Neuroprotective Effects on Somatotopic Maps Resulting from Piracetam Treatment and Environmental Enrichment After Focal Cortical Injury

C. Xerri, Y. Zennou-Azougui, and J-O Coq

Abstract

Acute and chronic postlesion reorganization of the cortical maps was examined in adult rats using electrophysiological mapping of the forepaw area in the primary somatosensory cortex. Recordings were made before and after (first 12 hr and 3 wk) induction of a focal thermal-ischemic lesion to a restricted part of the forepaw area. The influence of an anti-ischemic substance (piracetam) and housing in an enriched environment (EE) or impoverished environment (IE) on the organization of the spared regions of the cortical maps adjacent to the lesion was investigated. The results revealed (1) a gradual expansion of the injured zone and a cellular loss that were smaller in the piracetam-treated (PT) rats than in the placebo (PL) rats; (2) a better preservation of the somatotopic organization and the neuronal responsiveness in the maps of the PT rats during the first 12 hr after the lesion; (3) a gradual compression and fragmentation of the remaining forepaw map over the first 3 postlesion wk. These changes were found in all PL rats, with the most detrimental effects in the animals exposed to an IE. In the PT-EE animals, a contrasting substantial preservation of the map was observed. (4) Cortical responsiveness was diminished in the PL rats, whatever the environment, and in the PT-IE rats; but it was not significantly affected in the PT-EE animals. The findings demonstrate the protective function of acute piracetam treatment on electrophysiological properties of cortical neurons within the peri-infarct tissue and highlight the neuroprotective effects of an environmental therapy combined with the piracetam treatment during the weeks after ischemic damage.

Key Words: anti-ischemic substance; cortical plasticity; cutaneous forepaw representation; electrophysiological mapping; enriched/impoverished environment; ischemia; piracetam

Introduction

Cutaneous and proprioceptive inputs are encoded by peripheral mechanoreceptors and are transmitted in a topographic order through sensory fibers and integrated within different somatosensory brain structures, where they constitute somatotopically organized representational maps. Their organizational features are assessed by determining the properties of cortical neuron responses to peripheral stimulation (receptive field sensory submodality, location, and size).

The maps in the primary somatosensory (S1[1]) cortex areas are dynamic constructs that retain a capacity for remodeling throughout life. These representational maps undergo changes after peripheral denervation (Byrne and Calford 1991; Calford and Tweedale 1988, 1991a; Kelahan and Doetsch 1984; Merzenich et al. 1983; Rasmusson and Turnbull 1983; Salimi et al. 1994; Silva et al. 1996; Turnbull and Rasmusson 1990). It is well established that these map changes can occur within minutes and continue over weeks and years. Few reports have documented representational map remodeling after cortical damage. Furthermore, despite the importance of early cortical changes for later stages of map reorganization, and hence for functional recovery, alterations in the organizational features of the somatosensory cortical maps that occur immediately after induction of a focal cortical injury are largely unknown.

It has been shown that over a period of weeks after focal ischemic lesion to area 3b of the S1 cortex, small clusters of neurons in the peri-infarct cortical sectors begin to respond to subthreshold inputs from skin regions formerly represented in the ischemic zone (Doetsch et al. 1990; Jenkins and Merzenich 1987; Xerri et al. 1998). We have shown that after a focal infarct in area 3b, which damaged a large area of the hand representation, the skin surfaces stimulated during a manual dexterity task regained a representation in areas 1 and 3a, implying that neurons formerly excited dominantly by deep inputs responded to cutaneous inputs from the trained skin surfaces after the lesion (Xerri et al. 1998). This map reorganization paralleled behavioral recovery and was ascribed to the rehabilitative effects of training through experience-dependent mechanisms of neuroplasticity.

Indeed, it is well established that alteration of the organizational features of the somatosensory maps are determined by the spatiotemporal pattern of ascending sensory

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[1]Abbreviations used in this article: ACh, acetylcholine; ANOVA, analysis of variance; EE, enriched environment; GABA, gamma-aminobutyric acid; IE, impoverished environment; lsmeans, least square means; NMDA, *N*-methyl D-aspartate; n.s., not significant; PL, placebo; PT, piracetam-treated; RF, receptive field; SD, standard deviation; SI, primary somatosensory.

input associated with new sensory experience and learning (Byl et al. 1996; Coq and Xerri 1998, 1999a; Jenkins et al. 1990; Recanzone et al. 1992; Wang et al. 1995; Xerri et al. 1994). Interestingly, numerous studies have shown that housing in an enriched environment, which provides a large variety of stimuli that promotes physical exercise and social interaction (Bennett et al. 1964; Diamond et al. 1964; Globus et al. 1973; Holloway 1966), or in an impoverished environment that induces sensorimotor deprivation and social isolation (Bryan and Riesen 1989; Martin et al. 1991), results in biochemical and morphological alterations in the neocortex. In previous studies, we showed that exposure to an enriched or impoverished environment induced specific alterations in the forepaw representation in the S1 cortex of adult rats (Coq and Xerri 1998, 1999a).

The influence of an enriched environment on functional outcome after various brain injuries has long been investigated (Galani et al. 1997; Kelche et al. 1987; Will 1981; Will et al. 1977). In particular, several reports have described the effects of environmental enrichment on the behavioral recovery after focal ischemia (Biernaskie and Corbett 2001; Grabowski et al. 1995; Johansson and Ohlsson 1996; Ohlsson and Johansson 1995). However, little attention has been paid to the question of how sensorimotor experience through enriched versus impoverished housing conditions can facilitate the remodeling or preserve the functional organization of the somatotopic maps after injury to the S1 cortex. Yet, this issue is particularly relevant to the elucidation of the specific neurophysiological mechanisms underlying functional compensation or restitution after brain damage and to the evaluation of the impact of rehabilitative procedures on these mechanisms.

In this article, we report the main results obtained in previous cortical mapping studies (Coq and Xerri 1999b; Xerri and Zennou-Azogui 2003). We document the following: (1) the immediate changes in the somatotopic representation in the cortical sectors adjacent to a focal ischemic injury to the S1 cortex; (2) the effects of the early administration of an anti-ischemic substance, Piracetam (UCB-Pharma; Brussels, Belgium), a cyclic derivative of gamma-aminobutyric acid ($GABA^1$), on the extent of the lesion and on the lesion-induced reorganization in the cortical sectors surrounding the site of injury; (3) the influence of housing in enriched or impoverished environments on the functional remodeling of cortical zones adjacent to the lesion; and (4) the effects of chronic administration of piracetam on the functional organization of the remaining intact sectors of the somatotopic map in animals housed in enriched or impoverished environments.

Methods

Experimental Protocol

Electrophysiological mapping was performed on 52 male, 3-mo-old adult Long-Evans rats weighing from 300 to 350 g. Twenty rats were used to investigate the map alteration occurring during the first 12-hr after lesion induction (group I), and 32 rats were used to study the map changes taking place over the first 3 wk after lesion induction (group II). After weaning (30 days after birth), rats from different litters were housed for 2 mo in groups of three in Plexiglas cages (26.5 cm wide × 42.5 cm deep × 18 cm high) (i.e., in a standard laboratory environment). We recorded neuronal activity within the cortical area of the forepaw representation to obtain a control map and then induced a small neurovascular lesion within this area. Recordings were made during the first hour (T1) following the lesion to assess its initial boundaries.

Group I

Spread of the lesion was evaluated by recording from 3 to 4 hr (T2), 7 to 8 hr (T3), and 11 to 12 hr (T4) after the injury. Postlesion maps of the forepaw were elaborated from data obtained between 2 to 12 hr after the lesion induction. Whenever possible, postlesion recordings were made at the same cortical sites sampled before the lesion as identified by stable vascular landmarks. Piracetam (2-pyrrolidone-acetamide, $C_6H_{10}N_20_2$, Nootropyl; UCB 6215) or placebo substances (physiological saline, 200 mg/100 g, i.p.) were injected three times, at 1 hr, 5 hr, and 9 hr after induction of the lesion, according to a random procedure in which the experimenters did not know the identity of the injected substance. This identity was revealed after the statistical analysis was completed.

Group II

Piracetam or placebo substances were injected (200 mg/100 g, i.p.) 1 hr after the lesion induction. After the boundaries of the lesion were determined, the surgery was completed, and the animals were returned to the animal care facility and assigned to two housing environments, enriched (EE^1) or impoverished (IE^1). The rats received the piracetam (PT^1 groups: 8 EE and 8 IE rats) or the placebo substance (PL^1 groups: 8 EE and 8 IE rats) twice a day for 3 wk. Postlesion electrophysiological recordings were made on the 22nd day to map the spared regions of the forepaw representation.

Postlesion Housing Conditions

The operated rats housed in EE lived with a group of 10 male rats in a spacious cage (76 cm wide × 76 cm deep × 40 cm high). To promote tactile exploration and thus cutaneous stimulation, the EE cage contained mobile and immobile objects of various shapes and textures. These objects were renewed daily to maintain the animals' exploratory behavior. Rats in IE lived singly in small cages (23.5 × 35.5 × 19 cm) without objects.

Surgical Preparation

Experimental procedures were in accordance with *the Guide for the Care and Use of Laboratory Animals* (NRC 1996). Anesthesia was induced with halothane and maintained with sodium pentobarbital (50 mg/kg, i.p.). Animals were kept at an areflexive level of anesthesia throughout the experiment by supplemental administration of diluted pentobarbital (5 mg/kg, i.p.) as needed. The core body temperature was continuously monitored by a rectal thermistor probe and was maintained between 37° and 38°C by a heating pad. The head was held in a stereotactic frame. Posterior neck muscles were resected, and cerebrospinal fluid was drained through an opening in the dura covering the foramen magnum. After a scalp incision and the retraction of attached muscles, a craniotomy exposed part of the somatosensory cortex. For the animals in group II, the surgical and recording procedures were performed under sterile conditions and the bone flap was kept in physiological saline at 4°C. The dura was incised and resected, and the surface of the exposed parietal cortex was bathed in a thin layer of warm silicone fluid to prevent drying and edema. In group II, at the end of the first recording session, the silicone was removed with a wash of warm saline, and the dura was repositioned and covered with gelatin film (Gelfilm, Upjohn, Kalamazoo, MI). The bone flap was then reinserted and stabilized with dental acrylic. The animal's temperature was monitored until the recovery from anesthesia was complete.

On the 22nd day after the lesion, the anesthesia procedure was repeated. The bone flap was removed to allow access to the cortical zone to be mapped. After completion of the remapping procedures, the animal received a lethal dose of sodium pentobarbital (150 mg/kg, i.p.) and the brain was prepared for histological processing.

Induction of Cortical Lesion

The silicone fluid was removed and the exposed cortex was bathed in warm physiological saline. A 500-Hz radiofrequency current was delivered through the tip of a temperature-monitoring electrode in contact with the surface of the cortex. The lesion targeted the center of the forepaw representational zone. The temperature measured at its tip was gradually increased to 70°C within 1 min and maintained at this level for 1 min. In addition to destroying neural tissue, the heat generated in the brain tissue induced a focal infarct characterized by a visible occlusion of the vessels, along with a blanching of the cortical zone within the vicinity of the electrode tip.

Care was taken to preserve major arteries and veins while occluding their local branches. Radiofrequency-induced hyperthermia produces cellular injury (i.e., coagulation necrosis, neuronal shrinkage, nuclear pyknosis, and perineuronal astrocytic swelling) (Ohmoto et al. 1996), which is associated with cerebral ischemia. Moreover, localized hyperthermia induces increased extracellular glutamate concentrations that reach neurotoxic levels (Adachi et al. 1995). The functional impact of the lesion was assessed using electrophysiological recordings.

Electrophysiological Mapping Procedures

Magnified images of the exposed parietal cortex and the ventral and dorsal surfaces of the forepaw contralateral to the cerebral hemisphere to be mapped were digitized by using a high-resolution camera mounted on an operating microscope. The recording sites were located relative to the cortical surface vasculature on the digitized image of the cortex, and the cutaneous receptive fields (RFs[1]) were drawn on the forepaw images. Neurons were recorded with parylene-coated tungsten microelectrodes (~1 MΩ at 1 kHz). The responses of clusters of two to four neurons in layer IV were recorded at a depth of approximately 650 to 700 μm. At each recording site, large bursts of activity elicited by natural stimulation enabled us to classify neuronal responses as cutaneous or noncutaneous. Cutaneous RFs were defined as the skin area where just-visible skin indentation or hair deflection elicited reliable changes in multiunit activity. This stimulation was produced with a fine-tipped, hand-held glass probe and monitored by using magnifying glasses (×4). The ridges running along the glabrous skin of the digits and palm were used as landmarks to delineate the RFs. In our classification, responses to nail movements were considered as cutaneous. Noncutaneous responses were identified by more intense stimuli such as taps, pressure on muscles, tendons, or joint manipulations, when no cutaneous response was found. Cortical sites not exhibiting stimulus-evoked responses but only spontaneous discharges, were classified as unresponsive.

We elaborated maps of the forepaw representation by drawing boundaries enclosing the cortical sites in which RFs shared a common skin subdivision (i.e., finger, palmar pad). The areal extent of each region of the cutaneous map was then calculated.

To estimate changes in the responsiveness of somatosensory cortex neurons, in the group I animals, we classified the responses to just-visible skin indentation along a three-level ordinal scale (weak, clear, and strong). In the group II rats, the thresholds of neuronal responses to skin stimulation were determined using von Frey monofilaments (Semmes-Weinstein aesthesiometer; Stoelting, Wood Dale, IL), which apply indenting stimuli at a relatively constant, predetermined force. The most commonly used filaments were 3.22 (diameter: 0.152 mm; bending force: 0.166 g; bending pressure: 9.15 g/mm^2), 3.61 (0.178 mm; 0.407 g; 16.36 g/mm^2), and 3.84 (0.203 mm; 0.692 g; 21.39 g/mm^2). The stimulation consisted of pressing a filament gently against the skin, perpendicular to its surface at the center of the RF, until the filament began to bend. We repeated this procedure 5 to 10 times for each filament, using a stimulus series of increasing and decreasing strengths to determine the mechanical threshold evoking noticeable changes in neuronal discharge.

Experimental Measurements and Statistical Analysis

In an attempt to evaluate the postlesion loss of cortical tissue, we used stable vascular landmarks on the surface of the cortex. On the intact cortex image obtained in each rat in group II, we used these landmarks to demarcate a reference polygon with the lesion at its center. The polygon area surrounding the lesion was twice as large as the injured area determined on the basis of the neuronal recordings. The same vascular landmarks were used to delimit a new polygon on the images of the cortex obtained 3 wk after the lesion. The tissue loss was estimated by measuring the difference between the two polygons measured in each rat, thereby allowing the calculation of a relative decrease in the area of the reference polygon.

Cortical areas devoted to the forepaw representation (i.e., excited by stimulation of glabrous or hairy skin surfaces, or by nail movement) were calculated. For each rat, we evaluated the areal extent of the injured zone determined during the second hour following the lesion. For the group II rats, the spared portion of the representation was referred to as an "acute" postlesion area and was estimated by subtracting the injured area from the area of forepaw representation recorded before the lesion. The spared portion of the representation as measured from the map obtained 3 wk after the lesion was referred to as a "chronic" postlesion area. Relative changes in the area of the representation were expressed as follows: [(chronic area – acute area)/ acute area] × 100.

To assess more specifically the alteration of representations of glabrous, hairy skin surfaces and nail movement as parts of the forepaw representation, the corresponding parts of the map were expressed as a percentage of the whole area of the cutaneous map of the forepaw. The differences in these relative values of the size of the cortical maps (chronic area – acute area) were then calculated. The absolute sizes of glabrous and hairy RFs were measured in mm^2, normalized relative to the ventral and dorsal skin forepaw areas, respectively, and expressed as percentages. The relative RF areas measured in each rat were averaged (geometrical means), and the mean size of the RF was then computed for each group of rats. For each rat, postlesion changes in RF size were expressed as the chronic – acute differences between the corresponding mean sizes. We processed the stimulation thresholds obtained using the von Frey filaments by calculating the percentages of threshold responses obtained for a given filament's strength (3.22, 3.61, or 3.84) in each rat. Individual chronic – acute values were then calculated and averaged for each experimental group.

After we administered the Shapiro-Wilk normality test, we used the student's *t*-test, Mann-Whitney-U test, Chi-square test, analysis of variance (ANOVA[1]), which was supplemented with multiple comparisons post hoc test (Newman-Keuls) and least square means by using SAS 6.12 software. Statistical significance was set at $p < 0.05$.

Results

Cortical Map Alteration 12 Hr After Lesion

During the first hour after the lesion induction (T1), before the administration of the PL and PT substances, the injured areas determined electrophysiologically were similar for the two groups (PL: 0.18 ± 0.08 mm^2 [standard deviation (SD[1])]; PT: 0.18 ± 0.11 mm^2 [SD]). The size of lesion increased from T1 to T2 but did not differ significantly between the PL (0.42 ± 0.34 mm^2) and the PT groups (0.31 ± 0.26 mm^2) (Figure 1A) (Mann-Whitney; *p:* not significant [n.s.[1]]). The PL group showed a significant outward expansion of the silent cortical zone between T2 (0.42 ± 0.34 mm^2) and T4 (0.91 ± 0.52 mm^2) ($p < 0.007$), whereas the lesion in the PT rats remained relatively stable (T2: 0.31 ± 0.26 mm^2 vs. T4: 0.24 ± 0.36 mm^2).

The topographic organization of the forepaw representational map in the S1 cortex has been described in detail in a previous report (Coq and Xerri 1998). Briefly, the cortical sectors serving the forepaw surfaces were organized in somatotopic sequences along a rostrolateral (thenar eminence and digit 1) to caudomedial (digit five and hypothenar eminence) direction. The palm representation was situated along the medial edge of the map, and the hairy skin of the digits along the lateral margin of the map. Typically, the cortical zones representing skin surfaces were interspersed with islets displaying changes in the firing rate in response to taps, joint manipulations (noncutaneous responses), or nail movements.

The average areas of the forepaw representational maps before the lesion did not differ between the PL and the PT groups (2.14 ± 0.30 mm^2 and 2.05 ± 0.31 mm^2, respectively) (*p:* n.s.). Consistent with the lesion expansion described above, the mean net loss in cutaneous representational area calculated 12 hr after the lesion induction was significantly smaller in the PT rats (0.37 ± 0.37 mm^2) than in the PL rats (1.35 ± 0.53 mm^2) ($p < 0.0001$). Comparison of the maps derived before and 12 hr after the lesion induction revealed extensive remodeling of the representations within the spared territories of the forepaw map (Figure 1, B and C). This general reorganization was common to each map regardless of the treatment.

Indeed, substantial alterations were observed in the area devoted to a particular sensory submodality (cutaneous or noncutaneous), the location, the size, and the shape of zones serving a particular body part, not only in cortical sectors adjacent to the lesion but also in regions distant from the site of injury. Moreover, all postlesion maps showed the emergence of noncutaneous zones creating discontinuities in formerly contiguous sectors of cutaneous representation. As a consequence of these disruptions of the internal somatotopic order, the spared territories of the cutaneous maps appeared more fragmented 12 hr after the lesion induction. Despite conspicuous restructuring of organizational features in all postlesion maps, the somatotopic order seen before the lesion was better preserved in PT maps than in maps of the PL

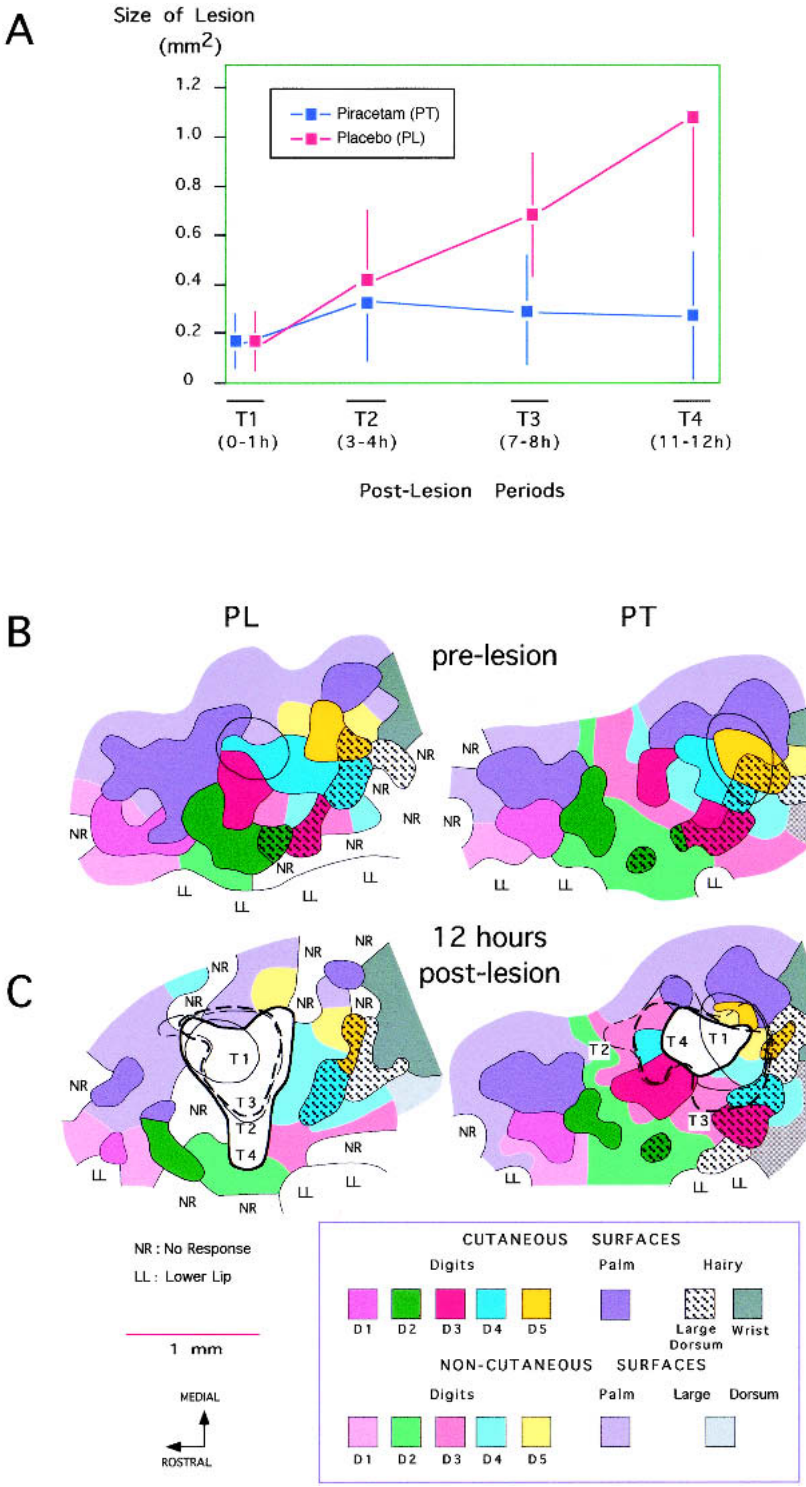


Figure 1 Effect of piracetam treatment on the acute remodeling of the forepaw representation in the S1 cortex. (A) Changes in the mean area of functional cortical lesions delimited on the basis of electrophysiological recordings over various time windows (T1 through T4) after cortical lesion induction in control (PL; red squares) and piracetam-treated rats (PT; black squares). Vertical bars represent confidence intervals ($p < 0.01$). (B-C) Somatotopic maps of representation of the forepaw obtained before (B) and up to 12 hr after a focal cortical injury (C) in typical PL and PT cases characterized by a silent zone within the injured area from T1. The silent cortical zones recorded during T1 are outlined on the prelesion maps (thin solid line). The electrophysiologically silent zones delimited over various time windows for T2 (thin broken line), T3 (thick broken line), and T4 (thick solid line) are outlined on the postlesion maps. Note that for this PT rat, the size of the silent area decreased from T3, whereas in the PL rat, the lesioned area increased over time. Modified from Coq JO, Xerri C. 1999b. Acute reorganization of the forepaw representation in the rat S1 cortex after focal cortical injury: Neuroprotective effects of piracetam treatment. Eur J Neurosci 11:2597-2608.

group, particularly in cortical sectors remote from the injured area (e.g., see rostral parts of the typical maps illustrated in Figure1C). In addition, those parts of the map located within the area damaged at T1 or T2 tended to re-emerge, at least partially, over time in sectors surrounding the lesion in the PT rats (Figure 1C).

Among the electrode penetration sites sampled before and after the lesion was induced, some were placed (PL: 532; PT: 735) at cortical sites judged to be similar by reference to stable vascular landmarks. Comparing RF submodalities (cutaneous or noncutaneous) at these paired-cortical sites allowed us to describe changes induced by the lesion and/or anti-ischemic treatment more precisely. After the lesion, in the PT rats, the cortical sites that exhibited unchanged submodality were more numerous (64.4%; 473/735) than those with a different submodality (35.6%: 262/735), whereas in the PL rats, the number of cortical sites falling within these two categories were identical (50.0%; 266/532) ($p < 0.0001$). For changes in submodality, comparison of the PL maps (N = 266) with the PT maps (N = 262) showed significant differences ($p < 0.0001$). Indeed, fewer cortical sites exhibited lesion-induced mutation from cutaneous to noncutaneous submodality in the PT rats (50.7%) than in the PL rats (62.4%). Moreover, more cortical sites exhibited changes from noncutaneous to cutaneous in the PT rats (27.5%) than in the PL rats (12.8%). Few recording sites without a reliable stimulus-evoked change in neuronal discharge before the lesion exhibited clear-cut responses to cutaneous or noncutaneous inputs after the lesion. More cortical sites classified as unresponsive before the lesion displayed cutaneous or noncutaneous responses after the lesion in the PT group (16.8%) than in the PL group (9.4%), whereas cutaneous or noncutaneous cortical sites that became unresponsive after the lesion were relatively more numerous in the PL group (15.4%) than in the PT group (5.0%).

We classified the responses to just-visible skin indentation along a three-level ordinal scale (weak, clear, and strong) to represent changes in the responsiveness of somatosensory cortex neurons. The PT and PL populations did not differ in this dimension before the lesion (Mann Whitney, *p:* n.s.). During the postlesion period examined, within the subpopulations of sites recorded at similar locations before and after the injury, the number of sites showing decreased sensitivity was smaller in the PT rats (33.0%; 77/233) than in the PL rats (58.9%; 63/107), those showing an increased sensitivity were greater in the PT rats (18.5%; 43/233) than in the PL rats (6.5%; 7/107), and those with unchanged sensitivity were greater in the PT rats (48.5%; 113/233) than in the PL rats (34.6%; 37/107) ($p < 0.0001$).

Cortical Reorganization/Preservation 3 Wk After the Focal Lesion

The percentage of tissue loss induced by the lesion was evaluated in the group II rats as a relative decrease in the reference polygon area delineated using stable vascular landmarks on the images of the cortex surface. On the average, the postlesion decrease was greater in the placebo rats (PL-IE: 29.97 ± 8.16%, SD; PL-EE: 26.12 ± 7.46%) than in the piracetam-treated rats (PT-IE: 20.86 ± 11.77%; PT-EE: 20.43 ± 8.98%) ($p < 0.03$), regardless of the postlesion environment (*p:* n.s.) (Figure 2).

The area of the injured zone, assessed on the basis of electrophysiological recordings during the second hour postlesion, was expressed in a percentage of the whole cutaneous zone of the corresponding forepaw map, which was defined as the sum of glabrous skin, hairy skin, and nail movement representational sectors of the map. The average area of the functionally defined lesion that represented approximately 0.65 ± 0.30 mm^2 was similar for the four groups of rats (PL-IE, 29.36 ± 5.89%; PT-IE, 30.17 ± 15.40%; PL-EE, 30.51 ± 15.69%; PT-EE, 29.02 ± 5.16%). As the lesion was centered within the forepaw map, the cortical sectors serving the glabrous skin surfaces and nail movements were more extensively injured (~ 0.55 ± 0.15 mm^2) than were those serving the hairy skin (~ 0.10 ± 0.10 mm^2) located along the lateral margin of the forepaw map.

Examination of the individual chronic maps obtained 3 wk after the lesion showed representational changes within the spared territories of the somatotopic representation of the forepaw (Figure 3A). The most obvious alteration consisted of an overall decrease in, and a

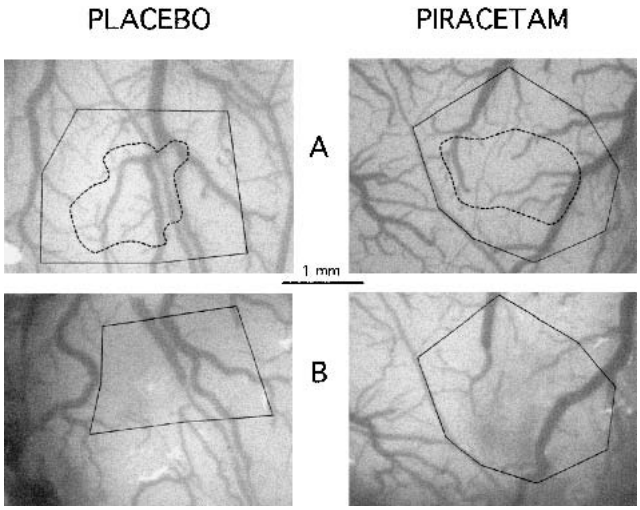


Figure 2 Effect of piracetam treatment on postlesion cellular death. Video images of the cortical surfaces taken (A) before and (B) 3 wk after focal ischemic lesion to the forepaw area of the primary somatosensory (SI) cortex of two representative placebo- (left) and piracetam-treated (right) rats housed in an impoverished environment. Polygons based on stable vascular landmarks were used to estimate the tissue loss induced by the lesion. Note that the tissue loss was greater in the placebo than in the piracetam-treated rat. Modified from Xerri C, Zennou-Azogui Y. 2003. Neuroprotective effects of chronic piracetam treatment and environmental therapy after focal injury to cortex. Neuroscience (In press).

fragmentation of, the cortical zones serving skin surfaces in the area surrounding the ischemic infarct. The spared areas of the cutaneous maps were 0.52 ± 0.30 mm^2, 0.70 ± 0.30 mm^2, 0.70 ± 0.40 mm^2, and 1.29 ± 0.41 mm^2 for the PL-IE, PT-IE, PL-EE, and PT-EE rats, respectively. The postlesion decrease was found to be significant in all animals except the PT-EE rats (Figure 3B).

The most deleterious effects of the focal injury were observed in the PL-IE rats, in which the remaining cutaneous zones were interspersed with large sectors displaying either noncutaneous responses or depressed spontaneous activity and no stimulus-evoked responses (Figure 3A). When the relative decrease in the spared area of cutaneous representation ([chronic area − acute area] / acute area × 100) was calculated, the ANOVA disclosed a significant effect of both treatment ($p < 0.016$), and environmental condition ($p < 0.011$), with no interaction between these two factors (*p:* n.s.). Analysis with the method of least square means (lsmean[1]; H0: lsmean = 0) confirmed that a significant substantial loss of representation occurred during the 3 wk postlesion in all groups (lsmean: −63.19 ± 10.31% [standard error of the mean calculated on residuals], −51.84 ± 10.31%, and −53.55 ± 10.31% for PL-IE, PL-EE, and PT-IE, respectively; $p < 0.0001$) with the exception of the PT-EE rats (−8.74; *p:* n.s.). Indeed, in contrast with the other groups, most representational zones were largely preserved in the PT-EE rats. A conspicuous effect of the enriched environment, which was particularly obvious when piracetam treatment was combined with this housing condition, was that some representational sectors located within the site of cortical damage tended to re-emerge in the peri-infarct zones (see Figure 3A).

The areas of glabrous or hairy skin RFs were measured in mm^2, expressed as a percentage of the corresponding ventral or dorsal skin area of the forepaw and averaged for each rat. Acute postlesion RFs were obtained by removing the RFs corresponding to the cortical sites located within the boundaries of the lesion from the prelesion populations of RFs. A chronic postlesion–acute postlesion value was calculated for the normalized RF sizes recorded for each rat, and a mean size difference was obtained for each experimental group. The glabrous or hairy skin surfaces "lacking" a cortical representation in the S1 cortex were estimated by measuring the skin areas devoid of cutaneous RFs on the individual forepaw images. For each rat, the total skin area with no RF was expressed as a percentage of the corresponding skin area. For the acute postlesion period, a skin area with no cutaneous RFs was measured after exclusion of the RFs recorded at the cortical sites within the lesioned zone. Then, a chronic area–acute area difference was calculated for each rat.

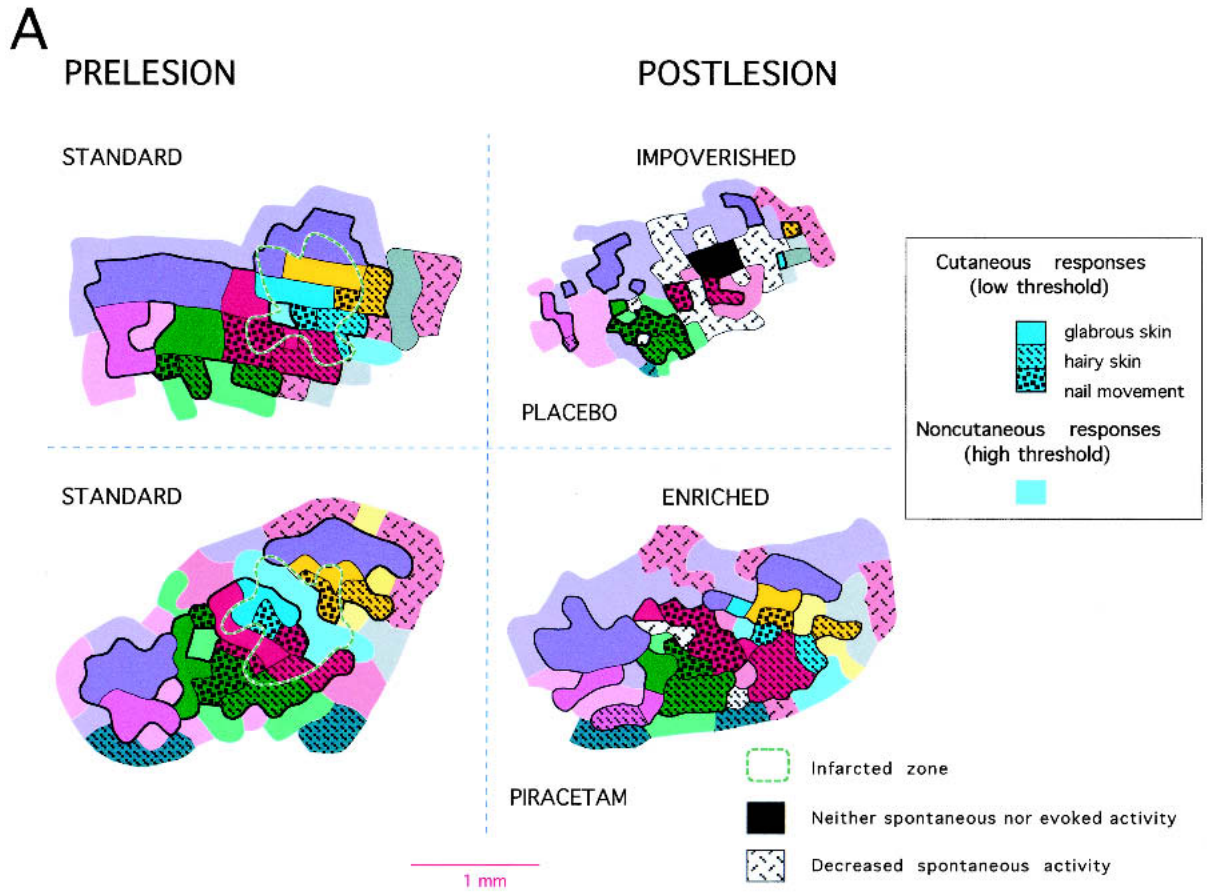
The ANOVA yielded a significant effect of the treatment ($p < 0.017$), but no effect of the environment and no interaction between these two factors. The lsmean method showed that over the 3 postlesion wk, a significant increase in the skin surfaces lacking RFs occurred in both the PL-IE (18.89 ± 6.60%, $p < 0.008$) and PL-EE rats (21.41 ± 6.60%, $p < 0.003$). A nonsignificant tendency for an increase was observed in the PT-IE (13.10 ± 6.60%, $p < 0.057$), whereas no change was found in the PT-EE rats (−6.32 ± 6.60%, *p:* n.s.). The same results were recorded when the ventral and dorsal skin surfaces were considered separately. However, the difference in ventral skin surfaces devoid of RF was found to be significantly different from zero in the PT-IE rats (11.56 ± 5.62%, $p < 0.049$).

The chronic-acute differences in percentages of neuronal responses displaying the highest stimulation threshold (21.39 g/mm^2, 692 mg) were 24.34 ± 3.94%, 23.37 ± 23.15%, 25.75 ± 14.26%, and 8.29 ± 21.61% in the PL-IE, PL-EE, PT-IE, and PT-EE rats, respectively. ANOVA yielded no significant effects of environment and treatment and no significant interaction between these two factors. Nevertheless, analysis with the lsmean method revealed that these increases were significantly different from zero for the PL-IE ($p < 0.050$), PL-EE ($p < 0.012$), and PT-IE ($p < 0.040$) rats, but not for the PT-EE rats (*p:* n.s.). As for the 16.36 g/mm^2 (407 mg) mechanical threshold (−4.84 ± 14.78%, −7.40 ± 14.62%, −5.24 ± 22.78%, and −0.07 ± 15.83% in the PL-IE, PL-EE, PT-IE, and PT-EE rats, respectively), no significant differences in percentages were found with either ANOVA or lsmean. The chronic-acute differences in percentages of responses exhibiting the lowest threshold (9.15 g/mm^2; 166 mg) were −19.50 ± 13.59%, −15.97 ± 13.04%, −0.51 ± 20.44%, and −8.22 ± 7.97% in the PL-IE, PL-EE, PT-IE, and PT-EE rats, respectively. ANOVA showed no significant effects of environment or treatment and no significant interaction between these two factors. The lsmean method indicated that the percentages of response with the lowest threshold decreased significantly in the chronic condition in the PL-IE ($p < 0.035$), PL-EE ($p < 0.018$), and PT-IE rats ($p < 0.028$) but not in the PT-EE rats.

Discussion

The present mapping study describes electrophysiological changes that occurred after a restricted ischemic infarct to the forepaw area of the S1 cortex in adult rats. This lesion produced both immediate and prolonged, experience-dependent, functional alteration in the representational zones surrounding the lesion. Over the first 12 hr after the infarct, we observed the following: (1) The cortical injury expanded, but this expansion was limited by piracetam treatment started 1 hr after the lesion induction. (2) The forepaw map began to reorganize immediately in the cortical sectors around the site of injury. (3) In the PT animals, the somatotopic organization and neuronal responsiveness in the peri-infarct cortical zones were better preserved than in the PL animals.

Three weeks after the lesion, we documented the following observations: (1) The cellular death induced by the ischemic injury was less severe in the PT rats regardless of their postlesion environmental condition. (2) The remaining



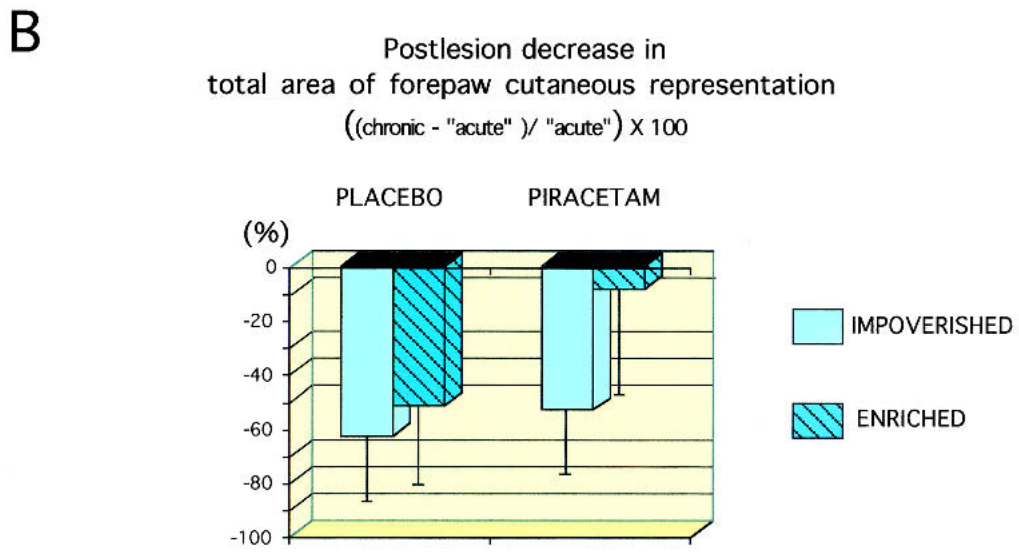


Figure 3 Effects of housing conditions and piracetam treatment on the organization of the somatotopic maps of the forepaw. (A) Forepaw representations obtained before (left) and 3 wk after (right) the lesion induction in a placebo (PL) rat housed in an impoverished environment (IE) after the lesion, and in a piracetam-treated (PT) rat housed in an enriched environment (EE) after the lesion. (B) Histograms showing the mean postlesion decrease in cortical area serving the forepaw cutaneous area including skin surface and nail movement representational zones (vertical bars: standard deviation [SD]). A spared representational zone referred to as "acute" postlesion area was estimated by subtracting the injured zone from the area of forepaw representation recorded before the lesion induction. The spared representational area measured from the map obtained 3 wk after the lesion was referred to as chronic postlesion area. The representational loss was calculated as follows: [(chronic area – acute area)/acute area] × 100. Note that the best preservation of representation was recorded in the PT rat housed in an enriched environment EE, whereas the most degraded representation was found in the PL rats housed in an IE. Modified from Xerri C, Zennou-Azogui Y. 2003. Neuroprotective effects of chronic piracetam treatment and environmental therapy after focal injury to cortex. Neuroscience (In press).

forepaw map exhibited a fragmentation of the cutaneous representational zones and an enlargement of the noncutaneous zones in the spared cortical sectors surrounding the lesion. These alterations were found in all PL rats (with the most detrimental effects in the animals exposed to an IE) and in the PT-IE rats. These changes were not significant in the PT-EE, in which most representational sectors of the forepaw map were well preserved. (3) Consistent with the representational loss, the skin surfaces, which lacked cutaneous RFs and were therefore presumed to be insensate, increased after the lesion in all animal groups, except in the PT-EE rats. (4) In the PT-EE rats, cortical responsiveness as assessed quantitatively with graded mechanical stimuli was diminished in the PL rats, regardless of the environment, and in the PT rats housed in the IE condition. However, cortical responsiveness was not significantly affected in the PT rats exposed to the EE.

Effects of Piracetam Treatment and Sensorimotor Experience on the Lesion Expansion

The electrophysiologically silent cortical zone gradually expanded over the 12 hr examined in the first study. The rate of this expansion is compatible with a slowly developing process leading to neuronal damage. That such a process occurs following focal ischemia is well documented and is known to be accompanied by hypoperfusion of the peri-infarct region. Gradual spread of the ischemic zone has been attributed to a "cascade" of biochemical events, which includes free-radical formation, large ionic fluxes, and other perturbation of cellular metabolism including glutamate neurotoxicity and inhibition of protein synthesis (for reviews see Bodsch et al. 1985; Choi 1990; Siesjö 1981). Interestingly, the injured area remained stable after an initial expansion in the PT rats, whereas the lesion expansion was continuous in the PL rats during the 12-hr time window examined. Based on stable vascular landmarks on the cortical surface, our measurements made 3 wk after the lesion induction indicate that a tissue loss centered on the core of the infarct resulted from the expansion of the initial lesion. The cellular death was less extensive in the PT rats, whether the animals were housed in IE or EE conditions. Our data are consistent with a histological study showing that acute piracetam treatment started 30 min after the induction of a focal ischemia in the rat hindlimb representation of the sensorimotor cortex reduced the infarct size (Chleide and Deberdt 1994). Moreover, consistent with a gradual outward expansion of the ischemic lesion, our electrophysiological results show that the remaining cortical zone devoted to the forepaw representation, as assessed early after the injury (acute postlesion), had further decreased by approximately 40 to 50% 3 wk after the initial lesion. This decrease occurred in the rats exposed to an IE, whether they received a PL substance or PT, as well as in the rats housed in an EE and receiving a PL.

In our study, the loss of representational zones serving cutaneous input in the spared forepaw area shows that the postlesion deterioration consisted not only of a defect in neuronal rescue and survival but also of an alteration of the physiological properties of the cortical neurons within the peri-infarct zone. It is worth mentioning a recent study, which demonstrates that piracetam produces a reduction of permanent middle cerebral artery occlusion-induced brain infarct volume (Tortiglione et al. 2002). In the present study, the loss in cutaneous representation measured early after the lesion induction did not increase significantly when EE promoting social interaction and sensorimotor experience was combined with PT, indicating that a substantial amount of the cortical tissue in the peri-lesion zone escaped the infarction process and displayed normal physiological responses.

Our findings from measures of vascular landmarks suggest that daily PT limited the cellular death, regardless of environmental conditions; however, our electrophysiological data suggest that piracetam failed to limit the representational loss in the IE. In addition, although environmental enrichment alone did not impede the cellular death and representational loss, the smallest lesion expansion, documented as the smallest map compression, was observed when EE and PT were associated. Piracetam and "environmental therapy" through social and physical enrichment seem to conjugate their actions, perhaps through interdependent mechanisms (see below). Indeed, as mentioned above, piracetam's beneficial effects can start within the first few hours after the ischemic infarct (i.e., during a critical time of brain tissue vulnerability), whereas the effects of the postlesion environment depend on a use-dependent, rehabilitation-like process. One can assume that, in the immediate stage of the lesion, early protective action of piracetam facilitated the onset and/or efficiency of an experience-driven reactivation of cortical neuronal circuitry. It thus minimized the loss of responsive cortex and contributed subsequently to preserving the electrophysiological properties of the cortical neurons in the area surrounding the lesion.

Early Map Remodeling in the Spared Representational Zones and Influence of Acute Piracetam Treatment

Restricted cortical lesion in the S1 cortex led to immediate reorganization in sectors of the forepaw representation surrounding the cortical area directly affected by the lesion. Such a remodeling process, which took place throughout the forepaw map, resulted from rapid alteration in the RF properties of the constituent neurons. There was an extensive mutability in both the submodality and the location of the cortical neuron's RFs. Changes in RF location were consistent with those reported in previous studies of cortical reorganization after peripheral nerve injury (Calford and Tweedale 1991a,b; Cusick et al. 1990; Merzenich et al.

1983; Salimi et al. 1994; Silva et al. 1996), amputation (Byrne and Calford 1991; Calford and Tweedale 1988; Kelanan and Doetsch 1984; Rasmusson and Turnbull 1983; Turnbull and Rasmusson 1990), local anesthesia (Byrne and Calford 1991; Calford and Tweedale 1991a,b; Rossini et al. 1994), or epidural block (Metzler and Marks 1979), all of which demonstrate immediate shifting of cutaneous RFs for neurons located in the deafferented cortical zone. Previous microelectrode mapping studies dealing with RF plasticity following cortical injury had documented only long-term changes. Those studies showed that many cortical neurons in representational zones adjacent to (Doetsch et al. 1990; Jenkins and Merzenich 1987; Xerri et al. 1998) or remote from (Xerri et al. 1998) the core of the ischemia responded to stimulation of previously ineffective skin regions. Immediate shifts in cutaneous RF of cortical neurons reveal the existence of unexpressed inputs from nearby skin surfaces. Consistently, intracellular recording has shown that suprathreshold and subthreshold cutaneous inputs from one or more forepaw digits and pads converge on spiny neurons within individual barrels in the rat's forepaw representational zone (Li and Waters 1996) even though a barrel serves only one digit or one pad. Furthermore, using biocytin labeling, the same authors observed that dendritic fields of cortical neurons extend into neighboring barrels. This morphological interdigitation provides the necessary neuroanatomical substrate for immediate postlesion reorganization.

Besides the changes in cutaneous RF location discussed above, emerging cutaneous representations in noncutaneous (i.e., high-threshold) cortical zones contribute to cortical map remodeling. Changes in the dominant submodality at a given cortical site that were assessed by multiple-unit recordings may be due to cells previously excited at a subthreshold level now brought to firing level and thus newly expressed in extracellular responses of cortical neurons by the strengthening or unmasking of pre-existing but ineffective synapses. Our data on postlesion changes in dominant RF submodality are consistent with extensive convergence of cutaneous and noncutaneous afferents within the rat S1 cortex (Chapin and Lin 1984; Gioanni 1987; Lamour and Jobert 1982).

Interestingly, relative to control rats, more cortical sites that were unresponsive before lesion became responsive after lesion in PT rats. Furthermore, fewer cortical sites changed from cutaneous to noncutaneous response, and more cortical sites changed from noncutaneous to cutaneous response in the latter group of rats, suggesting that the cutaneous submodality was best preserved under piracetam treatment. These findings also suggest that piracetam favors remodeling of functional connectivity in the residual cortical regions surrounding a restricted injury. Our data show that many cortical sites in the spared cortical territories of the forepaw map displayed a reduction in neuronal excitability during the first 12 hr after the lesion was examined. However, PT animals exhibited a significantly smaller postlesion decrease in responsiveness to cutaneous stimulation than control rats.

The electrophysiological effects recorded in PT rats can be tentatively ascribed to its influences on neural transmission (see Goulaiev and Senning 1994, for a review). An increase in cholinergic neurotransmission was observed in old animals compared with younger animals (Cohen and Müller 1993; Müller et al. 1990). Maillis et al. (1988) have shown that piracetam can affect neuronal activity either by depression or facilitation of the spontaneous firing rate of cortical neurons and that it interacts with the synaptic effects of glutamate, acetylcholine, and GABA. Piracetam produces an increase in the release and turnover of dopamine (Nybäck et al. 1979; Rägo et al. 1981), has an agonistic effect on serotonin receptors (Bhattacharya et al. 1989; Galeotti et al. 2000), and has an inhibiting influence on glutamate receptors (Bering and Müller 1985).

Effects of Postinjury Environments and Chronic Piracetam Treatment on the Map Organization in the Spared Cortical Sectors

In the present study, the substantial preservation of cutaneous representational zones after postinjury enrichment is consistent with the fact that in intact rats, this housing condition induces an enlargement of the cutaneous representation in the forepaw map, mainly in the cortical sectors serving the glabrous skin more likely to be stimulated through object palpation and manipulation (Coq and Xerri 1998). We hypothesize that with the beneficial effects of chronic piracetam treatment, the enrichment-induced expansion of skin representation tended to offset the deleterious effects of the ischemic infarct in the region at risk. One can assume that the preservation of electrophysiological representation depends on the increased acetylcholine (ACh^1) liberation suggested by the augmented rate of acetylcholinesterase synthesis in the somatosensory cortex of enriched rats (Bennett et al. 1964).

ACh release in the cortex is consistently associated with exploratory behavior induced by environmental novelty (Acquas et al. 1996). In addition, ACh liberation contributes to a long-lasting strengthening of neuronal connections (Verdier and Dykes 2001). An enriched environment promotes a wide range of changes in cortical connectivity that can potentially promote a sparing of cortical functional circuitry. Indeed, enrichment results in a larger number of synapses per neuron (Sirevaag and Greenough 1988), an increase in dendritic branching and dendritic length (Greenough et al. 1973; Uylings et al. 1978; Withers and Greenough 1989), greater numbers of dendritic spines (Globus et al. 1973; Schapiro and Vukovich 1970), and an augmentation in the size and number of synaptic junctions (Möllgaard et al. 1971; Vrensen and Cardozo 1981).

In the impoverished groups of rats, large cortical zones of noncutaneous (i.e., high-threshold) responses substituted for cutaneous cortical zones, which appeared as fragmented

patches scattered in the forepaw map. This effect of impoverishment on the forepaw map was also reported in our earlier study in intact rats (Coq and Xerri 1999a). Impoverishment may decrease ACh liberation through a diminution of acetylcholinesterase synthesis within the neocortex (Bennett et al. 1964), a result in agreement with a diminished ACh release in the hippocampus, after restriction of environmental space (Mitsushima et al. 1998). Interestingly, activation of cholinergic pathways in the basal forebrain induces long-term changes in excitability of somatosensory cortex neurons consisting of an increased magnitude and a reduced threshold of their response to cutaneous stimuli (Metherate et al. 1988; Tremblay et al. 1990). Particularly relevant to the present study is the finding that cells originally excited by high-threshold inputs (i.e., by slightly tapping the forepaw and thus exhibiting noncutaneous responses) fired in responses to light cutaneous stimulation when ACh was applied (Metherate et al. 1988). It is thus conceivable that impoverishment-induced diminution in ACh content tended to render larger cortical zones of the forepaw area responsive only to high-threshold inputs. In the present study, the representational zones recorded in the impoverished rats were consistently interspersed with cortical sectors in which spontaneous neuronal activity was strongly depressed and stimulus-evoked activity was virtually absent.

The lack of a protective action of the impoverished housing on the physiological properties of cortical neurons may also be attributed to the fact that this environment diminishes the number and volume of dendritic spines (Globus et al. 1973; Sirevaag and Greenough 1988), shortens the extent and branching of dendrites (Greenough and Volkmar 1973; Greenough et al. 1973; Uylings et al. 1978), reduces the number of synapses per neuron (Greenough et al. 1985; Sirevaag and Greenough 1985, 1987; Turner and Greenough 1985), and decreases the size of synaptic contacts (Diamond et al.,1975; Greenough et al. 1978; Sirevaag and Greenough 1985, 1987; Turner and Greenough 1985). Besides these alterations, sensory impoverishment may aggravate the effects of the infarction process through a reduction in perikaryal volume (Rosenzweig and Bennett. 1972), RNA/DNA ratios (Rosenzweig and Bennett 1978), total RNA content (Ferchmin and Eterovic 1986), and mitochondria number (Black et al. 1991).

Influence of Early Sensorimotor Experience on Postinjury Functional Changes

Several studies have underscored the importance of sensorimotor activity and behavioral training on postlesion remodeling of cortical representations. Using intracortical microstimulation techniques, Nudo and Milliken (1996) showed that a focal ischemic infarct confined to a limited territory of the motor representation of the hand leads to a further loss of this representation in the adjacent, initially undamaged cortex in spontaneously recovered monkeys. However, rehabilitative motor skill training prevented this subsequent loss of the hand representation within the motor area (Nudo et al. 1996). Indeed, retraining to a skilled reaching task started within 5 days after the infarct and following a behavioral procedure identical to that used before the lesion favored the expansion of the hand representation partially destroyed by the cortical lesion in the adjacent undamaged cortex. This functional reorganization of the motor representation was accompanied by full recovery of hand motor skill.

In our earlier study cited above in which we used the same manual dexterity task in the monkey (Xerri et al. 1991, 1998), we showed that retraining involving tactual motor skill and initiated a few days after focal cortical infarct to area 3b induced representational changes in the somatosensory areas 1 and 3a ipsilateral to the lesion. This retraining-induced reorganization was specifically related to the skin surfaces involved in the sensorimotor ability and paralleled a complete manual skill recovery that appeared to result from a relearning process. Training was postulated to remodel neuronal circuitry within the cortical area surrounding the lesion as well as in remote, functionally connected areas. It is of interest that 15 days after middle cerebral artery occlusion, a combination of environmental enrichment with daily training on a motor skill task promoted a better functional recovery in skilled use of the impaired forelimb than that observed after housing in standard condition without training (Biernaskie and Corbett 2001).

Recent studies have generated questions regarding whether early sensorimotor experience is beneficial or detrimental to functional outcome after ischemic injury. These studies have provided compelling evidence for potentially deleterious effects of excessive use during the early period after ischemia. For example, intensive behavioral training started 24 hr after a focal brain ischemia resulted in cortical infarct larger than that recorded when training was started 7 days after the lesion in hypertensive rats (Risedal et al. 1999). Moreover, during the first 2 wk after ischemic damage to the forelimb area of the sensorimotor cortex in rats, forced use of the affected forelimb through immobilization of the unimpaired limb induced an exaggeration of the initial neuronal injury (Humm et al. 1998 ; Kozlowski et al. 1996), but not when this overuse was imposed beyond this postlesion period (Humm et al. 1998). It is of interest that functional recovery was delayed and remained incomplete after the early overuse. Tissue survival in the peri-infarct zone might be compromised by excessive behavioral demand and usage during the early period after ischemia, when better recovery can be initiated. This vulnerability may depend on a use-dependent increase in glutamate release and *N*-methyl D-aspartate (NMDA[1]) receptor number (Bland et al. 1999), and a peri-infarct cortical hyperexcitability (Que et al. 1998).

Conversely, our data indicate both that the lack of environmental stimulation favoring disuse may be detrimental to the viable brain tissue and that environmental enrichment tends to exert a protective function through moderate sen-

sorimotor experience promoted by social interaction and spontaneous environment exploration. One can speculate that increased levels of neurotrophic factors measured after focal ischemia in enriched rats (Dahlqvist et al. 1999: nerve growth factor; Zhao et al. 2000: brain-derived neurotrophic factor) contribute to the protective effects revealed in the present study.

Effects of Postinjury Environments and Piracetam Treatment on Responsiveness of the Somatosensory Neurons and RF Properties in the Spared Cortical Sectors of the Forepaw Area

Our data indicate that in all groups of rats except the PT-EE rats, many cortical sites in the intact cortical territories of the forepaw map exhibited a significant reduction in neuronal excitability, as assessed by alterations in mechanical thresholds of response to cutaneous stimulation. We previously reported a diminution in neuronal responsiveness to light tactile stimulation in intact rats housed in an impoverished environment for 3 mo (Coq and Xerri 1999a). Moreover, as described above, decreased neuronal excitability was already recorded during the 12 hr after injury in the peri-infarct zone. This decrease was less pronounced in rats treated with piracetam during the same postlesion period. However, chronic administration of piracetam for 3 wk was not sufficient to prevent alteration in cortical excitability, nor was housing in an EE, whereas both conditions appeared to complement each other in maintaining cortical excitability and presumably resulting in a better balance between inhibitory and excitatory transmission.

One cannot rule out that a longer period of piracetam treatment or enriched housing could have restored normal levels of excitability in the somatosensory cortex of our rats. Nevertheless, the complementary effects of the antiischemic substance and environmental enrichment on neuronal responsiveness corroborate their efficiency in preserving or restoring other neurophysiological properties such as RF location and submodality within the neuronal networks underlying cortical representations.

Action Mechanisms Mediating the Neuroprotective Effects of Piracetam

The protective action of early and chronic piracetam administration presumably involves the hemorheological and antithrombotic properties of this substance. Indeed, piracetam improves local cortical cerebral blood flow (Vlahov et al. 1980) not only by increasing red blood cell deformability, and decreasing platelet aggregation through inhibition of thromboxane synthetase, but also by reducing plasma levels of fibrinogen (Moriau et al. 1993). Piracetam also ameliorates the microcirculation at the periphery of acute infarction by increasing compromised regional blood flow (Heiss et al. 1993; Platt et al. 1992), thereby favoring reperfusion of peri-infarcted tissue and promoting recovery of cellular metabolism Moreover, the protective action of piracetam on the physiological properties of cortical neurons could also depend on its capacity to augment oxygen (Danilov et al. 1994) and glucose consumption in cerebral tissue (Depresseux et al. 1986). Furthermore, by decreasing glutamate content in the neocortex (Bering and Müller 1985) and presumably by increasing calcium efflux (Rapin 1993), piracetam could limit excessive intracellular influx of calcium due to hyperstimulation of NMDA receptors, which leads to secondary neuronal death.

Interestingly, environmental enrichment also affects the microvasculature of the cortex by inducing an increase in capillary branching and surface area (Black et al. 1990; Sirevaag et al. 1988). In our experiments, these effects may have complemented the vascular properties of piracetam to promote cortical tissue reperfusion and neuronal survival over the first days after the ischemic infarct. These results are pertinent to the observation that early reperfusion of peri-infarcted brain tissue within 18 hr after ischemic injury correlates with the long-term recovery of neurological function (Furlan et al. 1996; Marchal et al. 1996).

In addition, previous studies have shown that piracetam administration increased cortical excitability (Dimov et al. 1984; Moyanova et al. 1985: cat) and improved electroencephalographic abnormalities in patients with acute stroke (Herrschaft 1978). These effects can be attributed to the ability of piracetam to improve cerebral blood flow, particularly following acute cerebral ischemia (Herrschaft 1978), as suggested by correlated increases in cerebral blood flow and somatosensory-evoked potentials in cats subjected to hypotensive ischemia and treated with piracetam (Sato and Heiss 1985). According to Mailis et al. (1988), piracetam acts as a neuromodulatory substance that can interact with the synaptic effects of glutamate, ACh, and GABA. The increase in cortical excitability induced by piracetam can be ascribed to the facilitatory effect that piracetam exerts on cholinergic transmission (see Goulaiev and Senning 1994, for a review). For example, Pivovarov et al. (1987) have reported that piracetam induced a shift in membrane potential toward depolarization in cholinoreceptor neuronal membrane. Piracetam also synchronizes the electrocorticograms of the somatosensory cortex in rats, an effect attributed to the fact that piracetam decreases GABA content in the cerebral cortex (Ostrovskaia et al. 1982; Raevskii and Nerush 1993) It is conceivable that in our multiunit recording study, the effects of piracetam treatment on neuronal sensitivity were due to a better recruitment of neurons synchronously firing in their cortical sites.

It is worth mentioning that Mittmann et al. (1994) showed that a transient neuronal hyperexcitability occurs in the area surrounding neocortex thermolesion between 1 and 5 days after injury. This effect, which may account for delayed reorganization of intracortical information processing, was attributed to a reduced GABA inhibition associated with prolonged NMDA receptor-mediated activity.

The presumed influence of piracetam on neurotransmission mechanisms could be potentiated by use-dependent mechanisms. For example, it is conceivable that the improvement in cholinergic transmission recorded in the cortex of enriched rats, and the increase in neuronal responsiveness to cutaneous input found in the forepaw area of the somatosensory cortex in these animals (Coq and Xerri 1998), was reinforced by the cholinergic effects of piracetam, thereby contributing to maintain cortical neuron excitability to nearly normal levels.

Summary

The study described herein highlights the potential for acute and chronic administration of piracetam, a neuroprotectant substance with hemorheological, antithrombotic, and pharmacological properties for possible use when neuronal survival in the area surrounding a focal ischemic injury to the cortex is compromised. The anti-ischemic effects of early piracetam administration may favor the onset of mechanisms of plasticity and improve later, use-dependent, adaptive changes. The possible benefits of its use have been indicated by the observation that environmental therapy promoting social interactions and sensorimotor activity combined with piracetam treatment exerts neuroprotective effects by preventing further loss of representational territories and by preserving the functional properties of constituent neurons of the sensory representations. Conversely, the lack of environmental stimulation is detrimental to the neuronal survival and the functional organization of sensory representations in the spared cortical area. Future studies should attempt to determine whether the combination of environmental therapy and anti-ischemic substance can promote sparing and/or recovery of neurological functions and behavior.

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