SYNAPTIC RESPONSIVENESS OF NEOCORTICAL NEURONS TO CALLOSAL VOLLEYS DURING PAROXYSMAL DEPOLARIZING SHIFTS

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Abstract—Based on intracellular recordings in vivo, we investigated the responsiveness of cat neocortical neurons to callosal volleys during different phases of spontaneously occurring or electrically induced electrographic seizures, compared with control periods of slow sleep-like oscillations. Overt seizures, with spiking, triggered by pulse-trains to the callosal pathway, started with a latency of approximately 20 s after cessation of stimulation, thus contrasting with paroxysmal activity elicited by ipsilateral cortical or thalamic stimulation that is initiated immediately after electrical stimulation. During the rather long preparatory period to callosally triggered seizures, cortical neurons displayed subthreshold depolarizing runs at 4–7 Hz, associated with increased amplitudes of excitatory postsynaptic potentials. The sequential analysis of neuronal responsiveness during different components of spike-wave complexes revealed progressively increased amplitudes of callosally evoked postsynaptic excitatory responses in regular-spiking and fast-rhythmic-bursting neurons, over a period of approximately 20 ms prior to the generation of paroxysmal depolarizing shifts. These data support the concept that seizures consisting of spike-wave complexes originate within the neocortex through a progressive synaptic buildup and that their synchronization is achieved, at least partially, by cortical commissural synaptic linkages. © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: callosal pathway, neocortex, seizures, spike-wave complexes, synaptic responsiveness, intracellular recordings.

The neocortex is the minimal brain substrate that can generate electrographic seizures consisting of spike-wave (SW) complexes at approximately 3 Hz, as in petit-mal epilepsy, or SW and polyspike-wave (PSW) complexes at slightly lower frequency, intermingled with fast runs at 10–20 Hz, as in the Lennox-Gastaut syndrome (Steriade, 2003). Experimental studies demonstrated the presence of such cortical paroxysms in animals with thalamocortical (TC) neurons during SW seizures is consequent to the faithful following of each paroxysmal depolarizing shift (PDS) of neocortical neurons by thalamic reticular GABAergic neurons (Steriade and Contreras, 1995; Timofeev et al., 1998; Slaght et al., 2002). Clinical studies have also shown that some SW seizures are locally generated in neocortex, resulting from multiple and independent foci (Petsche, 1962), that their synchronization is due to intracortical and interhemispheric synaptic linkages (Lemieux and Blume, 1986; Kobayashi et al., 1994), and that callosotomy or focal corticectomy are useful in the treatment of cortically generated seizures by preventing their spread (Miyauchi et al., 1988; Reutens et al., 1993).

The responsiveness of cortical neurons during the electroencephalogram (EEG) “spike” and “wave” components of SW complexes has been investigated using intracellular recordings in vivo. In essence, antidromic responses and excitatory postsynaptic potentials (EPSPs) are diminished or completely obliterated during the PDS associated with the EEG “spike,” whereas testing stimuli reliably elicit EPSPs and PDS patterns during the hyperpolarization related to the EEG “wave” (Steriade and Amzica, 1999). The decreased responsiveness of cortical neurons during the EEG “spike” is corroborated by a significant decrease in the apparent input resistance (R∞) during this component of SW complexes (Neckelmann et al., 2000) that is partially ascribable to a powerful inhibitory component during the PDS (Timofeev et al., 2002). These experiments used thalamic and/or ipsilateral cortical testing stimuli that could have trigger complex, uncontrolled events in reciprocal thalamocorticothalamic pathways.

In the present study, we used callosal volleys during spontaneously occurring or electrically induced seizures of the purely SW or Lennox-Gastaut type, and recorded from regular-spiking (RS) and fast-rhythmic-bursting (FRB) neurons. Morphological and electrophysiological studies show that callosal neurons originate and contact target neurons in homo- and heterotopic sites of the contralateral cortex, within supragranular layers II/III and infragranular layers V/VI (Innocenti et al., 2002; Cisse´ et al., 2003). It was reported that FRB neurons, which are located in both supragranular layers (Steriade et al., 1998b), display EPSPs in response to callosal volleys whose amplitudes are three-fold larger, and latencies two- to three-fold shorter, than those found in other neuronal classes (Cisse´ et al., 2003). One of the major results in the present study of neuronal responses during the EEG “spike” and “wave” is corroborated by a significant decrease in the apparent input resistance (R∞) during this component of SW complexes (Neckelmann et al., 2000) that is partially ascribable to a powerful inhibitory component during the PDS (Timofeev et al., 2002). These experiments used thalamic and/or ipsilateral cortical testing stimuli that could have trigger complex, uncontrolled events in reciprocal thalamocorticothalamic pathways.

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Abbreviations: EEG, electroencephalogram; EPSP, excitatory postsynaptic potential; FRB, fast-rhythmic-bursting; IPSP, inhibitory postsynaptic potential; PDS, paroxysmal depolarizing shift; PSW, polyspike-wave; R∞, apparent input resistance; RS, regular-spiking; SW, spike-wave; TC, thalamocortical.

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Fig. 1.
study is that RS and FRB neurons progressively increased their excitatory responses and decreased their latencies approximately 20 ms before the onset of PDSs during spontaneous or electrically induced seizures. This new evidence that recurrent excitatory intracortical neuronal processes lead to the development of SW and Lennox-Gastaut electrographic seizures.

EXPERIMENTAL PROCEDURES

Experiments were conducted on 23 adult cats, under pentobarbital (35 mg/kg, i.p.) (n = 15) or ketamine–xylazine anesthesia (10–15 mg/kg and 2–3 mg/kg i.m., respectively, n = 8). The animals were paralyzed with gallamine triethiodide after the EEG showed typical signs of deep general anesthesia and supplementary doses of anesthetics were administered at the slightest changes toward activated (high-amplitude and low-frequency) EEG waves. The cats were ventilated artificially with the control of end-tidal CO₂ at 3.5–3.7%. The body temperature was maintained at 37–38 °C and the heart rate was approximately 90–100 beats/min. Stability of intracellular recordings was ensured by hip suspension, drainage of cisterna magna, bilateral pneumothorax, and filling the hole made for recordings with a solution of 4% agar.

Experiments conducted to guidelines of the committee for animal care of Laval University and also conformed to international guidelines. The number of animals used and their suffering was kept to a minimum.

Intracellular recordings from the anterior part of the suprasylvian association cortex were performed using glass micropipettes filled with a solution of 3 M potassium-acetate (KAc). A high-impedance amplifier with active bridge circuitry was used to record the membrane potential (V_m) and inject current into the neurons. Field potentials were recorded in the vicinity of impaired neurons, using bipolar coaxial electrodes, with the ring (pial surface) and the tip (cortical depth) separated by 0.8–1 mm. Stimulating electrodes (similar to those used for field potential recordings) were inserted in homotopic points of the contralateral area 5.

At the end of experiments, the cats were given a lethal dose of pentobarbital (50 mg/kg, i.v.).

RESULTS

Long-latency initiation of callosally elicited seizures

In an overwhelming majority (88%) of seizures triggered by electrical stimulation of callosal pathway, the onset of overt paroxysms, consisting of rhythmic PDSs interrupted by hyperpolarizations, was delayed by 13 s to 30 s (mean 21±2.3, S.E.), and the period leading to full-blown seizure consisted of subthreshold, rhythmic depolarizations at 4–7 Hz (Fig. 1A, B, and Fig. 2). This stands in contrast with the immediate occurrence of full-blown seizures when triggered by ipsilateral cortical or thalamic stimulation (see single and dual intracellular recordings in Figs. 10–12 from Steriade et al., 1998a). The striking difference between the latencies of ipsilaterally and contralaterally recorded seizures, when using callosal stimulation, is also obvious by comparing in Fig. 2 (upper two traces) the EEG recorded from the field where stimuli were applied (right area 5) and the EEG recorded from the homotopic site in the contralateral cortex. In the former case, the seizure started even during stimulation, depth-EEG negative field potentials’ amplitudes increased immediately after cessation of stimulation, and the seizure stopped well before that recorded in the contralateral cortex, at which level the seizure’s full development took almost 30 s. Testing neuronal responsiveness with callosal volleys showed a progressive increased amplitudes of EPSPs, up to five-fold compared with control values, during the period of subthreshold depolarizations that led to full-blown electrographic seizure (Fig. 1B). Similar, while less spectacular, enhancement is illustrated in Fig. 2 (compare control responses in A1 to those recorded in B1 during the subthreshold depolarizations that preceded the seizure).

Progressively increased amplitudes and shortened latencies of callosally evoked EPSPs preceding the PDSs

Single stimuli were applied every 2 s to the depth of the suprasylvian site that was contralateral to the intracortically recorded RS (n = 15) and FRB (n = 9) neurons. Single testing volleys were continuously delivered, before the development of seizures as well as during and after cessation of spontaneously occurring paroxysms or electrically induced seizures using rhythmic pulse-trains, as depicted in Figs. 2 and 4. The amplitudes of callosally evoked EPSPs were measured as follow. We obtained averaged EPSPs from periods outside seizures; the amplitude was estimated as the difference in voltage that occurred just before the stimulus artifact and voltage achieved by neuron during at the maximal depolarization. Thereafter, we went back to individual responses and measured the amplitude of individual EPSPs during the epochs chosen for averages. The multiple peaks seen in individual responses (Fig. 2) could be due to the monosynaptic activation of fibers with different conductance velocities (see Cisseé et al., 2003) or could be due to oligosynaptic circuits. The vast majority of EPSPs was not contaminated or only slightly affected by IPSPs, as the probability of eliciting of IPSP by callosal stimulation is quite low (Cisseé et al., 2003) and the estimated reversal potential for the callosal EPSPs was more positive than 30 mV.

Fig. 1. “Phantom,” subthreshold SW runs (6–8 Hz) preceding the seizure elicited by rhythmic pulse-trains to the callosal pathway. (A) and (B). Two different cats under barbiturate anesthesia. In both cases, pulse-trains at 10 Hz were applied to right area 5 every 2 s and recordings were made from left area 5. (A) Intracellular recording of RS neuron and depth-EEG field recording from area 5. Below, expanded details of the periods marked by horizontal bars and arrows. Note rhythm, subthreshold depolarizations at 6–7 Hz preceding the seizure in left expanded period, preceding by approximately 13 s the full-blown seizure that consisted of SW complexes at approximately 3 Hz (at right). Seizure, consisting of subthreshold depolarizations, started after the third pulse-train (see intracellular trace). (B) Another RS neuron. Similar type of stimulation as in (A) led to prolonged (approximately 32 s) period of subthreshold depolarizations. Subthreshold depolarizations marking the seizure onset started approximately 2 s after the end of stimulation and full-blown seizure occurred later, as in (A). Single stimuli were applied to the contralateral area 5 before pulse-trains (1) and during the prolonged period of subthreshold depolarizing events (2 and 3). Note progressively increased amplitudes of EPSPs during the subthreshold rhythmic activity that preceded the overt paroxysmal activity. In this and following figures, membrane potential is indicated (arrows).
As a rule, tested EPSPs (a) increased their amplitudes during the period announcing the full-blown seizure, consisting of subthreshold depolarizing potentials (Figs. 1B and 2B); (b) gave rise to PDSs during the hyperpolarizing component of SW complexes (Figs. 2C* and 4); (c) were obliterated when stimuli fell during PDSs (Figs. 2D** and 4); and (d) returned to control levels approximately 30–40 s after the cessation of seizures (Fig. 2E). This picture was valid in all tested neurons, during either electrically evoked (Fig. 2) or spontaneously occurring (Fig. 4) seizures.

The sequential analysis of callosally evoked EPSPs' amplitudes and latencies at different time intervals between 100 ms preceding and 200–300 ms succeeding the time 0 of PDSs (taken as the first action potential) allowed us to detect a dramatic increase in amplitude and decreased in latency of tested EPSPs occurring progressively during the approximately 20 ms preceding...
the PDSs, compared with the virtual absence of responses during the approximately 150 ms before the PDSs (due to the effects of preceding PDSs). Thus, during the approximately 20 ms preceding the PDSs, the EPSPs displayed the same amplitudes as during control periods (before and after the seizures). This is documented in Fig. 3 (electrically induced seizure), Fig. 4 (spontaneous seizure), and is averaged from six neurons in Fig. 5. The dramatic decrease in latency of the EPSP illustrated in Fig. 3 (from approximately 80 ms to approximately 2 ms; see top superimposition) may be regarded as a transformation from a multi-synaptic EPSP (possibly including inhibition-rebound sequences in the activated neuronal chain) to a monosynaptic EPSP, due to short-circuit of intercalated relays (especially those implicating inhibitory linkages) during the progressively increased excitability prior to seizure.

At variance with the contrasting aspects of neuronal responsiveness during the two components of SW complexes, namely, evoked PDS during hyperpolarization and absence of overt responses during the depolarizing phase (see Fig. 4), callosal volleys invariably evoked firing (especially in FRB neurons) during the epochs of fast runs in the Lennox-Gastaut-like syndrome (Fig. 6). This is ascribable to the steady depolarization during this epoch and the fact that membrane conductance of cortical neurons is lower during fast runs than during the PDSs of SW seizures (Neckelmann et al., 2000).
Fig. 4. Responsiveness of cortical neuron to callosal volleys during different epochs of spontaneously occurring SW seizure. Top two traces depict depth-EEG and intracellular activity of RS neuron from area 5. Below, two superimposed traces illustrate expanded responses during the control period (before seizure), the hyperpolarization (corresponding to the EEG “wave” component) succeeding each PDS, and the depolarizing PDS. At the bottom, four responses (1–4) correspond to those in the right plot, constructed similarly to that depicted in Fig. 3. Note markedly increased EPSPs' amplitudes during the approximately 20 ms preceding the time 0 of PDS.
the neuron was intracellularly recorded) did not exhibit present experiments (from the same area as that where 2002). However, the field potentials recorded in the vicinity of the homotopic site in the contralateral hemisphere, where stimuli were applied, displayed clear-cut paroxysmal activity (Fig. 2). This suggests that the electrographic seizure started at the stimulated site and locally entrained neuronal pools into paroxysmal activity before a critical mass of projection cells was synchronously driven to transfer the seizure along the callosal pathway.

**DISCUSSION**

The major findings in the present study are (a) the delayed initiation of seizures elicited by trains of electrical pulses applied to callosal pathway, and (b) the progressively increased amplitudes of callosally evoked EPSPs during a period of approximately 20 ms prior to the generation of PDSs.

**Delayed initiation of callosally evoked seizures**

In contrast to seizures evoked by ipsilateral cortical or thalamic stimulation, which are initiated during or immediately after pulse-trains, callosally evoked seizures started with a latency of approximately 20 s after cessation of stimulation. During that preparatory period, cortical neurons displayed subthreshold depolarizing runs at 4–7 Hz, associated with increased amplitudes of EPSPs (see Fig. 1). In EEG clinical studies, such events are termed “phantom SW bursts” at approximately 5–6 Hz (Pedley, 1987), which do not necessarily indicate epileptic seizures but, if their amplitudes exceed approximately 50 μV, may predict the occurrence of seizures in more than half of subjects investigated (Hughes, 1980).

A possible factor accounting for the rather long delay in initiating overt seizures, which are accompanied by PDSs and action potentials, could be the spiking in neighboring and/or distant neurons, reflected as only subthreshold EPSPs in the neuron under observation. Indications that, before the occurrence of overt seizures, some neurons display increased discharge frequencies are found in our previous intracellular studies (see Fig. 4 in Neckelmann et al., 1998; and Fig. 6 in Timofeev et al., 2002). However, the field potentials recorded in the present experiments (from the same area as that where the neuron was intracellularly recorded) did not exhibit any change which would suggest that neurons others than that under observation were highly activated during the period leading to overt seizure; by contrast, the field potentials recorded in the vicinity of the homotopic site in the contralateral hemisphere, where stimuli were applied, displayed clear-cut paroxysmal activity (Fig. 2). This suggests that the electrographic seizure started at the stimulated site and locally entrained neuronal pools into paroxysmal activity before a critical mass of projection cells was synchronously driven to transfer the seizure along the callosal pathway.

**Responsiveness during the two components of PDSs, precursor increased EPSPs' amplitudes before the onset of PDSs**

The diminution up to complete obliteration of callosally evoked EPSPs during the PDSs (Figs. 2D** and 4) is in line with similar results using antidromic and synaptic responses elicited by ipsilateral cortical or thalamic stimuli (Steriade and Amzica, 1999) and can be mainly accounted for an important GABAAergic component during the PDSs (Cohen et al., 2002; Timofeev et al., 2002). The decreased responsiveness of cortical neurons during the PDS, corresponding to the EEG “spike” of SW complexes, is corroborated by a significant decrease in Rin during this component of SW complexes (Neckelmann et al., 2000; Timofeev et al., 2002). On the other hand, during the hyperpolarization that follows each PDS callosal stimuli reliably elicited powerful responses that often reached paroxysmal patterns (Figs. 2C* and 4) and, indeed, Rin is enhanced during this phase of SW complexes (Neckelmann et al., 2000; Timofeev et al., 2002). These data, combined to similar results from experiments conducted in a genetic rat model of absence epilepsy (Charpier et al., 1999; Staak and Pape, 2001; Crunelli and Leresche, 2002), are in sharp contrast with the idea of a role played by GABA receptors in the generation of the hyperpolarization associated with the EEG “wave” component of SW complexes (Giaretta et al., 1987; Destexhe, 1998). As to the possibility that GABA<sub>α</sub>-mediated IPSPs underlie the “wave” component of SW seizures, including QX-314 in the recording pipette to block the G-protein-coupled GABA<sub>α</sub>-evoked K<sup>+</sup> current (Jensen et al., 1993; Deisz et al., 1997) did not significantly affect the hyperpolarization in our experiments (I. Timofeev, F. Grenier and M. Steriade, unpublished data).

**Probable causes of increased responsiveness**

Probably, the most interesting finding, which was consistently obtained in all our recordings, was a progressively increased amplitudes and shortened latencies of callosally evoked EPSPs over a period of approximately 20 ms preceding the onset of PDSs (Figs. 2–5). As well, [Ca<sup>2+</sup>]<sub>i</sub>, progressively increased during the depth-positive EEG field potential (corresponding to the post-PDS hyperpolarization) until a maximum concentration was found simultaneously to the onset of PDS (unpublished data). This result, concerning the phasically increased [Ca<sup>2+</sup>]<sub>i</sub>, in advance of PDSs, corroborates recent data by Amzica et al. (2002). We hypothesize that an increase in [Ca<sup>2+</sup>]<sub>i</sub> toward...
the seizure termination leads to a higher Ca$^{2+}$ gradient and, consequently, more Ca$^{2+}$ entry into the neurons during PDSs. This should significantly enhance $I_{K(Ca)}$ that would overwhelm all excitatory influences, eventually leading to seizure cessation.

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