Increased Propensity to Seizures After Chronic Cortical Deafferentation
In Vivo

Dragos A. Nita, Youssouf Cissé, Igor Timofeev, and Mircea Steriade
Laboratoire de Neurophysiologie, Faculté de Médecine, Université Laval, Québec, Canada

Submitted 14 July 2005; accepted in final form 17 October 2005

Nita, Dragos A., Youssouf Cissé, Igor Timofeev, and Mircea Steriade. Increased propensity to seizures after chronic cortical deafferentation in vivo. J Neurophysiol 95: 902–913, 2006. First published October 19, 2005; doi:10.1152/jn.00742.2005. Cortical injury may lead to clinical seizures. We investigated the changing patterns of the sleeplike slow oscillation and its tendency to develop into paroxysmal activity consisting of spike-wave (SW) complexes at 2–4 Hz after partial deafferentation of the suprasylvian gyrus. Experiments were carried out in anesthetized cats, at different time intervals (wk 1 to wk 5, W1–W5) after cortical undercut. Multisite field potentials and single or dual intracellular recordings from the whole extent of the deafferented gyrus were used. The field components of the slow oscillation increased in amplitudes and were transformed into paroxysmal patterns, expressed by increased firing rates and tendency to neuronal bursting. The incidence of SW seizures was higher with transition from semiacute (W1) to chronic (W2–W5) stages after cortical undercut. The propagation delay of low-frequency activities decreased from W1 to W5, during both the slow oscillation and seizures. The initiation of seizures took place in territories contiguous to the relatively intact cortex (area 5 in the anterior part of the gyrus), as shown by cross-correlations of field potentials from different sites and simultaneous intracellular recordings from the anterior and posterior parts of the gyrus. The increased amplitudes of both slow oscillation and SW seizures, and their enhanced synchrony expressed by shorter time of propagation, are ascribed to increased neuronal and network excitability after cortical undercut.

INTRODUCTION

Acute cerebral cortical trauma may lead to paroxysmal activities. After severe brain injury the incidence of acute seizures was 95 times higher than that in general population (Annegers et al. 1998). Ten to 15 yr after the trauma about 50% of patients with penetrating cranial wounds would develop epilepsy characterized by recurring seizures (Marcikic et al. 1998; Salazar et al. 1985). In experimental animals, chronic neuronal hyperexcitability and epileptogenesis have been demonstrated in isolated neocortical islands with intact pial circulation in vivo (Burns 1951; Sharpless 1969; Sharpless and Halpern 1962) and in neocortical in vitro slices after chronic cortical injury (Hoffman et al. 1994; Li and Prince 2002; Li et al. 2005; Prince and Tseng 1993; Prince et al. 1997).

The origin of these seizures is unclear. Computational models of posttraumatic epileptogenesis in isolated cortical islands concluded that paroxysmal discharges possibly arise from changes in intrinsic properties of pyramidal cells and enhanced excitatory synaptic conductances without altering synaptic inhibition (Bush et al. 1999; Houweling et al. 2005). In vitro experimental studies on models of acute and chronic cortical deafferentation revealed an increased synaptic and intrinsic neuronal responsiveness after decrease in input signal (Prince and Tseng 1993; Prince et al. 1997; Turrigiano et al. 1998), which may favor the development of epileptogenesis. After a few days of pharmacological blockade of activity in cortical cell cultures, the amplitudes of excitatory postsynaptic currents (EPSCs) and miniature EPSCs (mEPSCs) in pyramidal cells increase (Turrigiano et al. 1998; Watt et al. 2000) as well as the quantal release probability (Murthy et al. 2001). Synaptic scaling occurs in part postsynaptically by changes in the number of open channels (Turrigiano et al. 1998; Watt et al. 2000), although all synaptic components may increase (Murthy et al. 2001), including the numbers of postsynaptic glutamate receptors (Liao et al. 1999; Lissin et al. 1998; O’Brien et al. 1998; Rao and Craig 1997).

There is a similar activity-dependent regulation of N-methyl-D-aspartate (NMDA) currents (Watt et al. 2000). Interestingly, miniature inhibitory postsynaptic currents (mIPSCs) are scaled down with activity blockade, in the opposite direction to excitatory currents. This effect is reversible (Rutherford et al. 1997) and is accompanied by a reduction in the number of open γ-aminobutyric acid type A (GABA_A) channels and GABA_A receptors clustered at synaptic sites (Kilman et al. 2002). Not only synaptic but also intrinsic excitability is regulated by activity. After chronic activity blockade, Na⁺ currents increase and K⁺ currents decrease in size, resulting in an enhanced responsiveness of pyramidal cells to current injections (Desai et al. 1999b). These observations suggest that homeostatic mechanism may regulate the average levels of neuronal activity. Some of these processes, collectively termed “homeostatic plasticity” (Turrigiano 1999), may also occur in vivo (Desai et al. 2003). Thus we hypothesized that deafferentation caused by the trauma may upregulate neuronal and network excitability, leading to seizures.

Acute experiments performed in vivo showed that partially deafferented neocortex displays increased local cortical synchrony in areas surrounding the undercut cortex, leading to paroxysmal activity that occurs 2–3 h after the undercut and arises from enhanced intrinsic and synaptic neuronal responsiveness, increased incidence of intrinsically bursting neurons, and slight reduction of inhibitory influences (Topolnik et al. 2003a,b). We have now investigated the evolution of electrical paroxysms after cortical deafferentation (induced by penetrating wound piercing the dural membrane) ≤5 wk after the undercut, to determine the spatiotemporal development of
posttraumatic seizures with respect to the initial cortical insult, and to quantify at different stages the transformation from the normal sleeplike slow oscillation (0.5–1 Hz) to increased amplitudes of phases building up this cortical rhythm, eventually reaching the level of paroxysmal activity.

METH O D S

Animals and cortical deafferentation

Experiments were performed on 33 adult cats of both sexes. Surgical procedures were carried out under sterile conditions, after a premedication with acepromazine (0.3 mg/kg, administered intramuscularly (im)), butorphanol (0.3 mg/kg im), atropine (0.05 mg/kg im), and ketamine (20 mg/kg im), under isoflurane anesthesia (1–2%). The level of anesthesia was continuously monitored by the EEG, heart rate, oxygen saturation of the arterial blood (aiming over 90%), and end-tidal CO2 (about 3.5%). Isoflurane concentration was adjusted correspondingly to maintain a cardiac frequency at 90–110 beats/min. General surgical procedures included: cephalic vein cannulation for systemic liquid delivery (lactated Ringer solution 5–10 ml·kg−1·h−1) and lidocaine (0.5%), infiltration of all pressure points or incision lines. Body temperature was maintained at 37–39°C with a heating pad.

A craniotomy was used to expose the cerebral cortex and a large undercut of the white matter below the suprasylvian gyrus (13–15 mm posteroanteriorly and 3–4 mm mediolaterally) was used to produce partial cortical deafferentation (Fig. 1). A custom-designed knife was inserted in the posterior part of suprasylvian gyrus perpendicular to its surface for a depth of 3–4 mm, then rotated 90° and advanced rostrally along the gyrus parallel to its surface for a total distance of 13–15 mm, then moved back, rotated 90°, and removed from the same place where it was entered. Thus the anterior part of the undercut cortex was relatively intact because it had preserved intracortical connectivity and ascending and descending connections were only partially damaged, whereas the white matter below the posterior part of the gyrus was completely transected, creating conditions of partial cortical deafferentation. The skull was reconstituted using acrylic dental cement and the skin of the scalp sutured. Animals were kept under observation up to full recovery and they received analgesic medication (anafen 2 mg/kg, subcutaneously) for the next 48–72 h.

Semichronic experiments

At different time intervals after the initial surgery, the different subgroups of animals were anesthetized with ketamine and xylazine (10–15 mg/kg and 2–3 mg/kg, respectively, im) and recorded at 1 wk from the undercut (W1, n = 5), 2 wk (W2, n = 5), 3 wk (W3, n = 5), 4 wk (W4, n = 5), and 5 wk (W5, n = 5). Control recordings were performed under ketamine and xylazine anesthesia in cats with no deafferentation of the suprasylvian gyrus (n = 4) at W1 after the sham surgical procedures. In addition, electrophysiological experiments were done on two cats at W1 and two cats at W5 anesthetized with sodium pentobarbital (30 mg/kg). EEG and heart rate were monitored continuously to maintain the anesthesia, and additional doses of anesthetic were given at the slightest tendency toward an activated EEG pattern or accelerated heart rate. All pressure points to be incised or infiltrated with lidocaine (0.5%). Muscle paralysis was induced correspondingly to maintain a cardiac frequency at 90–110 beats/min. Body temperature was maintained at 37–39°C with a heating pad.

FIG. 1. Experimental paradigm. A: placement of the array of EEG electrodes and of 2 intracellular pipettes over the left suprasylvian gyrus. Area in gray represents cortical deafferentation. Cortical deafferentation was performed from posterior to anterior. B (frontal) and C (sagittal) sections of cat brain; Nissl staining. Trace left by the knife in the white matter is expanded in the inset of B. Extent of the undercut is indicated by red arrows. Black arrows indicate the position of the recording electrodes confirmed by electrolytic lesions.

dose of sodium pentobarbital (50 mg/kg, iv). After experiments brains were removed and the extension of the undercut was verified on Nissl-stained (thionine) 80-μm brain sections (Fig. 1B). The location of recording electrodes relative to the undercut was evaluated using electrolytic lesions produced by a 20-mA continuous DC current for 1 min applied at the end of the experiments (Fig. 1C). All experi-
tal procedures were approved by the committee for animal care of Laval University and in accordance with the guidelines published in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Extracellular recordings**

Field potential (EEG) recordings were obtained by means of an array of eight monopolar tungsten electrodes (impedance 8–12 MΩ), about 2.5 mm apart, placed on the whole length of the suprasylvian gyrus at a depth of about 1.5 mm (Fig. 1A). The reference electrode was fixed to the neck muscles. We stereotaxically positioned the array of electrodes to have two electrodes in the relatively intact cortex, two electrodes over the area corresponding to the anterior limit of the undercut, and four electrodes in the deafferented cortex.

**Intracellular recordings**

Intracellular recordings in the partially deafferented area 21 and relatively intact area 5 of the suprasylvian gyrus were obtained with glass micropipettes (tip diameter <0.5 μm) filled with potassium acetate (3 M, in situ impedance 35–50 MΩ). Only stable recordings with resting membrane potentials more negative than ~60 mV and overshooting action potentials were accepted for analysis. The intracellular signals were passed through a high-impedance amplifier with an active bridge circuitry (bandwidth DC to 9 kHz). All signals were digitized (20-kHz sampling rate) and stored for off-line analysis.

**Data analysis**

Data analysis was performed using Wavemetrics’s Igor Pro software. To estimate changes in slow-wave activities after cortical undercut, the power in the 0– to 4-Hz range was quantified by the area under the graph of the fast Fourier transform (FFT) of field EEGs. The EEG amplitude was estimated from 1-min artifact-free periods as a difference between the most positive and most negative values. Means of comparative data were statistically evaluated with paired Student’s t-test. Differences between means were considered significant at P < 0.05. Auto- and cross-correlograms of different EEG channels were computed from periods of 2–3 min of stable activity and averaged for each different group (see Fig. 7B). The electrode where the seizures first occurred was taken as time reference. Time delays extracted from serial cross-correlograms between EEG electrodes, computed on successive 1-s epochs of filtered EEG (0–10 Hz), were averaged for each experiment and between experiments and used to calculate the speed of propagation.

Histograms of membrane potential (V_m) distribution (see Fig. 9C) were created for successive periods of 10 s by counting the number of samples with bins of 1 mV. The peaks of distribution that corresponded to the most probable mode of V_m were taken as the level of V_m. A time-delay histogram (as in Fig. 9D) was obtained from differences in time between the two intracellular recordings of the points corresponding to 10% of the amplitude of the depolarization between the V_m during hyperpolarizing states of the SW and the V_m at the onset of burst.

**RESULTS**

**Changes in slow oscillation and development of seizures in the undercut cortex**

The normal slow cortical oscillation (<1 Hz) constitutes two different activity levels: an active ("up") state in which cortical neurons are depolarized and tonically fire action potentials, and a silent ("down") state in which cortical neurons are hyperpolarized by a disfacilitation process (Contreras et al. 1996; Steriade et al. 1993).

All cats with chronic cortical deafferentation displayed a paroxysmal pattern of the slow oscillation, different from the one observed in cats with intact suprasylvian gyrus (Fig. 2A). More than 90% of animals anesthetized with ketamine–xylazine (23 out of 25) displayed low-frequency SW and polyspike-wave (PSW) complexes (3–4 Hz), intermingled with fast runs (10–20 Hz), which were similar to the waveforms seen in humans with severe epileptic encephalopathies (Fig. 2B). One of the two cats that did not develop seizures was part of the W1 group and the other part of the W2 group.

The FFT of EEG showed a unimodal distribution with a peak of activity corresponding to the frequency of the slow oscillation, 0.5–1 Hz (Fig. 2C, during both W1 and control). EEG recordings of the slow oscillation in the undercut cortex, performed from W1 to W5, showed that, although the frequency was similar at different electrodes, the EEG amplitude and the peak of FFT activity were higher for electrodes placed over the anterior limit of the undercut, at the border between the intact and deafferented cortex (EEG3), compared with posterior electrodes, in the most deafferented cortex (EEG8) (Fig. 2C). The higher EEG amplitude and peak of FFT activity were also observed during SW/PSW seizures. After cortical deafferentation, the power of slow activities appreciably augmented with time, mainly by the increase in the 0- to 4-Hz frequency domain and the change from a unimodal to a multimodal distribution (compare W1 with W5 in Fig. 2C).

It is known that ketamine–xylazine anesthesia favors development of seizures (Steriade and Contreras 1995; Steriade et al. 1998); however, the proportion of anesthetized animals with acute undercut (Topolnik et al. 2003b) and chronic undercut (present study) displaying seizures is much higher. To completely avoid the influence of ketamine–xylazine anesthesia on the development of paroxysmal activities, we recorded electrical activities from four cats with cortical undercut, anesthetized with barbiturates (two on them at W1 and the other two at W5). Barbiturates enhance GABAergic inhibition by prolongation of time of opening of Cl⁻ channels (Twyman et al. 1989). Similarly to our previous study (Topolnik et al. 2003b), we did not expect to see the development of full-scale seizures in those experiments, but we expected to see an enhanced activity in areas surrounding the undercut cortex. This was indeed the case in W1 animals (not shown). W5 animals revealed some different patterns (Fig. 3). During the early phases of anesthesia, in the relatively intact anterior part of suprasylvian gyrus, spindle activity was prominent, whereas it was absent in the partially deafferented areas. In the partially deafferented areas field potentials demonstrated aperiodic, asynchronous EEG “spikes” (see arrowheads in expanded parts of Fig. 3A). Five to 8 h later, when the level of anesthesia decreased, the activity in the relatively intact areas was dominated by 2- to 3-Hz paroxysmal discharges that merged with spindle oscillations (Fig. 3B). Probably, the barbiturate-related enhancement of inhibition prevented propagation of paroxysmal activities into partially deafferented cortical areas. Isolated EEG spikes still persisted in the partially deafferented cortex (Fig. 3B, EEG7).

The quantification of data on EEG amplitude in cats under ketamine–xylazine anesthesia is shown in the Fig. 4.
the slow oscillation, the maximal EEG amplitudes grew up in different electrodes as a function of time after the undercut, suggesting the increase in local synchrony. The maximal values of amplitude were recorded around electrode EEG3, i.e., the electrode that in most cases was located between relatively intact and partially deafferented areas, except for the control animals without cortical deafferentation in which the EEG amplitude during slow oscillation was rather uniform. During electrographic seizures, the EEG amplitude was higher than that during slow oscillation at all studied time samples and statistically significant differences were mainly found between W1 and W5 (Fig. 4).

FIG. 2. Patterns of EEG activity under ketamine–xylazine anesthesia. A: EEG field potentials showing slow oscillation in cat with intact suprasylvian gyrus. B: EEG field potentials during slow oscillation and SW/PSW seizures at W1 and W5 after cortical deafferentation. C: fast Fourier transform (FFT) of EEG activity shows an increased power of slow activities (<4 Hz) in anterior electrodes (EEG3) compared with posterior ones (EEG8), and in time from W1 to W5, both during slow oscillation and spike-wave (SW) seizures. Control data (Ctrl) are presented for slow oscillation epochs.
To quantify the power spectra of EEG recorded from eight electrodes placed over the undercut suprasylvian gyrus (see Fig. 1; EEG1, relatively intact cortex, through EEG8, partially deafferented cortex), the area under the FFT graph for the 0- to 4-Hz domain was computed for each period ranging from W1 to W5 (Fig. 5A) and in control experiments during slow oscillation. The increased power of slow activities in time, after deafferentation, was observed for both the slow oscillation and SW/PSW seizures in animals with cortical deafferentation. During seizures, the power of slow activities was consistently higher than that during the slow oscillation and the electrodes in the anterior part of the undercut (mainly EEG3) contained more slow activities than electrodes placed in the posterior part of the undercut (EEG8), suggesting that the occurrence of seizures is initiated at the border between the relatively intact and the deafferented cortex. Figure 5B presents comparatively the evolution of power spectra, from W1 to W5, in EEG activity recorded from posterior electrode 8 (in black) and from the electrode with the highest levels of slow activities, usually electrode 3 (in red), for both the slow oscillation and the SW/PSW seizures, together with the SD in each group. For the slow oscillation, statistically different values were obtained for the anterior part of the undercut in W3, W4, and W5 compared with control values in sham-operated animals, and in W5 for the posterior electrode. Between W1 and W5 a significant variation was observed for both anterior and posterior parts of the undercut, whereas the difference between anterior and posterior electrodes was significant only at W5. With respect to seizure activities, a significant difference was found between the anterior and posterior electrodes starting from W3 to W5, and from the same electrode from W1 and W5 (Fig. 5B).

As illustrated above (see Fig. 2C), the increase in power spectra of slow oscillation evolved in time from the peak around 1 Hz (at W1, more ample in anterior electrode EEG3 than in the posterior EEG8) to the expression of multiple frequencies in the FFT and the widening of the peaks over the entire 0- to 4-Hz domain (at W5).

Out of 38 neurons recorded intracellularly, 12 were recorded from the anterior part of the undercut, before the occurrence of overt SW/PSW seizures. The intracellular recording in the chronic deafferented cortex (Fig. 6) contained normal periods of slow oscillation (expanded in left) alternating with periods of paroxysmal slow activities. During paroxysmal–like activities the $V_m$ of neurons revealed slight ($2.6 \pm 1.4$ mV) depolarization calculated from the maximum of membrane potential distribution (Fig. 6E). This depolarization was accompanied with increased firing rates and reduction in action potential amplitudes. The other two distinct features were the presence of brisk hyperpolarizing waves (100–300 ms) and spontaneous bursts action potential generated by regular-spiking neurons (see Fig. 6, inset). The cell’s bursts were revealed by short intervals (5–10 ms) in the interspike histogram (see Fig. 6D) and were absent during normal spontaneous activities.
pared with SW/PSW discharges (Fig. 7B). Cross-correlation between activities recorded from different cortical sites ranged between the electrodes during slow oscillation, as compared with SW/PSW discharges (Fig. 7B). The time lags between activities recorded from different cortical sites ranged from about 6 to nearly 190 ms and were shorter during SW/PSW seizures than during propagation of the cortical slow oscillation (Fig. 7B). Pooled data from all animals (five cats in each group) with electrodes placed in the positions indicated in Fig. 1, and from the control group during the slow oscillation. Highest EEG amplitudes were found around EEG3 in animals 1–5 wk after the undercut. Stars above plots indicate statistically significant difference in the EEG amplitude.

Spatiotemporal properties of seizures in the deafferented cortex

In all cases the seizures started, and their amplitudes were most conspicuous, in the anterior part of the suprasylvian gyrus (electrodes EEG1 to EEG4), and propagated to the deafferented cortex (Fig. 7A). Usually, seizures started with SW/PSW complexes at 2–3 Hz, followed by episodes of fast runs at 10–20 Hz. Cross-correlation between activities recorded through different electrodes indicated a distinctive time-lag range between the electrodes during slow oscillation, as compared with SW/PSW discharges (Fig. 7B). The time lags between activities recorded from different cortical sites ranged from about 6 to nearly 190 ms and were shorter during SW/PSW seizures than during propagation of the cortical slow oscillation (Fig. 7B). Pooled data from all animals (five cats in each week, from W1 to W5) showed that the velocity in activity propagation for 0- to 10-Hz frequencies in the undercut cortex increased from W1 to W5, both during the slow oscillation (from 0.063 ± 0.004 to 0.3257 ± 0.0404 m/s between EEG4 and EEG8) and seizures (from 0.1182 ± 0.006 to 0.7751 ± 0.3107 m/s between EEG4 and EEG8); and the time lag of propagation was persistently shorter during SW/PSW seizures compared with that during the slow oscillation (Fig. 8). The level of correlation between the electrodes followed the same rule: it increased for both condition from W1 to W5 and it was steadily greater during SW/PSW seizures than during the slow oscillation (not shown).

Dual intracellular recordings (n = 9) from neurons located in the area around the anterior (relatively intact) area 5 and the posterior (deafferented) area 7, in conjunction with field EEGs recorded over the whole extent of the suprasylvian gyrus (with a space resolution of 1.5 mm) were used to analyze the pattern of synchrony during seizures and their propagation. Such simultaneous intracellular data showed that neurons in the anterior part of the suprasylvian gyrus, in the relatively intact cortex, fired consistently before the neurons in the posterior part of the undercut (Fig. 9). Time detection was made at 10% amplitude between the resting $V_m$ and the firing threshold of the first spike (Fig. 9, B and D). Histograms of the $V_m$ of two neurons are depicted in Fig. 9C. Both displayed a bimodal distribution with peaks corresponding to the resting $V_m$ and to the value of the depolarizing plateau. The mean membrane potential of neurons in relatively intact and partially deafferented areas was not statistically different, but the membrane potential of neurons had a tendency to be more hyperpolarized during silent network states (“up” states in relatively intact area 69.4 ± 3.9 mV; “up” states in partially deafferented area 53.5 ± 4.2 mV, “down” states in partially deafferented area 74.3 ± 4.8 mV).

The average membrane resistances of recorded neurons were 19.3 ± 7.5 MΩ (ranging from 13.5 to 32.7 MΩ) in W1, 20.2 ± 9.6 MΩ (ranging from 16.2 to 37.3 MΩ) in W2, 18.5 ± 8.2 MΩ (ranging from 14.9 to 40.4 MΩ) in W3, 21.3 ± 5.7 MΩ (ranging from 15.5 to 28.6 MΩ) in W4, and 23.1 ± 9.4 MΩ (ranging from 15.1 to 33.2 MΩ) in W5. The differences were not statistically significant (Student t-test, $P < 0.05$) either between the different groups or compared with previously reported values from in vivo acute preparations (Topolnik et al. 2003a).

**Discussion**

We found that the chronic stages of cortical undercut are characterized by I) increase in amplitudes of field potentials

---

**FIG. 4.** EEG amplitudes in the undercut cortex during slow oscillation (left) and paroxysmal discharges (right) in ketamine–xylazine anesthetized cats. Mean amplitude of EEG traces was obtained from different animals ($n = 5$ in each group) with electrodes placed in the positions indicated in Fig. 1, and from the control group during the slow oscillation. Highest EEG amplitudes were found around EEG3 in animals 1–5 wk after the undercut. Stars above plots indicate statistically significant difference in the EEG amplitude.
that build up both the slow oscillation and SW/PSW seizures; 2) increased velocity of low-frequency activity propagation from W1 to W5, during both the slow oscillation and seizures; and 3) initiation of seizures in territories contiguous to the relatively intact cortex, as shown by both field potentials and intracellular recordings.

The ketamine–xylazine anesthesia used in this study induced a sleeplike slow oscillation, which is similar to that recorded during natural sleep in cats and humans (Achermann and Borbély 1997; Amzica and Steriade 1997; Molsberg et al. 2004; Mölle et al. 2002; Steriade et al. 1993). Under this type of anesthesia, 20–30% of animals can spontaneously develop seizures with SW/PSW complexes (Steriade and Contreras 1995; Steriade et al. 1998). However, the present and previous data rule out the main contribution of ketamine–xylazine anesthesia in the generation of seizures that followed cortical deafferentation, based on: 1) the much higher percentage (>90%) of seizures after the undercut, compared with the incidence of such paroxysms previously observed in the nonlesioned cortex (20–30%); 2) the presence in all recorded animals of a peculiar pattern of slow oscillation, containing alternating periods of normal activity and periods with paroxysmal oscillation in the 0- to 4-Hz domain (Fig. 2B, W5), which was never observed in previous experiments conducted under the same anesthesia, but without chronic cortical deafferentation; and 3) the highly increased amplitudes and sharp waves after cortical undercut in animals under barbiturate anesthesia (Fig. 3), whereas barbiturates normally prevent the occurrence of seizures by enhancing GABAergic inhibitory processes. The transformation of the slow oscillation into SW/PSW seizures was shown by the preferential occurrence of these paroxysms during slow-wave sleep and by similar relations between field and intracellular activities during slow oscillation and epochs with SW/PSW seizures (see also Fig. 4).
Several factors may account for the increased propensity to seizures after the cortical insult produced by undercut. Although acute epileptogenesis arising from increased $[K^+]_o$ (Moody et al. 1974) may be partially explained by the $K^+$-mediated increase in the hyperpolarization-activated depolarizing current ($I_H$) that leads to paroxysmal activity in neocortical networks (Timofeev et al. 2002), the progressively increased power of seizures over time, up to 5 wk after undercut (present data), would not favor the same mechanism. The same reasoning may apply to changes in extracellular glutamate that is increased after cortical trauma and was reported to promote epileptogenesis (Sakowitz et al. 2002). The changes in intrinsic neuronal properties after cortical injury or undercut, leading to seizures, have been first studied in vitro and some of these results have been corroborated in vivo. Prince and Tseng (1993) compared layer V neurons of epileptogenic slices with those in control slices and found no significant differences in action potential characteristics and resting $V_m$, but the value of input resistance ($R_{in}$) was more than double in injured than in control slices. The increased $R_{in}$ of neurons recorded from epileptogenic slices is likely behind the increased intrinsic and synaptic responsiveness found in neurons recorded from the relatively intact suprasylvian cortex, at which level seizures are initiated after acute deafferentation in vivo (Topolnik et al. 2003a). This result fits in well with injury-induced enhanced responsiveness of corticospinal neurons after their axotomy (Tseng and Prince 1996) and with the increased synaptic and intrinsic responses of cultured cortical neurons during chronic absence of spontaneous activity (Desai et al. 1999a; Turrigiano et al. 1998). Trauma-induced chronic hyperexcitability and focal epileptogenesis could occur when homeostatic plasticity mechanisms upregulate processes leading to increased excit-

FIG. 6. Intracellular recording in the anterior part of the undercut cortex during slow oscillation, 3 wk after deafferentation (ketamine–xylazine anesthesia), illustrating different cellular patterns of activity. Underlined epochs in A are expanded in B and C. Slow oscillation contained periods with normal up-and-down states (B) alternating with periods when the up-states were disrupted by short hyperpolarizing events (C) and the neuron displayed a tendency toward bursting as indicated by the interspike interval histograms from intracellular activity displayed in D. Burst spotted in gray in C is expanded in the inset below the trace. E: serial 5-s histograms of $V_m$ from the epoch depicted in A illustrating the shift in the bimodal distribution associated with paroxysmal epochs.
FIG. 7. Time correlations across different cortical leads and from W1 to W5 during slow oscillation and seizures. Ketamine–xylazine anesthesia. A: development of seizure during W4 consisting of SW/polyspike-wave (PSW) complexes and finishing with fast runs over electrodes EEG1 to EEG8. Beginning of the seizure is expanded in the right inset showing that seizure started in anterior leads (EEG3–EEG5) and then propagated more posteriorly. B: spatiotemporal properties of the slow oscillation (SO) and SW/PSW seizures in the deafferented cortex. One cat is depicted for each week (W1–W5). Cross-correlations were performed between the most anterior electrode where the seizures occur first (EEG4) and the rest of the electrodes placed over the suprasylvian gyrus. Signal was filtered in the 0- to 10-Hz range. For both the paroxysmal slow oscillation and the SW/PSW seizures the time lags diminished from W1 to W5, and the propagation was faster during seizures compared with the paroxysmal slow oscillation (during W1–W4).

FIG. 8. Quantification of time lags during paroxysmal SO and SW/PSW seizures. Pooled data from 5 cats in each week (W1–W5). Bottom axis: electrode from the anterior to posterior.
ability (Houweling et al. 2005). The high $R_{\text{in}}$, shown to characterize neurons in epileptogenic slices (Prince and Tseng 1993), is probably a factor promoting seizures by favoring transformation of regular-spiking into intrinsically bursting neurons whose incidence is much higher in disconnected cortical slabs in vivo (Timofeev et al. 2000) and in cortical slices in vitro (Nishimura et al. 2001) than that in the intact cortex (Steriade et al. 2001). Besides the above-mentioned changes in intrinsic properties, a shift in the balance between inhibitory and excitatory processes may be changed toward excitation because, in parallel experiments using immunohistochemical staining with GAD65 and 67 and anti-GABA antibodies, we detected an apparent reduction in GABAergic neurons in disconnected cortical areas (unpublished data). The presence of normal inhibitory connectivity in more intact cortical areas may favor spatial localization of excitatory neuronal networks.

![Dual intracellular recordings from the relatively intact (Intra-cell 1) and deafferented (Intra-cell 2) cortex, 1 wk after undercut (A) in ketamine-xylazine-anesthetized cat. Neuron 1 fired before neuron 2 (expanded in B). Histogram of time lags at 10% of the amplitude of depolarization between the 2 neurons (with anterior one as reference) is presented in D. C: histogram of the $V_{\text{m}}$ of the 2 neurons.](http://jn.physiology.org/)

FIG. 9. Dual intracellular recordings from the relatively intact (Intra-cell 1) and deafferented (Intra-cell 2) cortex, 1 wk after undercut (A) in ketamine-xylazine-anesthetized cat. Neuron 1 fired before neuron 2 (expanded in B). Histogram of time lags at 10% of the amplitude of depolarization between the 2 neurons (with anterior one as reference) is presented in D. C: histogram of the $V_{\text{m}}$ of the 2 neurons.
and thus prevent generalized epileptogenesis (Traub and Wong 1982). The above changes explain, at least partially, the increased synchrony and shorter time-delay propagation of low-frequency (slow oscillation and seizure) activities after cortical undercut. High-density EEG recordings of the slow sleep oscillatory waves in the human cortex indicate a wide range of conduction velocities, 1.2 to 7 m/s (Massimini et al. 2004). The present data indicate that, after deafferentation, the slow oscillation propagates with progressively shorter time lags from W1 to W5 (see Fig. 7B). The increased velocity of signal propagation indicates an increased neuronal excitability. Multisite field potential and intracellular recordings have shown that cortically generated SW seizures propagate from one to another area in the suprasylvian gyrus through mono-, oligo-, and multisynaptic linkages (Amzica and Steriade 1995; Neckelmann et al. 1998). After cortical undercut, the propagation of paroxysmal activity is also progressively shorter from W1 to W5 (Fig. 7B).

In summary, after partial deafferentation the neocortex displays progressively increased signs of paroxysmal activity, reflected in paroxysmal-like patterns of the slow oscillation and progressively enhanced amplitudes and synchrony of SW/PSW seizures, which are however relatively localized within disconnected territories adjacent to the relatively intact cortex.

ACKNOWLEDGMENTS
We appreciate the technical assistance of P. Giguère.

GRANTS
This work was supported by Canadian Institutes for Health Research Grants MT-3689, MOP-36545, and MOP-37862 and National Institute of Neurological Disorders and Stroke Grant NS-40522.

REFERENCES


