

3) TC cells from the VL nucleus were identified by antidromic activation from area 4 stimulation (Fig. 10), a hyper-
polarizing envelope of spindle sequences (Figs. 10 and 11), and hyperpolarization-activated clocklike delta oscillation (Fig. 9).

Cortical fast runs display various frequencies in different foci

As reported in a companion paper (Steriade et al. 1998a), 70% of cortically generated seizures consisted of both SW/PSW complexes at 2–3 Hz and fast components at 10–15 Hz. Most (80%) of cortical fast components had frequencies similar to those of polyspikes, which build up PSW complexes (see also Fig. 6 in the present paper). In the remaining 20%, cortical fast components started directly from sleep patterns (Fig. 4). In virtually all (95%) seizures induced by electrical stimulation of cortex, self-sustained fast paroxysmal oscillations immediately followed the pulse train while SW/PSW complexes occurred later (Figs. 3, 9, and 10).

The fast components of seizures had slightly different frequencies in various cortical foci, and the same neuron exhibited frequency variations as well as differences in time relations with field potentials during various fast oscillatory epochs (n = 44). A typical example of these variations among different epochs of the same spontaneously occurring seizure, consisting of both SW complexes and fast runs, is illustrated in Fig. 1, showing simultaneous intracellular and field potential recordings from different cortical sites. The area 7 neuron, electrophysiologically identified as an FRB neuron by depolarizing current pulses (see Fig. 7 in the companion paper by Steriade et al. 1998a), was intracellularly recorded together with field potentials from the depth of adjacent suprasylvian areas 5, 7, and 21 as well as the electrothalamogram (ETHG) from the related LP nucleus. During epoch A, the area 7 neuron discharged high-frequency spike bursts at ~14 Hz that were not fired, as expected, during the peak of depth-negative field potentials recorded from the same area, but during the descending positive phase. Distinctly, during epoch B, the same neuron discharged lower-frequency (~11 Hz) spike bursts that were closely related to the depth-negative cortical field potential, thus indicating synchrony between the single neuron and the neuronal pool giving rise to the field potential. However, during the same epoch B, with fast runs at ~11 Hz, wave-triggered averages (WTA) showed that field potentials from area 21 were not in phase with those from areas 5 and 7. The time lags between areas 7 and 21 was ~40 ms, and between areas 5 and 21 was ~50 ms, which may explain the absence of oscillation in the LP nucleus.

The frequency differences in fast runs during epochs A and B are likely due to slight differences in $V_n$ that was relatively depolarized (~60 mV) in A, with faster frequency, as compared with B (~67 mV), with lower frequency. These complex patterns, that were constant findings in our multisite recordings, indicate, on one hand, changes in frequencies of fast runs during different epochs of the same seizure, and, on the other, absence of perfect synchrony between cortical neuronal pools during different epochs of cortical seizures with fast runs. This conclusion is important for the thalamic reflection of cortical fast components. Indeed, as neurons in different neocortical areas (5, 7, and 21), all impinging on the LP nucleus, do not simultaneously fire during the fast runs, the corticothalamic inputs will not produce synchronous fast oscillations in the thalamus, but will rather induce a steady depolarization on different target LP neurons, with the consequence that local LP field potentials will not exhibit clear-cut fast oscillations (see section entitled, Membrane polarization of thalamocortical neurons during fast runs and SW complexes).

In line with the above findings, macroelectrodes recorded fast runs arising from different pools of neurons oscillating at a similar frequency, but not synchronously. Such fast oscillations (~9–10 Hz) were not in phase in some epochs (see depth-EEG in Fig. 2A) but could coalesce after a few seconds, thus resulting in fast EEG runs with higher amplitudes (Fig. 2B; amplitude of field potentials in A is magnified by 5). Correlatively, the simultaneously recorded EEG waves had low amplitudes of fast runs during the epochs in which the oscillations arising from different neuronal pools in the cortical depth were not in phase, but the amplitudes of EEG waves increased fivefold during the seizure epochs when the previously quasi-independent fast oscillators coalesced their rhythms. Spike-triggered averages (STA), using the action potentials of the intracellularly recorded neuron, demonstrated that the area 4 neuron discharged in close time relation with one, but not the other, neuronal pool before their coalescence (see bottom left panel).

Trains of fast stimuli (100 Hz) applied to cortical areas induced seizures that constantly (95%) started with fast runs. We used this type of faradic stimulation because cortical seizures often contain ripples at 100–120 Hz, both intracellu-larly and at the EEG level (see Figs. 8 and 14 in Steriade et al. 1998a), and such fast oscillations may have a strong impact on postsynaptic neurons. Intracellularly recorded neurons, located closely to the stimulation site, were strongly depolarized and exhibited self-sustained fast oscillations (10–20 Hz) that eventually diminished their frequencies in association with the declining plateau of depolarization. The dual intracellular recordings from neurons separated by only 0.2 mm within the posterior part of area 5 (Fig. 3) shows the initial seizure depolarization and the fast oscillations produced in both neurons by electrical stimulation of a more rostral site in area 5. Cell 2 was more depolarized at the onset of the seizure, immediately after the pulse train; accordingly, this cell took a leading role in the seizure that started with high-frequency tonic discharges. The STA, with cell 1 at time 0, demonstrates that the progressive repolarization in cell 2 was accompanied by the slowing frequency of fast runs. Also, the action potentials of cell 2 preceded those of cell 1 at the onset of the electrically induced seizure, but the reverse was observed with the declining depolarization of cell 2 (bottom right panel). The focal nature of the seizure resulted in a slight reflection of fast runs in the EEG lead from the adjacent area 7 and complete absence of such activity in the more posterior area 21.

Thalamic reticular neurons during cortical seizures with SW complexes and fast runs

Two main types of activities were observed in intracellularly recorded RE neurons (n = 95) during spontaneous or electrically induced seizures. In the first type, RE cells were
FIG. 1. Fast components of spontaneously occurring cortical seizures do not occur simultaneously in all recorded neocortical areas. Ketamine-xylazine anesthesia. Intracellular recording of fast-rhythmic-bursting (FRB) neuron from area 7 (see text for definition of FRB neurons) together with field potential recordings from the depth of cortical areas 5, 7, and 21, and from thalamic LP nucleus (see top right diagram). Two epochs with fast runs indicated A and B are expanded below (the 2 traces represent intracellular and field potential recordings from area 7). Wave-triggered averages (WTA) from the same epochs are depicted below (arrows) with all 5 recording sites (peaks of depth-negative field potentials from area 7 are taken as reference time). See text.
FIG. 2. Coalescence of 2 fast cortical oscillators into a single one with progression of seizure. Ketamine-xylazine anesthesia. Intracellular recording from area 4 together with surface- and depth-electroencephalogram (EEG) from the same area. The area 4 neuron fired high-frequency spike bursts at the onset of spontaneously occurring seizures and single action potentials during fast runs. The onset of seizure is expanded below the top panel. Further below, 2 panels (A and B), as indicated below the intracellular trace in the top panel, illustrate spike-triggered averages (STA, n = 20) showing that the intracellularly recorded neuron participated in 1 of the 2 oscillating pattern. Note that, at cortical depth, field potentials display 2 oscillations with a frequency of 9–10 Hz (A) that, later on, coalesce into a single oscillatory pattern (B). The amplitude of field potentials in A is amplified by 5.

progressively involved in seizures through increasing hyperpolarization with a maximum reached in the middle part or the end of the seizure, thus increasing the number of spikes in the rebound spike bursts. This pattern occurred when the spontaneous seizures started with fast runs or when seizures were triggered by pulse trains (see Fig. 5). As to the second pattern, RE neurons were relatively hyperpolarized during the SW/PSW complexes and recovered their $V_m$ close to the rest value during the fast runs (see below, Fig. 7). These aspects were also suggested by the discharge patterns of
FIG. 3. Changing time relations between 2 simultaneously recorded cortical neurons during fast seizure. Barbiturate anesthesia. Intracellular recordings from 2 neurons (0.2 mm apart) in the posterior part of area 5, together with depth-EEG from areas 7 and 21 (see brain figure). Stimulation (100 Hz, 1 s) was applied in front of cell 1. The elicited seizure consisted of fast runs at 10–20 Hz. Bottom left: each trace in STA of cell 2 was obtained by 10 successive action potentials from cell 1 (top to bottom). Note progressive repolarization of cell 2 and slowing down of fast runs’ frequency (from 20 to 10 Hz). Plot at bottom right (same epoch as in the STA) shows that excitation in cell 2 precedes excitation in cell 1 at the beginning of seizure and that, with progressive hyperpolarization of cell 2, time relations are reversed. Filled circles represent action potentials, and open circles excitatory postsynaptic potentials (EPSPs), in cell 2.
extracellularly recorded RE cells ($n = 33$) in conjunction with cortical field potentials or intracellular recordings of cortical neurons (see Figs. 4 and 6).

Figure 4 shows the progressive depolarization of a cortical neuron involved in a spontaneously occurring seizure that was initiated with fast runs at 8–9 Hz, eventually reaching the pattern of PSW complexes at 2 Hz. Simultaneously, the extracellularly recorded RE neuron discharged single action potentials or spike doublets during the fast runs (panel 2) and high-frequency spike bursts during the polyspike components of PSW complexes (panel 4). The increased number of action potentials, from single spikes or spike doublets to high-frequency spike bursts, probably occurred as a result of RE-cell hyperpolarization. Such an evolution of $V_m$ was indeed observed with intracellular recordings and triggering seizures with pulse trains applied to the dorsal thalamus (Fig. 5). As in virtually all other seizures induced by electrical stimulation, the self-sustained activity was initiated by fast (8–9 Hz) runs but, as the seizure evolved, a progressive hyperpolarization appeared and it was associated with PSW complexes at 2 Hz during the final stage of the seizure. The progressive hyperpolarization was clearly associated with an increasing number of spikes per burst. Averaged activities triggered by the depth-negative cortical waves during both the fast runs and PSW complexes showed the synchronous cortico-RE discharges in both components of the seizure.

With simultaneous recordings of cortical field potentials from area 4 and extracellular unit discharges from the rostromedial RE nucleus, WTA analyses (Fig. 6) demonstrated that both the spike bursts of RE neurons during PSW complexes (2.5–3 Hz) and single spikes or spike doublets during the fast runs (10–12 Hz) were time locked with the cortical field potentials. Because we know from intracellular studies of cat RE neurons that their high-frequency spike bursts occur at a hyperpolarized membrane potential, by contrast with their tonic single-spike firing that appears at a more depolarized level (Contreras et al. 1992; Mulle et al. 1986), the above extracellular data suggest two distinct levels of membrane potentials in RE neurons; this assumption was confirmed in intracellular recordings.

During spontaneously occurring seizures ($n = 33$), RE neurons underwent repeated transitions from hyperpolarized to relatively depolarized levels that corresponded to SW/PSW components and fast runs, respectively. In Fig. 7, the difference between the trough of hyperpolarizations in epochs with SW/PSW complexes and in epochs with fast runs was 8 mV (−85 and −77 mV, respectively). RE neurons discharged high-frequency, prolonged spike bursts that were associated with PSW complexes at 1.5–2 Hz (left part in Fig. 7) or SW complexes at 3 Hz (right part in Fig. 7). The fast runs were associated with single action potentials or, more often, with spike doublets or triplets followed by an afterdepolarization hump on which presumed dendritic spikes were superimposed, as seen in the expanded recordings at the bottom of Fig. 7 (see evidence for the dendritic origin of these events in Contreras et al. 1993; and Destexhe et al. 1996).

**Membrane polarization of thalamocortical neurons during fast runs and SW complexes**

As in RE neurons, the $V_m$ of TC neurons was hyperpolarized during SW/PSW complexes, compared with epochs with fast runs when the $V_m$ was close to rest values (−55 to −64 mV). In the spontaneous seizure shown in Fig. 8, the $V_m$ was on average 11 mV more negative during SW complexes at ~3–4 Hz than during fast runs at ~10 Hz. This was calculated from the trough of rhythmic inhibitory postsynaptic potentials (IPSPs) that immediately followed the excitatory postsynaptic potentials (EPSPs) that were time locked (~10-ms delay) with the spiky depth-negative component of cortical SW complexes (seen averaged activity in bottom left panel of Fig. 8). The IPSPs were exceptionally followed by rebound spike bursts: of ~80 IPSPs during the first 2 epochs with SW complexes in Fig. 8, only 1 low-threshold spike (LTS) crowned by a fast action potential was observed (see discussion). During the fast runs, the neuron was relatively depolarized, likely because it received less inputs from GABAergic RE neurons. Although field potentials from area 4 and VL nucleus oscillated in phase, the neuron displayed the same frequency of fast runs but out-of-phase (bottom right panel in Fig. 8), as if it received inputs from a different cortical source.

The seizures triggered by electrical stimulation displayed similar relations between the $V_m$ of TC neurons and the two major components of cortical seizures. The transition between the epoch with fast runs, that opened the seizures, and the epoch with SW/PSW complexes, that ended the seizures, was associated with a progressive hyperpolarization (Fig. 9). The analysis of $V_m$ distribution in six successive epochs dominated by fast runs indicated that, from initial $V_m$s with peaks between −65 and −70 mV (1 in bottom panel), the neuron had $V_m$s mainly between −70 mV and −75 mV (panel 4) to finally reach $V_m$s around −75 to −80 mV during the final epoch of fast runs, immediately preceding the PSW complexes (panel 6).

That fast runs in TC cells were mainly EPSPs of cortical origin was shown by analyzing these events at different levels of $V_m$ ($n = 9$). Again, seizures with initial periods of fast runs were triggered by faradic stimulation of cortical area 4 (Fig. 10). At slightly depolarized levels, the TC neuron from the VL nucleus, as identified by antidromic responses to stimulation of cortical area 4, displayed single spikes or, occasionally, spike doublets in close time relation to the depth-negative fast cortical runs. On hyperpolarization, the VL neuron oscillated at the same frequency (~12 Hz) as that of cortical field potentials, and its depolarizing events increased in amplitude (see Fig. 10, Wave-triggered-average). The reversal potential of these depolarizations at about −15 mV indicated that they mainly consisted of EPSPs (see bottom right panel), whereas their relation to the cortical fast runs indicated their corticothalamic origin. The peak of averaged thalamic EPSPs occurred with a delay of ~5 ms compared with the depth-negative peak of cortical field potentials. Since cortically elicited EPSPs of TC cells have a relatively long latency because of the distal dendritic contacts made by corticothalamic axons (see Steriade et al. 1997), this latency is compatible with monosynaptic activation.

To further demonstrate that TC neurons reflect the cortical seizures, we compared the temporal relations between cortical and thalamic events during thalamically and presumably cortically generated oscillations, respectively spindles and seizures ($n = 6$). To produce reliable spindle sequences, we
FIG. 4. Thalamic reticular (RE) neuron is progressively involved in spontaneous cortical seizure. Ketamine-xylazine anesthesia. Intracellular recording of area 4 neuron together with surface- and depth-EEG from area 4 and extracellular unit recording from rostrolateral part of RE nucleus. Seizure lasted for ~45 s. The RE neuron fired synchronously with cortical neuron and EEG only during the 2nd half of seizure when polyspike-wave (PSW) complexes at ~2 Hz prevailed. Periods marked by 1–4 are expanded below.
FIG. 5. RE neuron progressively hyperpolarizes during seizure induced by electrical stimulation of dorsal thalamus. Ketamine-xylazine anesthesia. Intracellular recording of rostral RE neuron together with depth-EEG from area 4. Stimulation consisted of pulse train (10 Hz, 5 s) applied to ventral lateral (VL) nucleus. Seizure started with fast runs at ~8–9 Hz and then shifted to spike-wave (SW) and PSW complexes at ~2 Hz. Parts indicated by horizontal bars below the intracellular traces are expanded below (arrows). Bottom panels: WTA and histograms of spike distribution from the 2 epochs of fast runs (left) and SW/PSW complexes (right). Reference time was the depth-negative field potentials from area 4.
FIG. 6. Two types of firing patterns in extracellularly recorded RE cell suggest different membrane polarization during PSW complexes at ~2.5 Hz and fast runs at ~10 Hz. Ketamine-xylazine anesthesia. Extracellular recording of rostral RE neuron together with focal field potentials picked up by the same microelectrode in the RE nucleus and surface- and depth-EEG from area 4. Spontaneously occurring seizure consisting of PSW complexes and fast runs. Part marked by horizontal bar in the top panel is expanded below. Note high-frequency spike bursts (~300 Hz) during virtually each polyspike of PSW complexes (left: typical accelerando-decelerando burst is expanded at left, arrow) and single action potentials during fast runs (right). This suggests that RE cell is more depolarized during fast runs (see text and following Fig. 7). Below, WTA triggered by the peak of depth-negative EEG waves (dotted line) during both PSW complexes and fast runs.
FIG. 7. Membrane potential of RE neurons is more depolarized during fast runs than during SW complexes. Ketamine-xylazine anesthesia. Intracellular recording of rostral RE neuron together with depth-EEG from area 4. Spontaneous seizure consisted of SW/PSW complexes at 2±3 Hz (left and right part) and fast runs at 10–11 Hz. Parts marked by horizontal lines during fast runs and SW complexes are expanded below (arrows). Two cycles of fast runs are further expanded below to depict presumed dendritic spikes (asterisk; see text).

used barbiturate anesthesia, whereas seizures with fast runs were elicited by single or multiple pulse trains applied to motor cortex. STAs obtained from dual intracellular recordings of cortical area 4 and thalamic VL neurons (Fig. 11) demonstrated that, during spindles, VL cell’s depolarization preceded the action potentials in area 4 neuron by ~30 ms and the group of high-frequency spikes (~300–400 Hz) in the VL neuron indicated that LTSs crowned by spike bursts gave rise to excitation in the cortical neuron. In fact, ~75% of VL-cell depolarization preceded cortical spikes. By contrast, during the seizure, when the leading events are located in cortex, corticothalamic axons produced EPSPs in the VL neuron, and 91.6% of the total VL depolarization followed the action potentials in area 4 cell. The short-lasting IPSP in the VL neuron was mediated by thalamic inhibitory (RE and/or local-circuit) neurons and this inhibition was truncated by a secondary depolarization which mainly resulted from multiple spike firing in area 4 neuron (see bottom
FIG. 8. Thalamocortical (TC) neuron is steadily hyperpolarized during SW complexes, and its membrane potential returns near resting value (dashed line) during fast runs of spontaneously occurring seizure. Ketamine-xylazine anesthesia. Intracellular recording of VL neuron together with focal waves (electrothalamogram) from VL nucleus and depth-EEG from area 4. Seizure lasted for 80 s. An epoch of ~30 s is shown in the top panel. During SW complexes at ~3–4 Hz, the neuron was steadily hyperpolarized by ~8 mV and displayed inhibitory postsynaptic potentials in close time relation with cortical SW complexes, whereas during the fast runs the neuron almost completely recovered its resting membrane potential (~62 mV) and displayed EPSPs. Averages triggered by sharp depth-negative EEG wave in area 4 show temporal relations between cortical and thalamic events during both SW complexes (left) and fast runs (right).
FIG. 9. TC neuron displays a progressive hyperpolarization from fast runs to PSW complexes during seizure elicited by cortical stimulation. Barbiturate anesthesia. Intracellular recording of TC cell from VL nucleus, together with depth-EEG from area 4. Top panel: from left to right: 1) DC current (−1 nA) applied to the cell, which induced a few cycles of clocklike delta waves; after 1.5 s, the depolarizing sag brought the neuron to a stable membrane potential of −70 mV; 2) stimulation of area 4 (pulse train at 100 Hz, 3 s); and 3) self-sustained seizure lasting ~15 s and consisting of fast runs at ~12 Hz followed by SW/PSW complexes at ~2.5–3 Hz. Periods marked by horizontal bars are expanded below (arrows) to depict the hyperpolarization-activated delta oscillation (left) and the fast runs developing into SW complexes (right). Note progressive hyperpolarization during fast runs (dotted line); horizontal line indicates −70 mV. Below, histograms of $V_m$ distribution during 1-s consecutive windows. Histograms 1–6 (corresponding to the epochs indicated above the intracellular trace in the top panel) were constructed by sampling neuronal activity at 10 kHz and counting the number of samples at each membrane potential. This gave the proportion of the time spent by the neuron at each membrane potential ($y$-axis, number of samples; $x$-axis, membrane potential).
FIG. 10. Depolarizing events during fast runs in TC cell are corticothalamic EPSPs. Barbiturate anesthesia. Intracellular recording of slow-conducting TC cell from VL nucleus together with depth-EEG from area 4. Local seizures were elicited by pulse trains applied to area 4 (100 Hz, 1 s). Three left panels: recordings under DC depolarization (+0.3 nA), rest, and DC hyperpolarization (−0.3 nA). Right column: from top to bottom: 1) antidromic invasion (latency 3.6 ms) during the initial part of the cortical pulse train and the subsequent failure of responses during cell hyperpolarization; 2) averaged activities of depolarizing events in VL cell at 3 levels of membrane potential (see left column), triggered by peak of depth-negative EEG waves during fast runs of seizures; and 3) plot representing reversal potential (~15 mV) of depolarizing events in VL cell, thus indicating that they mainly consist of EPSPs.
FIG. 11. Temporal relations between cortical and TC cells during spindles and fast components of cortically evoked seizure. Barbiturate anesthesia. Dual intracellular recordings from area 4 and VL nucleus, together with depth-EEG from area 4. Five brief pulse trains to area 4 (each of them 100 Hz, 0.2 s duration) elicited a cortical seizure lasting 10 s. Periods marked by 2 thick bars (the 1st during a spindle sequence before stimulation, the 2nd during the seizure) are expanded below, in the left column. At right, STA during spindles (above; small deflections in the VL cell are due to capacitive coupling from area 4 neuron) and seizure (below). The 1st action potential of spike train in area 4 neuron was taken as time 0. Note that, during spindles, the main depolarizing events in VL cell preceded cortical discharges whereas, during seizure, the main depolarizing events in VL cell occurred after cortical spikes.
traces in Fig. 11, during seizure). These data suggest that, at the level of TC cells, the cortical excitation during the fast runs may overwhelm the inhibition arising from RE neurons.

Seizures with fast runs and SW complexes are generated in isolated cortical slabs

All the above data from the intact brain congruently suggest the cortical origin of seizures consisting of two main components, SW/PSW complexes and fast runs. To directly demonstrate this idea, we isolated cortical slabs from suprasylvian areas 5 or 7 (n = 22). Figure 12 shows the completeness of transection leading to an isolated slab from area 7 (see METHODS), although pia remained intact, as well as the ability of the isolated cortex to generate a self-sustained seizure after a series of pulse trains applied within the slab. The seizure had features very similar to those of seizures induced by electrical stimulation in the intact brain, namely, it started with fast runs (~12 Hz) and ended with SW/PSW complexes (~2 Hz). The complete isolation of the slab was demonstrated, besides the histological control, by the absence of correlated activity between the electrically induced paroxysmal fast runs in the slab and the normal activity displaying the slow oscillation (~0.7 Hz) outside the slab.

DISCUSSION

Data show that 1) fast runs occur with slightly different frequencies during different episodes of spontaneous seizures, and they appear asynchronously in various cortical foci; 2) both RE and TC neurons are hyperpolarized during the SW/PSW complexes at 2–3 Hz and are relatively depolarized during the fast runs; 3) accordingly, RE neurons discharge prolonged high-frequency spike bursts in close time relation with the spiky component of cortical SW/PSW complexes, and single action potentials or shorter spike bursts during the fast runs; 4) the cortical fast runs are directly reflected as EPSPs in TC cells; and 5) the whole pattern of seizures elicited by electrical stimulation in intact-brain animals, namely fast runs followed by SW/PSW complexes, can be obtained in histologically and physiologically verified, completely isolated cortical slabs.

Different features of sleep and seizure oscillations in corticothalamic networks

Various sleep and paroxysmal rhythms use thalamic and cortical networks in quite similar ways. During natural sleep or under anesthesia, different types of oscillations are generated in either the thalamus or neocortex, but the cortex contributes to the grouping of various rhythmic sleep patterns within complex wave sequences. Spindles are generated within the RE nucleus (Steriade et al. 1987) and through RE–TC interactions (Bal et al. 1995a,b; Steriade et al. 1993b), but corticothalamic volleys have a decisive role in the synchronization of thalamically generated sleep spindles over widespread territories, in both cats and humans (Contreras et al. 1996, 1997). At later stages of resting sleep, spindles are replaced by an intrinsic, clocklike, delta oscillation of TC neurons (Nuñez et al. 1992; Steriade et al. 1991), which is due to the interplay between two inward currents of TC neurons (Leresche et al. 1991; McCormick and Pape 1990; Soltesz et al. 1991) at more hyperpolarized levels than those at which spindles occur. Although this delta oscillation is generated in TC cells even in decorticated animals (Curro Dossi et al. 1992; Timofeev and Steriade 1996), the corticothalamic inputs during the slow oscillation periodically dampens the intrinsic delta rhythm (Steriade et al. 1993a) because of the increased conductance of TC cells. Thus, although some sleep rhythms can be recorded in isolated structures, in the normal brain the cortex and thalamus are reciprocally related within a unified sleep oscillatory machine, and the global brain rhythms (EEG) result from interactions between the building blocks of cortical and thalamic neuronal networks.

Similarly to the normal sleep rhythms, the cortex has a leading role in the paroxysmal oscillations described in the present series of studies. 1) During the fast runs (10–15 Hz), the firing of cortical neurons acts on both RE and TC cells and, thus, shifts their Vm toward depolarized levels. In addition to rapid effects, the glutamatergic actions of corticothalamic neurons may also control, via metabotropic receptors, the excitability of thalamic neurons on a slower time scale (McCormick and von Krosigk 1992; but see Kao and Coulter 1997). The fact that different neuronal pools in neocortex display fast runs with slightly different frequencies and the out-of-phase relations between neurons or groups of neurons (Figs. 1–3) would predict that target thalamic neurons may not synchronously participate in this aspect of cortically generated seizures. This was indeed the case for the LP nucleus that, despite being a projection site of both cortical areas 5 and 7 (Jones 1985), which were together implicated in seizure, did not display paroxysmal activity within the frequency of fast runs at the field potential level (see Fig. 1). 2) During SW/PSW complexes, the majority of TC cells are tonically hyperpolarized and display phasic IPSPs in close time relation with spike bursts in cortical neurons, because of coalescing IPSPs arising in RE neurons driven by corticothalamic volleys at ~3 Hz (Lytton et al. 1997; Steriade and Contreras 1995). At adequately hyperpolarized Vm’s, however, some TC neurons display rebound spike bursts at ~3 Hz during the seizure, which are related to spike bursts in cortical neurons. These data are congruent with the results obtained in slices that preserve corticothalamic connectivity, in which a major property of corticothalamic projections is the selective amplification of responses to stimuli at 3 Hz (Kao and Coulter 1997).

Origin of depolarization during fast runs in cortical networks

Corroborative data on fast runs, and especially their presence in isolated cortical slabs, point to their origin in cortical neurons at depolarized levels. The depolarization may arise from three main sources (intense synaptic excitation, increased extracellular K+, and intrinsic depolarizing currents in cortical neurons) that are discussed below.

1) Fast runs occur at two epochs of cortical seizures: at their onset or in the middle of compound seizures when they are intermingled with SW/PSW complexes at 2–3 Hz. Fast runs generally appear at the onset of seizure when the parox-
FIG. 12. Electrically induced seizure in an isolated cortical slab from area 7. Ketamine-xylazine anesthesia. Coronal section shows the completeness of transection producing the slab. Three traces: from top to bottom: surface- and depth-EEG field potentials from the slab, and depth-EEG from area 5, outside the slab. Four brief pulse trains (each at 100 Hz) induced a self-sustained seizure, lasting 12 s and consisting of fast runs (~12 Hz) and SW/PSW complexes (~2 Hz). Averaged activity during paroxysmal fast runs (triggered by depth-negative EEG in the slab) shows reversal of fast activity at the surface and absence of correlation with EEG activity outside the slab. On the other hand, averaged activity triggered by the depth-negative wave of the slow oscillation (~0.7 Hz) in the intact brain shows absence of correlation with intraslab activity.
yssm is triggered by electrical stimulation (see Figs. 3 and 5) or after repetitive burst firing in a subset of neurons (Fig. 2). In both cases, postsynaptic neurons may easily be depolarized to the level of seizure. Synaptic excitation and burst firing were also reported to produce seizures with tonic onset in penicillin-triggered paroxysms (Fisher and Prince 1977). When fast runs are interspersed with PSW complexes, their frequency is similar to that of polyspikes building up PSW complexes (see Figs. 7 and 12 in Steriade et al. 1998a) and, thus, fast runs resemble prolonged PSW complexes that are not truncated by hyperpolarization. In some instances, the fast runs are preceded by an enhanced amplitude of fast ripples at ~100 Hz (Fig. 8 in Steriade et al. 1998a), which reflect the pattern of FRB neurons (Steriade 1997; Steriade et al. 1998b), whose fast rhythmic, high-frequency spike bursts provide favorable conditions for circulation of excitation within neocortical networks. The pyramidal-shaped and local-circuit FRB neurons send horizontal axons within the cortex (Gray and McCormick 1996; Steriade et al. 1998b) and could induce prolonged depolarization of postsynaptic neurons, either directly or, in the case of local inhibitory interneurons, through disinhibition mediated by contacts on other inhibitory interneurons (Kisvárday et al. 1993). That FRB neurons are among the best candidates to promote seizures was suggested, on the basis of experimental data, in a companion paper (Steriade et al. 1998a). Short-lasting inhibitory processes may survive during fast runs, as shown by evoked IPSPs during the depolarizing plateau in which paroxysmal fast runs occur and action potentials are inactivated (see Fig. 5B1 in Steriade and Amzica 1994). Overall, however, during fast cortical runs the intracortical excitation overwhelms inhibitory processes, which is an effective factor in depolarizing large populations of cortical neurons.

2) Seizures are accompanied by increased extracellular K⁺ concentration ([K⁺]o), with maximal increase during the tonic components (Moody et al. 1974; Zuckermann and Glaser 1968). The increase in [K⁺]o converts many regular-spiking CA1 hippocampal neurons to bursters (Jensen and Yaari 1997), mainly by increasing the depolarizing afterpotential (DAP). In FRB neurons, which were proposed to promote cortical seizures (Steriade et al. 1998a), the increase number of action potentials within a burst stems from DAPs (Steriade et al. 1998b). Thus regular-spiking neurons might be transformed into FRB neurons by elevated [K⁺]o during seizures.

3) The above factors may activate intrinsic depolarizing currents. An important one is the persistent Na⁺ current (INa(p)), which is activated in neocortical neurons at Vm of more positive than ~60 mV (Stafstrom et al. 1982). INa(p) is enhanced when the late outward rectifier is decreased (Stafstrom et al. 1984), and the latter seems to be suppressed during fast cortical runs (see Fig. 13 in Steriade et al. 1998a). This would allow a progressive depolarization of neocortical neurons up to the level of spike inactivation (see Fig. 5 in Steriade et al. 1998a). The INa(p) exists not only in the soma, but also in the apical dendrites of cortical neurons (Schwindt and Crill 1995), where it would provide a mechanism for the graded, voltage-dependent amplification of tonic excitatory inputs.

Therefore the increased synaptic activity during paroxysmal episodes is an important factor for triggering a cascade of extracellular and intrinsic neuronal processes resulting in the tonic depolarization underlying the fast components of seizures.

Concluding remarks

Synaptic activities resulting from spontaneously occurring sleep oscillations as well as responses to repetitive thalamic volleys result in spike bursts in many cortical neurons. This increases the [K⁺]o. In normal conditions, the [K⁺]o level is quickly restored and does not produce epileptiform activity. If the buffering by glial cells is impaired, cortical neurons will be excessively depolarized and produce stronger spike bursts, which will activate intrinsic currents that will further increase the depolarization. The neurons located close to the primary focus may be depolarized beyond the level of action-potential generation, but neurons in the area surrounding the focus will be less depolarized, and many of them will be able to display single spikes or spike bursts, depending on their intrinsic properties. Because of local differences in epileptogenic factors, such as synaptic activities and [K⁺]o, the leading times may continuously vary from one site to another. Although FRB neurons may fire rhythmic spike bursts around 40 Hz on depolarizing current pulses in normal conditions, their discharge properties are greatly affected by network synaptic activity (Steriade 1997; Steriade et al. 1998b), and, during the fast runs of seizures, their maximal frequency is close to 15 Hz. The discharges of FRB cells could decisively influence the neurons in the focus as well as, by their projections, in more distant, neocortical and thalamic structures.

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REFERENCES


Curro Dossi, R., Nunez, A., and Steriade, M. Electrophysiology of a...


