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DEFICITS AND RECOVERY OF BODY STABILIZATION DURING ACROBATIC LOCOMOTION AFTER FOCAL LESION TO THE SOMATOSENSORY CORTEX: A KINEMATIC ANALYSIS COMBINED WITH CORTICAL MAPPING

C. XERRI[1], M. BENELHADJ AND F. HARLAY

Laboratoire de Neurobiologie des Adaptations et Restaurations Fonctionnelles, Université de Provence/CNRS, UMR 6149 Neurobiologie Intégrative et Adaptative, 52, Faculté des Sciences "St. Jérôme", Case 361, 13397 Marseille Cedex 20, France

INTRODUCTION

Numerous studies have investigated functional changes after the induction of large neocortical lesions. By contrast, there are fewer reports of sensorimotor dysfunction after focal neocortical injury. In a previous report, we documented the effects of a restricted unilateral injury to the hand representational zone of area 3b in the primary somatosensory (SI) cortex of monkeys trained on a manual dexterity task (XERRI *et al.*, 1998). We found that this lesion induced transient purely tactile deficits, but enduring sensorimotor impairment. In the early postoperative period, deficits in the precision of ballistic movement, inaccurate hand positioning, disruption of grip formation and inability to execute independent and precisely coordinated finger movements were observed. These sensorimotor deficits subsided and manual dexterity gradually improved over a period of several weeks, until complete recovery was achieved. In addition, we reported correlative changes in the organization of electrophysiological maps in peri-lesion zones of area 3b, as well as in areas 1 and 3a interconnected with the region of the direct damage. This cortical map remodelling was clearly related to rehabilitative effects of training on the dexterity task, presumably through experience-dependent mechanisms of neuroplasticity.

In other studies, we reported evidence that a focal unilateral lesion in the forepaw area of the SI cortex of adult rats induced immediate and longer-term representational reorganization in the spared sectors of the forepaw map (COQ and XERRI, 1999; XERRI and ZENNOU-AZOGUI, 2003). Typically, such focal brain damage induces subtle deficits which are not disclosed when the animals are observed in their daily activities or when conventional tests devoted to assess sensory or motor impairments in rodents are used. In the rat, the SI cortex is topographically organized into a single map representing both cutaneous and proprioceptive inputs. Therefore, we anticipated that an appropriate paradigm using sensitive behavioral measures would allow detection of functional deficits that cannot be evaluated using current methods. To address this question, we used a rotating beam device that we

[1] Corresponding Author: Tel. 33 491 288464, Fax 33 491 288616, E-mail: xerri@up.univ-mrs.fr

ability to produce rapid postural adjustments in order to keep its body balance while compensating for the lateral shift of the continuously moving support. Preoperative training required 6 to 8 morning sessions of about 1h for the rats to stabilize their performance in terms of maximum rotation speed that did not lead to fall. Kinematic analysis was performed with a video motion analyzer (ELITE system, BTS, Milan, Italy). Two infrared video cameras positioned 3.6 m in front of the beam recognized infrared reflecting markers (diameter: 8 mm). Two markers were glued to the skin above the T13 and S4 vertebrae. The ELITE system detects these markers through shape recognition and computes their centroid coordinates for both video cameras. Calibration was performed before each recording session. Marker positions were sampled every 10 ms (100 Hz). The ELITE system elaborated marker trajectories with a 1/3000 accuracy and 3D reconstruction (FERRIGNO and PEDOTTI, 1985). In our experimental conditions, the analysis was restricted to orientation and movements of the rostro-caudal axis of the rat's body. The segment linking the 2 markers was used to measure the orientation and stability of the body axis in the coronal and horizontal planes (Fig. 1). The temporal evolution of the individual markers in the horizontal plane was also recorded as was the lateral angle of the body axis with the beam longitudinal axis.

Surgical preparation.

Experimental procedures were in accordance with the regulations of the NIH guide of Health Guide for the Care and Use of Laboratory Animals (NIH Publications N° 80-23) revised 1996. All efforts were made to minimize the number of animals used and their suffering. Anesthesia was induced with halothane and maintained with sodium pentobarbital (50 mg/kg, i.p.). The 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 °C and 38 °C by a heating pad. The head was held in a stereotactic frame. Surgical and recording procedures were performed under sterile conditions. 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 (about 16 mm^2) exposed part of the somatosensory cortex (ant.: 2.5 mm; post.: 1.5 mm; lat.: 2-6 mm with respect to bregma). 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 (30000 cenistokes; Accumetrics) to prevent drying and oedema. At the end of the recording session, after induction of the cortical lesion, the silicone was removed with a wash of warm saline, the dura was repositioned and covered with a gelatin film (Gelfilm, Upjohn). The bone flap was reinserted and stabilized with dental acrylic. Connective tissue was closed with absorbable sutures and the scalp with silk sutures. The animal's temperature was monitored until the recovery from anesthesia was complete. Prophylactic administration of antibiotic (Pyocefal, Takeda; 150 mg/kg, i.m., in two daily injections) was administered daily for 7 days. On the 21st day after the lesion, the anesthesia procedure was repeated. The bone flap was removed to allow access to the forepaw area of the SI cortex. Then, this cortical zone was mapped. After completion of the remapping procedure, the animal received a lethal dose of sodium pentobarbital (150 mg/kg, i.p.) and the brain was prepared for histological processing. Sham-operated rats were subjected to the same surgical procedure as the experimental animals. This procedure included the dura incision, except that the cortex was left intact. The sham-operated rats were also anesthetized for a similar duration.

Electrophysiological mapping.

A high-resolution camera mounted on an operating microscope was used to digitize images of the exposed parietal cortex, and the ventral and dorsal surfaces of the contralateral forepaw. The recording sites were located relative to the cortical surface vasculature on the digitized image of the cortex and the cutaneous RFs were drawn on the forepaw images, by using Map 0.925 software (PETERSON and MERZENICH, 1995). Neurons were recorded with parylene-coated tungsten microelectrodes (about 1 MΩ at 1 kHz). The electrode was moved perpendicular to the cortical surface in cartesian coordinates using a 3-dimensional micromanipulator driven by stepping motors (Märzhauser). The recording artifact generated by the microelectrode contact with

visible skin indentation or hair deflection elicited reliable changes in multi-unit activity. This stimulation was produced with a fine-tipped, hand-held glass probe and monitored by using magnifying glasses (x4). In many instances, Von Frey monofilaments (Stoelting, Semmes-Weinstein aesthesiometer) that apply indenting stimuli at a relatively constant, predetermined force were also used. The ridges running along the glabrous skin of the digits and palm were used as landmarks to delineate the RFs. The size of all cutaneous RFs was measured off-line by Map 0.925 software. Noncutaneous responses were identified by more intense stimuli such as taps, pressure on muscles 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 used Canvas software (Deneba) to elaborate 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. Boundaries were drawn midway between adjacent recording sites where RFs were restricted to distinct and separate skin subdivisions. A line crossed cortical sites at which a single RF included different but adjoining skin subdivisions of the forepaw. Borders were placed midway between responsive and unresponsive sites. Elaboration of each cortical map was based on a total sample of about 160 to 170 recordings. Canvas software was used to calculate the areal extent of each region of the cutaneous map.

Induction of Cortical Lesion.

The silicone fluid was removed and the exposed cortex was bathed in warm physiological saline. A temperature-monitoring electrode (Radionics TCZ; Kopf; USA) was moved perpendicular to the cortex surface by the micromanipulator and was slowly lowered so that its tip (diameter: 0.25 mm) was in contact with the cortical surface. The lesion was performed at about the center of the forepaw map to spare an amount of tissue that can allow a reorganization of the intact areas surrounding the lesion. A 500 MHz radiofrequency current was delivered through the tip of an electrode equipped with a thermistor. The temperature was gradually raised 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. This was 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. Radio-frequency induced hyperthermia produces cellular injury, i.e. coagulation necrosis, neuronal shrinkage, nuclear pyknosis and perineuronal astrocytic swelling, (OHMOTO *et al.*, 1996) that is associated with cerebral ischemia. Moreover, localized hyperthermia induces increased extracellular glutamate concentrations that reaches neurotoxic levels (ADACHI *et al.*, 1995).

As in previous studies (JENKINS and MERZENICH, 1987; XERRI *et al.*, 1998) the functional impact of the lesion was assessed using electrophysiological recordings during the 2nd hour postlesion. The boundaries of the cortical infarct were drawn midway between adjacent recording sites which displayed normal response characteristics, i.e. spontaneous discharges and clear responses to peripheral stimulation, and those which exhibited strongly or totally depressed spontaneous activity or stimulus-evoked responses. The surface area of the lesion was measured on the cortex image using Canvas software.

Histological procedure.

Several electrode tracks in each post-lesion experiment were marked with electrolytic lesions at the borders of the forepaw map by passing cathodal current (10 μA DC, 10 s) through the recording electrode. After the mapping session, the rats were given a lethal dose of pentobarbital and perfused transcardially with 0.9% physiological saline followed by a solution containing 4% paraformaldehyde in 0.1% sodium phosphate-buffered (pH: 7.4). The brain was removed and post-fixed in a 4% paraformaldehyde solution containing 10% sucrose in a phosphate buffer. Coronal sections 50 μm thick were cut on a freezing microtome and processed for Nissl staining. Histological sections were used to verify rearding locations and to assess cellular changes occurring within the lesioned zone. Extensive loss of cells and gliosis were found from layer I to layer IV, with little or no damage to deep layer V. The injured zone was surrounded by a region of normal architectonic appearance.

Statistical analysis.

ANOVA was used for statistical treatment of the data. This analysis was supplemented with the Newman-Keuls multiple comparison post-hoc test.

RESULTS

Kinematic analysis.

Figure 2 illustrates typical kinematics recordings obtained for 2 speeds of beam rotation, during the last prelesion training session in one rat. The various orientations and lengths of the coronal plane projections of the segment linking the anterior and posterior markers show that it remained nearly vertical at slow rotation speed (2 m/min) (Fig. 2A). This data indicates that the body rostro-caudal axis was maintained almost parallel to the beam longitudinal axis. By contrast, these segments were tilted and of greater length at a faster speed (17.5 m/min), indicating that the body was deviated with the posterior marker shifting toward the beam rotation direction.

In addition, the kinetic of the markers during beam walking shows that they moved out of phase at a low speed rotation (Fig. 2B). At the same time, we observed that the body waddled smoothly during locomotion on the beam. By contrast, at a faster speed, the markers tended to remain in phase, indicating that the anterior and the posterior parts of the body did not move independently, as the rat displayed a crabwise pattern of walking. At the low rotation speed, the mean lateral angle formed by the rostro-caudal axis of the body and the longitudinal axis of the beam, calculated for the group of 5 experimental rats was -9.8 ± 5.5 deg (450 measurements at a 10 ms sampling period over 4500 ms for each crossing). This value corresponds to a slight lateral deviation of the anterior part of the body toward the direction of beam rotation. The data illustrating the changes of lateral deviation during beam walking shows regular oscillations resulting from body deviations toward the rotation followed by transitory readjustments tending to a realignment of the body rostro-caudal axis with the beam longitudinal axis (Fig. 2C). At the faster rotation speed, the body was deviated toward the opposite direction of the rotation (Fig. 2C-D). The mean value of deviation recorded for the group of 5 rats was 25.7 ± 7.2 deg. As illustrated in Figure 2C, the body axis was no longer realigned with the beam axis, as the rat failed to produce postural adjustments compensating for the lateral shift of the body.

The kinematic analysis showed that unilateral focal cortical ischemia within the forepaw region of the somatosensory cortex induced deficits in gait during locomotion on the rotating beam. Examples of the kinematic data obtained for the 2 m/min rotation are illustrated for different postoperative days in Figure 3. The segments recorded in the coronal plane indicate that the body deviation increased from the 1st day postlesion and tended to return to control values at the end of the 2nd week. Furthermore, the trajectories of the body were not rectilinear, as indicated by the scattering of segments during this postoperative period. In addition, the data illustrating the lateral displacements of the 2 markers shows that the anterior and poste-

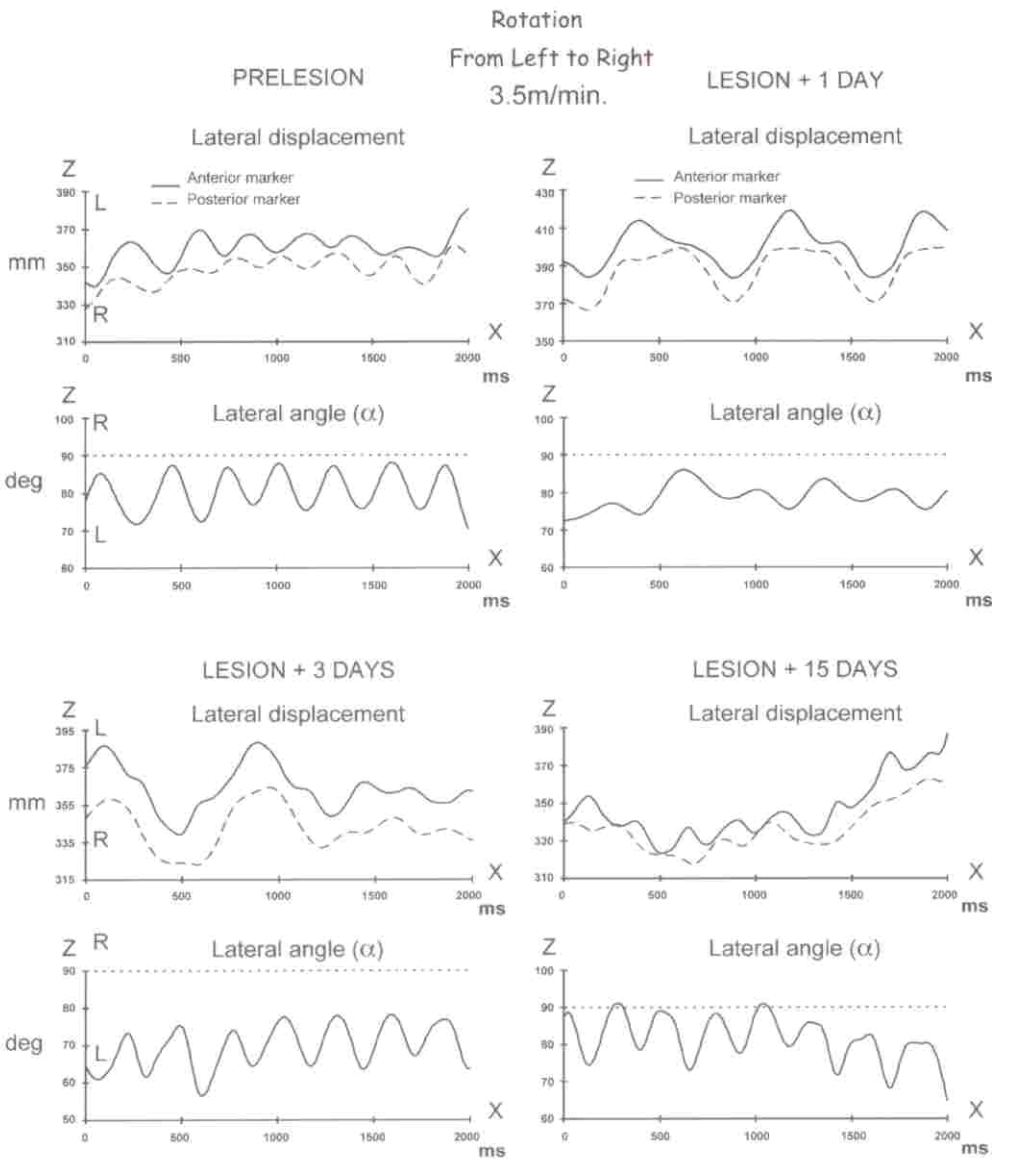

Fig. 4. *Postlesion deficits and recovery of body orientation and locomotion stability recorded in the horizontal plane at low speed of beam rotation.*

Example of recordings obtained in an individual rat. The anterior and posterior markers (upper traces) moved out of phase prior to the lesion, within the low range of speed rotation. These markers moved out of phase in large waves during the first 3 days postlesion. Normal phase opposition and movement periodicity were restored from the 15 day postlesion. The angular deviation of the body (lower traces) became irregular, as the capacity to reorient the body deviated toward the direction of rotation was impaired, only during the first 2 days postlesion. The data corresponds to that illustrated in Fig. 3.

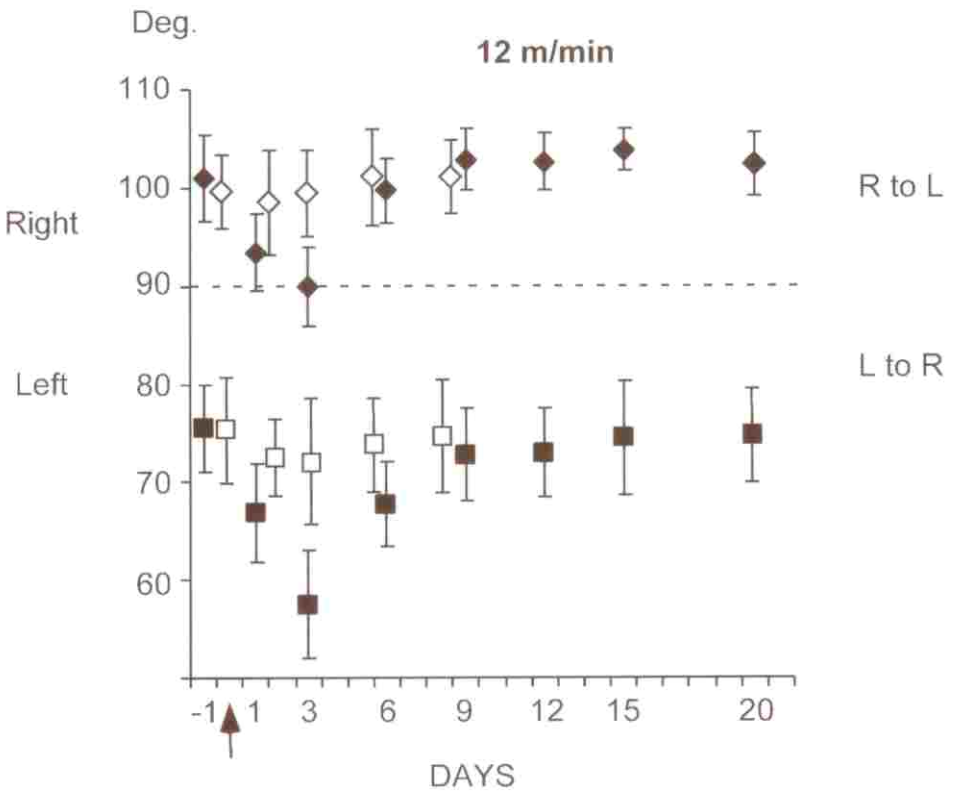

Fig. 5. *Postlesion deficits and recovery of lateral body deviation.*

Mean (± S.D.) lateral angle calculated for the experimental (filled symbols) and sham-operated rats (open symbols) at the 12 m/min rotation speed, prior to the lesion and during the first 3 postoperative weeks. Note a transient increase of the lateral deviation from the 1st to the 9th day postlesion (90 degrees corresponds to a perfect alignment of the rat's and beam axes). This deviation was greater for rotations to the lesioned side (right). The arrow indicates the day of lesion induction.

mm^2). The relative change in the spared area over the weeks following the injury was calculated as follows: [(chronic area - acute area)/acute area] X 100. The results show a further representational loss of -39.50 ± 9.40%, i.e. a total loss of about 60% of the intact forepaw area (Fig. 6C). However, reemergence of some skin surface representations was noted in the peri-lesion zone. Interestingly, the loss of representation recorded in the present study was smaller than that obtained in a previous investigation (-63.19 ± 10.31%; $F_{8,1} = 38.05$, $P < 0.001$) in which the rats underwent a similar lesion (29.36 ± 5.89%), were also housed singly, but were not subjected to behavioral training (Xerri and Zennou-Azogui, 2003). Consequently, the spared cortical areas recorded in the trained rats (0.95 ± 0.36 mm^2) were, on average, greater than those found in the untrained rats (0.52 ± 0.30 mm^2). It is also relevant to mention that the prelesion maps (2.27 ± 0.29 mm^2) obtained from our trained rats housed in groups of

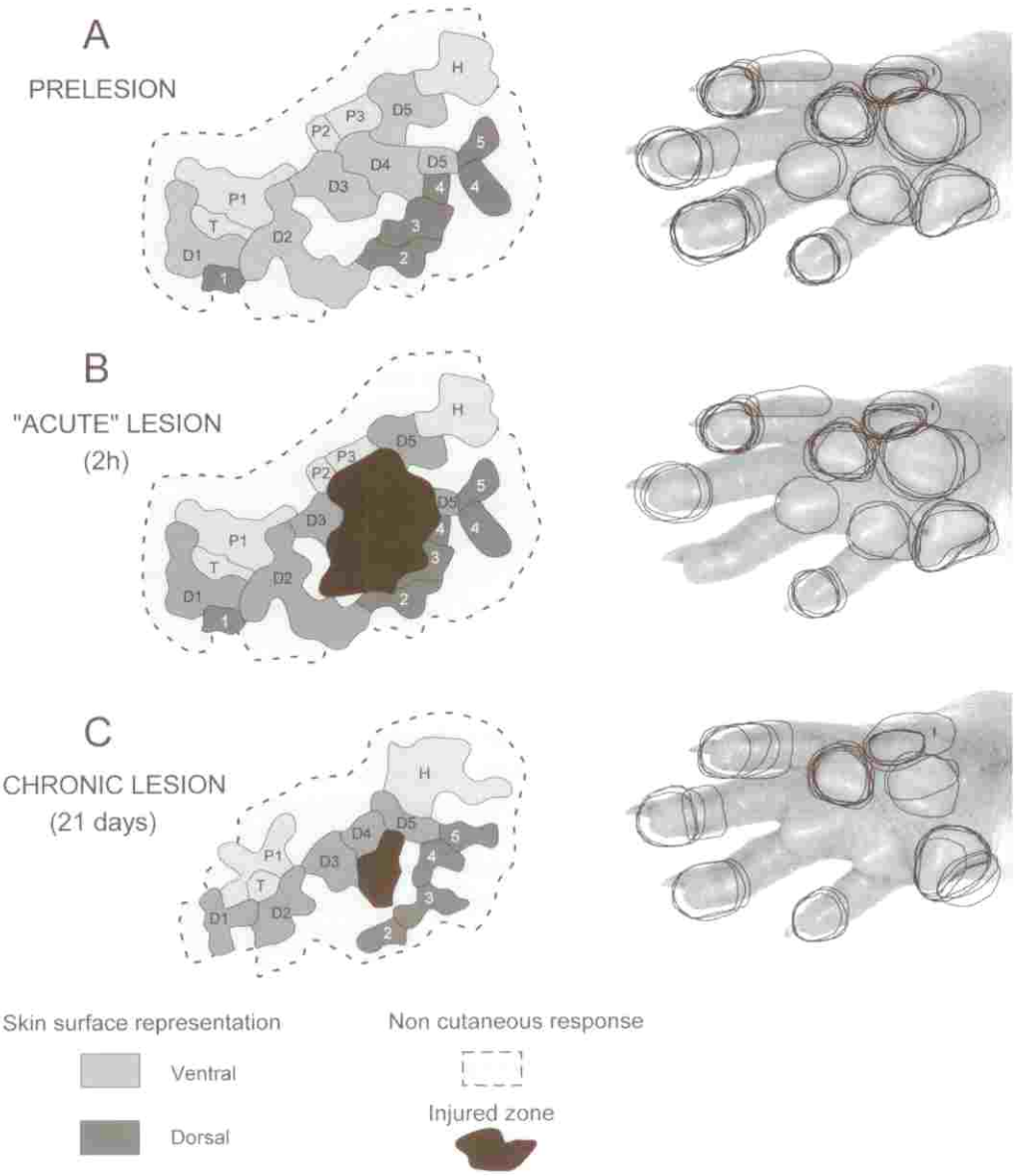

Fig. 6. *Remodeling of the somatotopic maps of the forepaw after focal cortical lesion.*

Left: Double electrophysiological mapping illustrating the forepaw representations recorded prior to **(A)** and 3 weeks **(C)** after the lesion induction in the same rat. The injured, electrophysiologically silent, area defined on the basis of neuronal recordings performed during the 2nd hour 1h after the lesion induction is shown on the prelesion map **(B).** Note that the injured area was smaller in the map elaborated 3 weeks after the lesion induction, due to substantial cellular loss. D1-D5: digit 1 – digit 5 ventral skin areas; 1-5: digit 1 – digit 5 dorsal skin areas; P1-P3: Pad 1 – Pad 3; T: thenar eminence; HT: hypothenar eminence.
Right: Cortical neurons' glabrous skin receptive fields used to elaborate the cortical maps shown on the left.

tion was fully recovered within a 2-3 week period. The functional recovery cannot be ascribed to a full restitution of the lost sensory representations since a permanent decrease of forepaw representation was recorded. It has long been documented that sensorimotor experience before and after brain injury can enhance functional outcome. For example, exposure to enriched environments after damage to various brain areas ameliorates behavioral recovery in rodents (WHISHAW *et al.*, 1984; HELD *et al.*, 1985; GALANI *et al.*, 1997; RISEDAL *et al.*, 2002). It is also of interest that 2 weeks after a middle cerebral artery occlusion, environmental enrichment combined with daily training on a motor skill task improves functional restoration of skilled use of the impaired forelimb (BIERNASKIE and CORBETT, 2001). Rats with small lesions to the sensorimotor cortex which experienced complex motor skill tasks, displayed improved coordination of the forelimbs, compared with rats receiving simple locomotor exercise (JONES *et al.*, 2003) Along the same line, rats having extensive locomotor experience following cortical lesions displayed reduced deficits and faster recovery in a behavioral test consisting in walking on a narrow, immobile beam (HELD *et al.*, 1985). Rehabilitative motor skill training has also been shown to improve recovery of hand motor skill after focal lesions to the motor (NUDO *et al.*, 1996) or somatosensory cortices (XERRI *et al.*, 1998). In the latter study, evidence was given that the manual skill recovery resulted from a relearning process. In our experimental conditions, as the locomotor task was resumed early after the injury, one can assume that repeated sensory feedback signals over successive trials were used to readjust the motor commands and were eventually integrated into the built-in motor programmes, as a substrate for sensorimotor relearning and recovery of skilled locomotion. These corrective signals may originate from somatosensory, vestibular or visual receptors. Conceivably, the functional recovery may involve an increased reliance on all theses sources of sensorimotor regulation. Direct connections between visual, somatosensory and motor cortices have been evidenced in the rat (MILLER *et al.*, 1984). These corticocortical pathways provide the basis for multisensory integration that may aid the rat in the coordination of visually and somatosensory guided behaviors. This multisensory integration may be useful to optimize adaptation to the damage and/or mediate compensation for the locomotor deficits through a substitution process, after injury to the SI cortex. In addition, one cannot exclude the developpement of compensatory movement patterns or the recourse to alternative behavioral strategies to circumvent functional deficits, like an increased use of intact forelimbs to ensure postural stabilization while traversing the rotating beam. Indeed, an enhanced use of the nonimpaired forelimb in a footfault task has been reported after unilateral damage to the forelimb sensorimotor cortex in rats (SCHALLERT *et al.*, 1997). Local anesthetization of the nonimpaired forelimb produced a reinstatement of the impaired forelimb deficits. The improvement in performances that involved the intact forelimb was enhanced in rats trained on an acrobatic task which required the animals to learn complex movement coordination to traverse a series of obstacles (JONES *et al.*, 1999).

It is plausible that early retraining limited the expansion of the cortical injury, and thus tended to partially preserve the forepaw representation in the peri-injury zone,