

# Permanent Reduction of Seizure Threshold in Post-Ischemic CA3 Pyramidal Neurons

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**Congar, Patrice, Jean-Luc Gaïarsa, Théodora Popovici, Yezekiel Ben-Ari, and Valérie Crépel.** Permanent reduction of seizure threshold in post-ischemic CA3 pyramidal neurons. *J. Neurophysiol.* 83: 2040–2046, 2000. The effects of ischemia were examined on CA3 pyramidal neurons recorded in hippocampal slices 2–4 mo after a global forebrain insult. With intracellular recordings, CA3 post-ischemic neurons had a more depolarized resting membrane potential but no change of the input resistance, spike threshold and amplitude, fast and slow afterhyperpolarization (AHP) or ADP, and firing properties in response to depolarizing pulses. With both field and whole-cell recordings, synaptic responses were similar in control and post-ischemic neurons. Although there were no spontaneous network-driven discharges, the post-ischemic synaptic network had a smaller threshold to generate evoked and spontaneous synchronized burst discharges. Thus lower concentrations of convulsive agents (kainate, high  $K^+$ ) triggered all-or-none network-driven synaptic events in post-ischemic neurons more readily than in control ones. Also, paired-pulse protocol generates, in post-ischemics but not controls, synchronized field burst discharges when interpulse intervals ranged from 60 to 100 ms. In conclusion, 2–4 mo after the insult, the post-ischemic CA3 pyramidal cells are permanently depolarized and have a reduced threshold to generate synchronized bursts. This may explain some neuropathological and behavioral consequences of ischemia as epileptic syndromes observed several months to several years after the ischemic insult.

## INTRODUCTION

The brain is critically dependent on its blood flow for a continuous supply of oxygen and glucose (Siesjö et al. 1978). Only a few minutes of severe global ischemia can induce selective damage to particularly sensitive brain structures, including the hippocampal formation. In this structure, an ischemic episode induces in human and experimental animals a delayed selective damage of CA1 pyramidal neurons (2 to 4 days after ischemic episode), whereas CA3 and dentate gyrus neurons are largely resistant (Petito et al. 1987; Pulsinelli 1985; Zola-Morgan et al. 1992). Most of the studies on ischemia have focused on short-term effects (up to 1 wk) (for review see Schmidt-Kastner and Freund 1991), and despite several immunohistological studies (Schmidt-Kastner and Freund 1991), the long-term modifications and the physiology of the post-ischemic adult hippocampal network are poorly known. Only recently, long-term abnormal activities have been reported in post-ischemic neocortical pyramidal neurons (Luhmann et al. 1995; Mittmann et al. 1998). The possibility of persistent changes of synaptic network activity after an ischemic stroke is

of major interest as patients surviving this type of insult often express delayed epileptic syndromes months or years after the initial insult (Cocito et al. 1982; Kilpatrick et al. 1990; McNamara 1979). The CA3 region is a good candidate to subserve these long-term alterations because this region is one of the most susceptible regions in the brain for the generation of seizures (Green 1964; Hablitz and Johnston 1981). In addition, the CA3 pyramidal cells have lost their principal target cells (CA1 neurons) but still receive most of their excitatory inputs including the granule cells of the fascia dentata (Schmidt-Kastner and Freund 1991), and it is known that the neurons deprived of their projecting sites or after axotomy are often hyperexcitable (Chung et al. 1993; Prince et al. 1997).

Using post-ischemic hippocampal slices, we now report that several months after the ischemic insult, CA3 pyramidal cells display a more depolarized resting membrane potential and have a lower threshold to generate synchronized bursts than controls. Therefore in addition to the short-term effect of ischemia, i.e., the degeneration of CA1 pyramidal neurons, there are long-term changes in the activity of CA3 pyramidal cells that may modify the hippocampal network properties and may have several important pathophysiological implications.

## METHODS

### *Animal models*

Experiments involving animals were approved by French ethical science committee (statement no. 04223). Rats had access to food and water ad libitum and were housed under a 12-h light–dark cycle at 22–24°C.

### *Forebrain ischemia*

The ischemic episode was achieved by four-vessel occlusion as described previously by Pulsinelli et al. (1979). On the first day, male Wistar rats weighing 180–200 g (Charles River, France) were anesthetized with intraperitoneal injection of 5% chloral hydrate (350 mg/kg). Both vertebral arteries were electrocauterized in the alar foramina of the first cervical vertebra and atraumatic clamps set on both carotid arteries. The animals were then allowed to recover from anesthesia overnight. The next day, the carotid arteries were clamped in the unanesthetized rats for 20–25 min. The carotid artery clamps were then released and animals were allowed to survive until experiments (2–4 mo later). Only rats that were unresponsive, had lost their righting reflex, and developed fully dilated pupils during carotid clamping were included in the study. Rats with only electrocauterized vertebral arteries (sham-operated rats) and controls rats of the same age were used as controls because we did not observe significant differences between the two populations of rats. Within the first 24 h after the ischemic insult, only 25–30% of the animals displayed a

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status epilepticus and subsequently died. After this critical postoperative period (around 48 h), we did not observe apparent chronic seizure activity in the surviving rats. However, we did not monitor [electroencephalograph (EEG) or video camera] these post-ischemic rats and we cannot exclude that they could have presented episodic seizures.

### Hippocampal slices preparation

Experiments were performed in CA3 hippocampal neurons in slices obtained from 350–500 g male Wistar rats, 2–4 mo after ischemia. The animals were intracardially perfused, under chloral hydrate (350 mg/kg) anesthesia, with an ice-cooled and oxygenated modified-artificial cerebrospinal fluid (mACSF, 0–3°C) containing (in mM): 2 KCl, 0.5 CaCl<sub>2</sub>, 7 MgCl<sub>2</sub>, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, 26 NaHCO<sub>3</sub>, 11 glucose, and 250 sucrose equilibrated with 95% O<sub>2</sub>-5% CO<sub>2</sub> (pH = 7.4). After decapitation, the brain was quickly removed from the skull, hippocampi were dissected free, and 400- $\mu$ m-thick transverse slices were prepared by using a Leica VT 1000E tissue slicer in the same 0–3°C ice-cooled mACSF equilibrated with 95% O<sub>2</sub>-5% CO<sub>2</sub> (pH = 7.4). The slices were then incubated at room temperature in an oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 3.5 KCl, 2 CaCl<sub>2</sub>, 1.3 MgCl<sub>2</sub>, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, 25 NaHCO<sub>3</sub>, 11 glucose, equilibrated with 95% O<sub>2</sub>-5% CO<sub>2</sub> (pH = 7.4), as previously described (Cherubini et al. 1987). After a 2-h recovery period, hippocampal slices were transferred one at a time to a submerged recording chamber and continuously superfused (2.5–3 ml/min at 30–32°C) with ACSF.

### Electrophysiological recordings

Electrophysiological recordings were obtained from CA3 pyramidal neurons by using the extracellular, intracellular, and whole-cell recording techniques. Extracellular recordings were performed by using a World Precision Instrument amplifier and glass microelectrode (2–3 MOhms) filled with ACSF. Intracellular recordings were performed in current-clamp mode by using an Axoclamp 2 amplifier (Axon Instruments) and KCl-filled glass microelectrode (3 M, 50–80 MOhms); bridge balance was checked repeatedly during the experiment and capacitive transients were reduced to a minimum by negative capacity compensation. Whole-cell recordings were performed in voltage-clamp mode by using an Axopatch 200A amplifier (Axon Instruments). Patch electrodes had a resistance of 4–6 MOhms when filled with a Kgluconate (Kglu) internal solution that contained (in mM): 120 Kglu, 10 KCl, 10 NaCl, 1 CaCl<sub>2</sub>, 2 MgATP, 0.5 GTP, 10 EGTA, 10 HEPES, pH 7.3 (intracellular free Ca<sup>2+</sup> = 100 nM). Biocytin (0.5%) was added to the patch pipette solution for morphological analysis and passively diffused into neurons. For synaptic recordings, slices were stimulated by a bipolar electrode placed into the hilus of dentate gyrus. Stimulation parameters were 20–50  $\mu$ s duration, 10–80 V intensity, and 0.033 Hz frequency.

### Histological procedures

The extent and the specificity of the lesion in the post-ischemic hippocampus was determined by cresyl violet staining. The avidin-biotinylated horseradish peroxidase complex (ABC) reaction was used to visualize the biocytin-filled cells. After recording, slices were fixed overnight at 4°C in a solution containing 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). After cryoprotection in 20% sucrose, slices were quickly frozen in dry ice and recut in 60- $\mu$ m-thick transverse sections by using a cryostat. After several rinsings in a Tris buffer saline (TBS, 0.1 M Tris; 1% NaCl, pH = 7.3), the sections were processed overnight with the standard ABC solution (Vectastain Elite ABC Kit, Vector Laboratories). After several rinsings in Tris-HCl (0.1 M, pH = 7.3), the sections were reacted with diaminobenzidine (DAB, 50 mg/100 ml Tris-HCl), as a chromogen, and H<sub>2</sub>O<sub>2</sub> (0.01%) for 15 min. The sections were then rinsed in Tris-HCl,

stained with Giemsa, dehydrated in graded ethanol, cleared in xylene, and mounted in Permount. The labeled cells were then visualized and drawn with the aid of a Camera Lucida by using either a  $\times 20$  or a  $\times 50$  oil-immersion objective.

### Materials

6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D-2-amino-5-phosphonovaleric acid (D-APV) were a gift from Novartis (Switzerland). Biocytin, kainate, and all other drugs were purchased from Sigma (St. Louis, MO). CNQX, D-APV, and kainate were diluted in oxygenated ACSF and bath-applied.

### Data analysis

Membrane responses were displayed on a Nicolet digital oscilloscope and digitized and stored with a personal computer by using a Labmaster interface card (DIPSI, Asnieres, France). Data were analyzed off-line by using Acquis1 software (G. Sadoc, France). To study passive membrane properties and spike parameters, the pyramidal cells were recorded intracellularly. The input resistance was measured by using small (20 pA) 300-ms-square negative-current pulses and calculated from the slope of the voltage-current (*V-I*) curve. The reference points used to measure functional spike parameters from digitized traces are depicted in Fig. 1*D*. The spike parameters included the following: 1) spike threshold (*a*); 2) spike amplitude (the voltage difference between *a* and *b*); 3) fast afterhyperpolarization (AHP) amplitude (the voltage difference between *a* and *c*); 4) afterdepolarizing potential (ADP) amplitude, if present (the voltage difference between *c* and *d*). Data are presented as means  $\pm$  standard error (SE). Statistical significance ( $P < 0.05$ ) was assessed by using the Student's *t*-test analysis (unpaired data).

## RESULTS

In the present study, the intrinsic and network properties of the post-ischemic CA3 neurons were examined 2–4 mo after a transient global ischemic insult when most of CA1 pyramidal neurons have degenerated (Fig. 1*A*). Some of those post-ischemic pyramidal cells were labeled with biocytin to be reconstructed ( $n = 7$ ). Reconstruction of these neurons showed that the CA3 post-ischemic cells conserved pyramidal features with basal and apical dendrites as the control neurons. Both long-shaft (with a long and thin apical dendrite, Fig. 1*B*) and short-shaft (with a short and thick apical dendrite, not shown) pyramidal neurons were observed in post-ischemic hippocampi as in control hippocampi (Fitch et al. 1989).

### CA3 pyramidal neurons are more depolarized several months after ischemia

With intracellular recordings, 2–4 mo after the ischemic insult, the resting membrane potential of CA3 pyramidal neurons shifted from  $-72.6 \pm 1.8$  mV ( $n = 17$ ) to  $-67.6 \pm 1.4$  mV ( $n = 16$ ,  $P = 0.0174$ , Fig. 2*A*). Therefore post-ischemic pyramidal cells displayed a resting membrane potential closer to the spike threshold than control neurons (see Fig. 2*B* and Table 1). In contrast, the input resistance of post-ischemic neurons was similar to that of control cells (see Table 1). A brief (3–5 ms) and a long (200–1000 ms) depolarizing pulse evoked similar responses in both control and post-ischemic cells, respectively, a solitary spike and a burst of action potentials (Fig. 1, *D* and *E*). Several other parameters were also not modified after ischemia including: spike threshold and amplitude, fast AHP and ADP amplitudes (see Table 1), and peak

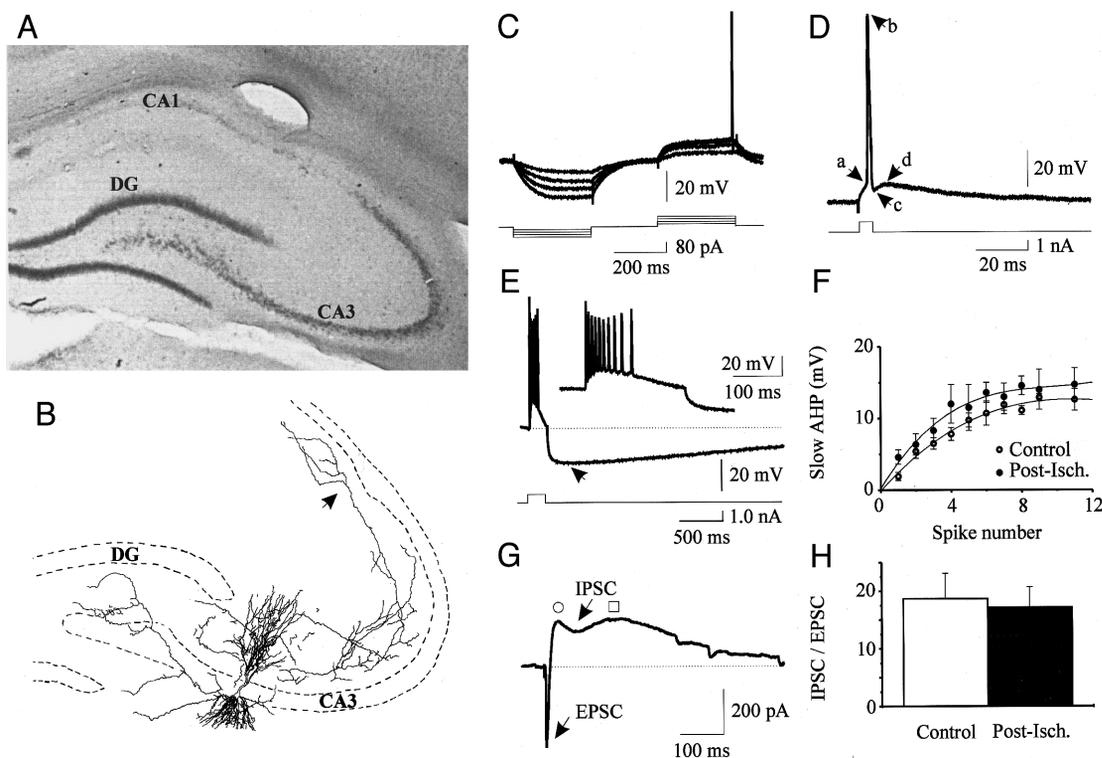


FIG. 1. Histological and electrophysiological properties of post-ischemic CA3 pyramidal cells. *A*: cresyl violet staining of a coronal section of rat hippocampus 3 mo after ischemia. Note that CA1 pyramidal layer disappeared, whereas the CA3 region and the dentate gyrus are preserved. *B*: camera lucida drawing of a 3-mo post-ischemic CA3 pyramidal neuron labeled intracellularly with biocytin. Arrow: Schaffer collaterals projecting to the CA2 area. Note that pyramidal features of this post-ischemic cell are preserved. *C–F*: intracellular recordings. *C*: hyperpolarization (*left*) and depolarization (*right*) of membrane potential in response to negative (*left*) and positive (*right*) current pulses in a post-ischemic CA3 pyramidal neuron ( $V_m = -65$  mV). Note that the last depolarizing current pulse evokes an action potential. *D*: action potential evoked by a brief positive current pulse ( $V_m = -65$  mV) in a post-ischemic CA3 pyramidal neuron; *a*, *b*, *c*, and *d* are the reference points used to measure the functional parameters of the action potential. *E*: burst of action potentials evoked by a long positive current pulse in a post-ischemic CA3 pyramidal neuron ( $V_m = -65$  mV). Arrow: the slow AHP that develops after the burst discharge. Insert (*top*) displays the burst discharge at a faster time scale. *F*: plot of mean amplitude of slow afterhyperpolarization (AHP) vs. spike number in control ( $n = 12$ ,  $\circ$ ) and post-ischemic ( $n = 13$ ,  $\bullet$ ) neurons. Note that slow AHP is on average not significantly different in control and post-ischemic neurons. *G*: whole-cell recording of synaptic response evoked by electrical stimulation of hilus of the dentate gyrus in a post-ischemic CA3 pyramidal neuron. Note that synaptic response displays a classical EPSC and IPSC sequence (including the GABA-A,  $\circ$ , and GABA-B,  $\square$ , receptor-mediated responses). *H*: histogram of the mean ratio of the inhibitory postsynaptic current (IPSC) vs. excitatory postsynaptic current (EPSC) (measured as surface area) in control ( $n = 11$ , open column) and post-ischemic ( $n = 11$ , closed column) neurons. Note that this ratio is not significantly different in control and post-ischemic neurons. In this and following figures, synaptic responses are evoked by hilar stimulations; bars, SE.

amplitude of the slow AHP (see Fig. 1*F*). Therefore post-ischemic CA3 pyramidal cells have a resting membrane potential closer to the spike threshold and may fire more easily than control neurons.

#### CA3 pyramidal neurons are more excitable several months after ischemia

Because post-ischemic CA3 pyramidal neurons have a more depolarized resting-membrane potential, we hypothesized that the CA3 pyramidal network may generate more readily synchronized burst discharges in post-ischemic slices than in control ones. To test this assumption, we first studied the evoked synaptic responses in normal conditions in the post-ischemic CA3 pyramidal cells by using whole-cell ( $V_H = -60$  mV) and extracellular recordings. The synaptic response was evoked by hilar stimulation and compared with the synaptic response recorded in hippocampal slices from control rats. In those conditions, the post-ischemic-evoked synaptic response presented a classical waveform without synchronized burst dis-

charges as in control slices ( $n = 23$ , see Fig. 1*G*, and Fig. 3*A*). In addition, there was not a significant change in the ratio of the inhibitory postsynaptic current (IPSC) (including the GABA-A and GABA-B receptor-mediated responses) versus the excitatory postsynaptic current (EPSC) in the post-ischemic cells ( $n = 11$ ) compared with the control neurons ( $n = 11$ ,  $P = 0.79$ , see Fig. 1*H*). Therefore in normal conditions, there was no apparent change in the excitability of the post-ischemic CA3 pyramidal network.

We then hypothesized that the post-ischemic pyramidal cells may have a lower threshold to generate synchronized burst discharges in the presence of convulsive agents. To test this assumption, different concentrations of  $K^+$  and kainate were bath applied (Ben-Ari and Gho 1988; Korn et al. 1987). When the external concentration of  $K^+$  was raised to 5 mM, the hilar stimulation evoked an all-or-none interictal burst of population spikes (50–100 ms duration) in the post-ischemic CA3 pyramidal cells ( $n = 6$ ) in contrast to the control CA3 pyramidal cells ( $n = 6$ , Fig. 3, *A* and *B*, and Fig. 4). In the presence of higher extracellular concentration of  $K^+$  (7.5 mM), which

generates network-driven synaptic events in CA3 area (Ben-Ari and Gho 1988; Korn et al. 1987), the synchronized all-or-none burst discharge was much more robust in the post-ischemic CA3 pyramidal cells ( $n = 5$ ) than the control cells ( $n = 5$ , Fig. 3, A and B). We also observed that, in presence of 5 and 7.5 mM  $[K^+]_0$ , spontaneous interictal burst discharges developed more frequently in post-ischemic CA3 pyramidal cells ( $n = 5$ ) than in control cells ( $n = 5$ ; Fig. 3B). Similarly, a low dose of kainate (50 nM, 4 min duration) triggered a burst of population spikes in post-ischemic pyramidal cells ( $n = 5$ ) but not in control neurons ( $n = 5$ , Fig. 3C). With higher concentrations of kainate (100 or 200 nM, 4 min duration), the number of population spikes increased more significantly in post-ischemic neurons ( $n = 5$ ) than in control cells ( $n = 6$ , Fig. 3C). Spontaneous interictal bursts of population spikes also developed more frequently in the post-ischemic CA3 pyramidal neurons ( $n = 5$ ) than in control cells ( $n = 6$ ) in the presence of 100 and 200 nM kainate (Fig. 3C). The burst discharges evoked by bath application of high concentration of  $K^+$  (5 and 7.5 mM) or in the presence of kainate were mediated by glutamatergic receptors, because they were abolished in the presence of 10  $\mu$ M CNQX and 50  $\mu$ M D-APV (not shown). Therefore several months after the ischemic insult, CA3 pyramidal neurons had a lower threshold to generate glutamatergic network-driven bursts than control cells.

#### Paired-pulse stimulation evoked a burst discharge in post-ischemic neurons

A paired-pulse protocol has been frequently used to test the excitability of CA1 pyramidal cells in epileptic rats in normal

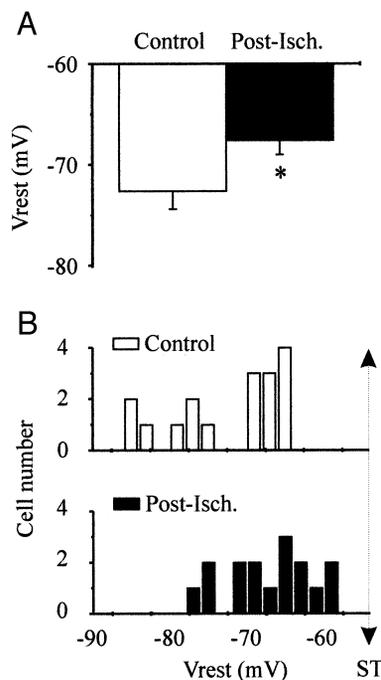


FIG. 2. Resting membrane potential of CA3 post-ischemic neurons is shifted to more positive value. A: histogram of the mean resting membrane potential ( $V_{rest}$ ) recorded intracellularly in control ( $n = 17$ , open column) and post-ischemic ( $n = 16$ , closed column) neurons. Note that  $V_{rest}$  is significantly more depolarized in post-ischemic vs. neurons than in control neurons. B: histogram distribution of cell number vs.  $V_{rest}$  (bin = 2 mV), in control (top, open column), and post-ischemic (bottom, closed column) neurons. Note shift of  $V_{rest}$  toward the spike threshold potential (ST) in post-ischemic neurons. In this and following figures, \*, significant difference ( $P < 0.05$ , Student's  $t$ -test).

TABLE 1. Long-term effect of global ischemic insult on intrinsic properties of resistant CA3 pyramidal neurons

| Parameters                       | Control Cells        | Post-Ischemic Cells    |
|----------------------------------|----------------------|------------------------|
| $V_{rest}$ (mV)                  | $-72.6 \pm 1.8$ (17) | $-67.6 \pm 1.4$ (16)*  |
| Input resistance (M $\Omega$ )   | $79.8 \pm 7.3$ (17)  | $84.3 \pm 9.5$ (16)    |
| Spike threshold (mV)             | $-55.5 \pm 1.0$ (17) | $-56.65 \pm 0.95$ (16) |
| Spike amplitude (mV)             | $74.5 \pm 1.6$ (17)  | $77.9 \pm 1.9$ (16)    |
| fAHP amplitude (mV)              | $4.5 \pm 0.3$ (15)   | $5.2 \pm 0.5$ (15)     |
| ADP amplitude (mV)               | $3.1 \pm 0.3$ (8)    | $3.05 \pm 0.7$ (5)     |
| Burst duration (ms)              | $50.7 \pm 6$ (9)     | $58.8 \pm 7.3$ (10)    |
| Spike frequency (Hz)             | $118 \pm 9.3$ (9)    | $130.6 \pm 9.9$ (10)   |
| $V_{rest}$ -spike threshold (mV) | $15.85 \pm 1.9$ (17) | $10.7 \pm 1.8$ (16)*   |

Values are means  $\pm$  SE with number of cells in parentheses. \*  $P < 0.05$ , Student's  $t$ -test.

conditions (Bernard and Wheal 1995; Cornish and Wheal 1989). In an attempt to test the capability of post-ischemic CA3 pyramidal cells to generate synchronized bursts in absence of any convulsive agent, we used the same type of paradigm. A conditioning (first stimulation) and a test (second stimulation) pulse of equal intensity of stimulation were applied to the hilar area with an interpulse interval ranging from 10 to 100 ms. The strength of the intensity of the first pulse was adjusted to evoke a single population spike in the conditioning response. In control CA3 pyramidal cells, only one or two population spikes were evoked by the test pulse at all interpulse intervals ( $n = 10$ , Fig. 5, A and B). In contrast, in post-ischemic CA3 pyramidal cells ( $n = 8$ ), the test pulse evoked a burst discharge when the interpulse interval ranged from 60 to 100 ms (Fig. 5, A and B). Interestingly, in two post-ischemic slices out of eight, a burst discharge was evoked by both the conditioning and the test pulse during the paired-pulse protocol; this bursting response could be still evoked by a single pulse after the protocol, suggesting a permanent alteration of the network properties (not shown). Using this protocol, we also studied the change of the slope of the synaptic transmission (recorded in the stratum radiatum) and the amplitude of the first population spike evoked by the test pulse (recorded in the pyramidal cell layer). In both control ( $n = 10$ ) and post-ischemic neurons ( $n = 8$ ), the paired-pulse protocol induced a statistically identical facilitation of the slope and the population spike (see Fig. 5, C and D).

Therefore several months after ischemia, it is possible to evoke a burst discharge in CA3 pyramidal cells by using a paired-pulse protocol without convulsive agent. This observation confirms that the post-ischemic network becomes hyperexcitable after an ischemic insult.

#### DISCUSSION

The principal result of the present report is that 2–4 mo after an ischemic insult, CA3 pyramidal cells have a more depolarized resting membrane potential and a greater susceptibility to generate all-or-none interictal burst discharges. Therefore in addition to the short-term effect of ischemia, i.e., the degeneration of CA1 pyramidal neurons, there are long-term changes in the activity of CA3 pyramidal cells that may modify the hippocampal network properties.

#### Specific intrinsic properties of the post-ischemic CA3 pyramidal neuron

Study of the intrinsic membrane properties of the post-ischemic CA3 pyramidal cells revealed that there was a clear shift of the

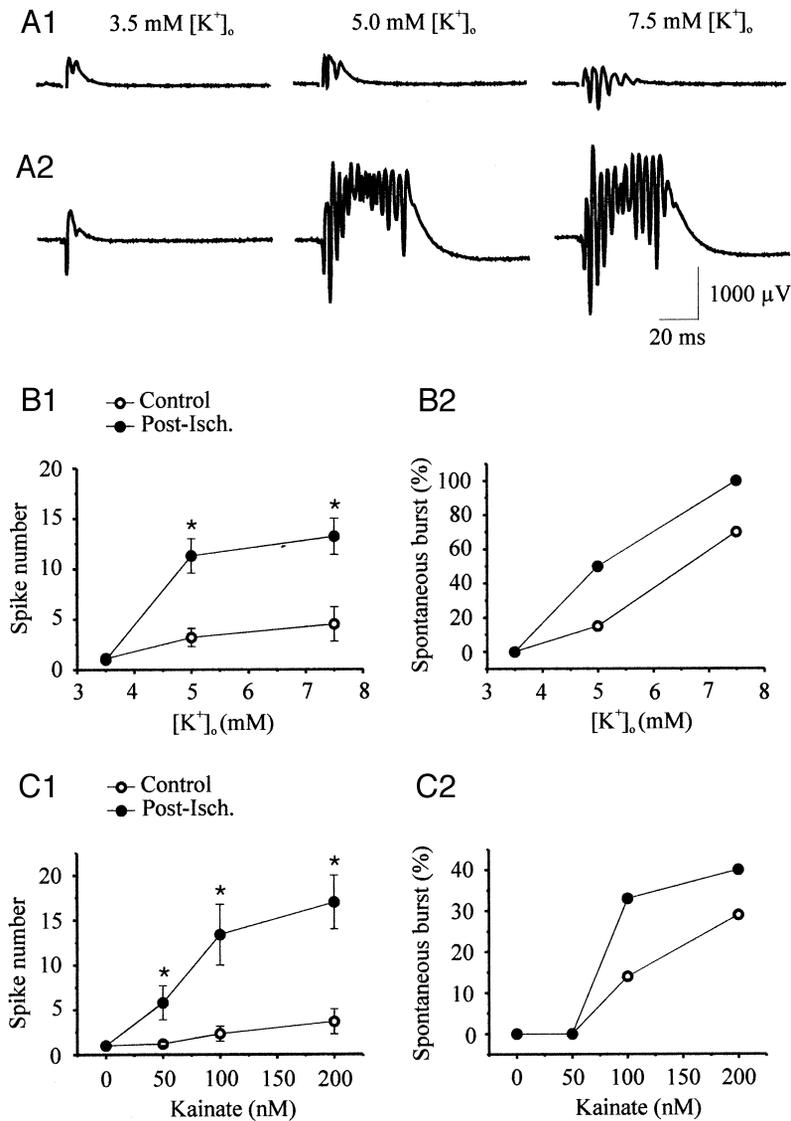


FIG. 3. CA3 post-ischemic neurons are more sensitive to convulsive agents than the control cells. *A*: somatic field potentials recorded in control (*A1*) and post-ischemic (*A2*) cells, in the presence of 3.5, 5, and 7.5 mM  $[K^+]_o$ . Note that 5 mM evoked a robust synchronized burst discharge in post-ischemic neurons in contrast to control cells. *B–C*: plot of the spike number of the synchronized burst (1) and percentage of spontaneous synchronized burst (2) vs.  $[K^+]_o$  (*B*) or different concentrations of kainate (*C*) in control ( $\circ$ ) and post-ischemic ( $\bullet$ ) neurons. Note that increasing  $[K^+]_o$  (*B*) or kainate concentration (*C*) evokes more robust synchronized burst discharges in post-ischemic neurons than in control cells, as well as more frequent spontaneous interictal synaptic responses.

resting membrane potential of CA3 pyramidal neurons toward positive values. In contrast, those cells did not display significant changes of the input resistance, the spike threshold, the spike

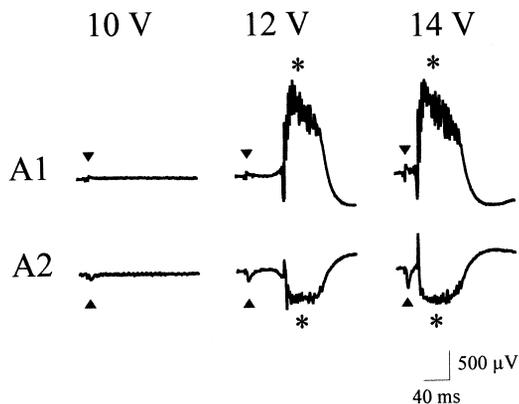


FIG. 4. Synchronized burst discharges are all-or-none events. *A*: somatic (*A1*) and dendritic (*A2*) field potentials evoked by different intensities of stimulation (10–14 V) in post-ischemic cells, in the presence of 5 mM  $[K^+]_o$ .  $\blacktriangle$ , evoked monosynaptic response; \*, polysynaptic response. Note that synchronized burst discharges have variable onset latency and all-or-none characteristics.

amplitude, the accommodation, the fast AHP, the ADP, and the slow AHP in comparison with control cells. The resting membrane potential of pyramidal neurons is a key factor in the regulation of the cell excitability and mainly depends on the  $Na^+/K^+$  ATPase (Haglund et al. 1985; Haglund and Schwartzkroin 1990) and potassium channel activities (for review see Storm 1990). Down-regulation of the  $Na^+/K^+$  ATPase and potassium channel activities has been described in different models of lesions. For example, a decrease of the  $Na^+/K^+$  ATPase has been reported in CA1 area several weeks after lesion of the CA3 pyramidal cells (Anderson et al. 1994) and postaxotomy mammalian motoneurons display down-regulated potassium currents (Gustafsson 1979; Laiwand et al. 1988). Therefore similar phenomena may take place in the partially deafferented post-ischemic CA3 pyramidal neurons and underlie the depolarization of the resting membrane potential. The reduction of the membrane potential (without a change of input resistance) after an ischemic insult is not a unique feature of CA3 pyramidal cells. Previous studies have shown similar results in neocortical neurons submitted to a fore-brain ischemic insult (Luhmann et al. 1995). In contrast, neocortical pyramidal cells recorded in the infarct border zone induced by a focal ischemia do not display modifications of their resting

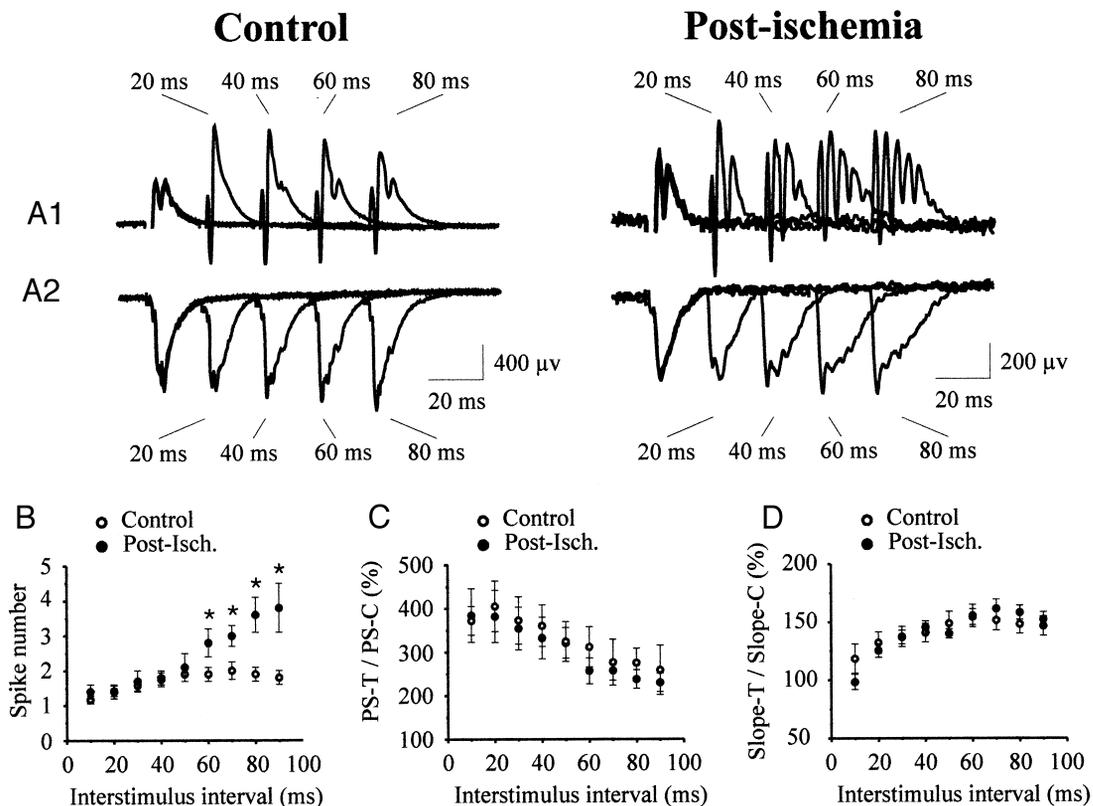


FIG. 5. Paired-pulse stimulation evokes a burst discharge in post-ischemic neurons. *A*: somatic (*A1*) and dendritic (*A2*) field potentials evoked by paired-stimulation with interpulse intervals ranging from 10 to 100 ms in control (*left*) and post-ischemic (*right*) cells. The first stimulation evokes the conditioning response and the second stimulation evokes the test response. Note that synchronized burst discharges are evoked by the paired-pulse protocol in post-ischemic neurons in contrast to control cells. *B*: plot of the spike number of the test response vs. interstimulus intervals in control ( $n = 10$ ,  $\circ$ ) and post-ischemic ( $n = 8$ ,  $\bullet$ ) neurons. Note that burst discharges develop, on average, for an interpulse interval ranging from 60 to 100 ms in post-ischemic neurons. *C*: plot of the amplitude of the test population spike (PS-T, first population spike of the test response) as the percentage of the conditioning population spike amplitude (PS-C, first population spike of the conditioning response) vs. interstimulus interval in control ( $n = 10$ ,  $\circ$ ) and post-ischemic ( $n = 8$ ,  $\bullet$ ) neurons. *D*: plot of the slope of the test dendritic field potential as the percentage of the slope of the conditioning dendritic field potential vs. interstimulus interval in control ( $n = 10$ ,  $\circ$ ) and post-ischemic ( $n = 8$ ,  $\bullet$ ) neurons. Note that the paired-pulse stimulation evokes a facilitation of the population spike and slope of the field potential which is not significantly different in control and post-ischemic neurons.

membrane potential (Mittmann et al. 1998). Therefore the modification of the membrane potential in the post-ischemic-resistant neurons will depend on the type of ischemic insult.

#### Synaptic network properties of the post-ischemic CA3 pyramidal cells

Examination of the synaptic transmission in the post-ischemic CA3 pyramidal cells showed that there is no seizure-like activity in normal conditions. However, we observed that those neurons are more susceptible than the control ones to display evoked and spontaneous synchronized burst discharges when low doses of convulsive agents (as  $K^+$  and kainate) were bath applied. We suggest that the positive shift of the resting membrane potential toward the spike threshold may facilitate the generation of the synchronized burst discharges in post-ischemic CA3 pyramidal neurons. However, we cannot exclude alternative hypothesis. Indeed, raising the extracellular concentration of  $K^+$  or bath application of kainate have pleiotropic effects in addition to inducing a depolarization (Ben-Ari and Gho 1988; Rutecki et al. 1985). This includes a reduction of GABAergic inhibition (Fisher and Alger 1984; Korn et al. 1987; Rovira et al. 1990; but see also Cossart et al. 1998;

Frerking et al. 1998) and a down-regulation of potassium current as  $I_Q$  and  $I_C$  (Gho et al. 1986). Therefore the generation of synchronized burst discharge may be due to a greater vulnerability of these conductances to the convulsive agents in post-ischemic neurons. We also showed that synchronized burst discharges could be induced in normal ACSF conditions when a paired-pulse protocol was used. We observed that these burst discharges developed when the interpulse interval ranged from 60 to 100 ms. At this interpulse interval the GABA-A receptor-mediated inhibition is reduced by 25–50% (Davies et al. 1990). We suggest that the same paired-pulse depression of GABAergic inhibition facilitates more efficiently the firing of the post-ischemic cells than the control neurons, because the post-ischemic cells have a more depolarized resting membrane potential. However, we cannot exclude that the paired-pulse depression of GABAergic inhibition is more robust in the post-ischemic neurons compared with the control neurons. Further experiments will be necessary to clarify this point. All together these different observations clearly demonstrate that the post-ischemic CA3 pyramidal cells are more excitable than the control ones and that synchronized burst discharges can be induced in normal ACSF if two synaptic events are successively evoked within the appropriate time window. Interest-

ingly, these results are reminiscent of previous observations performed in neocortical post-ischemic neurons. However, in contrast to the present study, these neurons display epileptiform activities evoked by a single shock of stimulation even in normal conditions, due to a drastic reduction of GABAergic inhibition in favor of excitatory responses (Luhmann et al. 1995; Mittmann et al. 1998). The role of the ischemic insult and/or the degeneration of the CA1 pyramidal cells in the change of excitability of the CA3 pyramidal cells remain at present unclear. A global hypoxic insult, performed between p10 and p12, induces seizure activity in CA1 and CA3 areas of the rat hippocampus but not at younger or older ages (Jensen et al. 1998). In this model, the hypoxic insult was not associated with lesion. It is possible that the change of excitability of CA3 pyramidal cells induced by ischemic insults in our study is partly a consequence of the lesion of CA1 pyramidal cells and the reactive plasticity that may take place, because the neurons deprived of their projecting sites or after axotomy are often hyperexcitable (Chung et al. 1993; Prince et al. 1997).

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