

# Recovery of Evoked Potentials, Metabolic Activity and Behavior in a Mouse Model of Somatosensory Cortex Lesion: Role of the Neural Cell Adhesion Molecule (NCAM)

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**Understanding the processes that underlie functional recovery after cortical injury is a major challenge for neurobiology and clinical neurology. The aim of the present study was to establish a mouse model of functional recovery that would facilitate the investigation of the molecular and cellular events involved in cortical dynamics. We show that a focal injury of ~0.5 mm of diameter and 1 mm depth made in the barrel cortex of adult mice induced a transitory deficit that could be characterized using somatosensory evoked potential (SEP), metabolic mapping and a behavioral test. SEP recordings of short latency responses using an epicranial multi-array system showed a decreased cortical activity in the peri-lesion regions 2 weeks after the injury and a partial recovery to normal pattern 6 weeks after the lesion. Delayed SEP signals over the motor cortex were not altered by the injury. Metabolic mapping with [<sup>14</sup>C]deoxyglucose uptake in the surround of the injury reproduced the time course of deficit and recovery. Finally, a deficit in vibrissae related performance in a gap-crossing test 1 week after injury was followed by a functional recovery in the following 2 weeks. We show in addition that the recovery process is deficient and significantly delayed in NCAM knockout mice lacking all isoforms of NCAM (neural cell adhesion molecule) and PSA-NCAM. These results support the hypothesis that impairment and recovery of functions after focal cortical lesion involves remodeling of intact circuits surrounding the lesion and that the NCAM molecule participate in this process. The model opens new possibilities for investigating the role of candidate molecules in functional recovery using genetically modified mice.**

**Keywords:** behavior, evoked potentials, metabolic activity, mouse model, neural cell adhesion molecule, somatosensory cortex

## Introduction

The transitory or permanent functional deficit produced by a traumatic brain injury is very often a consequence of focal cortical damage. A large body of experimental and clinical studies suggests that functional recovery after focal lesions is possible, though often limited (Geschwind, 1985; Jenkins and Merzenich, 1987). The degree and the time course of restoration is highly variable and appears to depend on a large number of factors, including the lesion size, patient age, sex, previous experience, stimulation, emotional status and motivation (Geschwind, 1985). An emerging view is that functional recovery after focal injury mainly relies on a dynamic reorganization of preserved networks (Buonomano and Merzenich, 1998; Witte, 1998). The bulk of evidence for this plasticity stems from experiments in the somatomotor cortex (Jenkins and Merzenich, 1987). Subtotal lesions in the primary somatosensory cortex of owl monkeys were shown to be followed by behavioral recovery due to remodeling of cortical representational maps (Jenkins and Merzenich, 1987). In this model,

intact zones in the neighborhood of a focal lesion took over the function of the damaged areas. Similar restorative mechanisms have been reported after unilateral focal lesions of the sensorimotor cortex in a number of species and the same observations were made in human cases (Geschwind, 1985). While the capacity for functional recovery after cortical damage is well documented, little is known about the physiology of recovering cortical networks and the molecular mechanisms contributing to the repair process. Molecules such as growth factors, proteases or cell-cell adhesion molecules, that shape the formation of neuronal networks during development, are particularly interesting candidates. Recent data suggest that these molecules play a role in several aspects of cellular and synaptic plasticity, mechanisms that are proposed to underlie the re-organization of cortical maps associated with functional recovery (Buonomano and Merzenich, 1998). The role of PSA-NCAM has been established in axon growth, lesion induced sprouting, cell migration and activity-dependent synaptic plasticity (Kiss *et al.*, 2001). Importantly, removal of alpha 2,8-linked sialic acid (polysialic acid; PSA) from NCAM prevents induction of long-term potentiation (LTP) and long-term depression (LTD) in the CA1 (Muller *et al.*, 1996) and CA3 regions of hippocampal slice cultures and NCAM knockout mice (Cremer *et al.*, 1998) and results in deficit in spatial learning (Cremer *et al.*, 1994; Becker *et al.*, 1996). Based on these data, PSA-NCAM has emerged as an attractive candidate for performing a role in functional recovery after lesion.

In an attempt to develop a clinically relevant analysis of cortical recovery in animals, we recently introduced an epicranial approach for multi-electrode recording of vibrissal sensory evoked potentials (SEP) in the mouse (Troncoso *et al.*, 2000). The vibrissae-barrel-field circuit is of particular interest in mice. Mystacial whiskers are sensitive tactile organs that selectively activate a predictable area, the barrel in the somatosensory cortex (Woolsey and Van der Loos, 1970). The topographic disposition of barrel-like structures in this cortical region obeys the same arrangement as the vibrissae on the mystacial pad. Due to this modular somatotopic characteristic, this sensory system has become one of the major models for assessing the cortical reorganization. Using an epicranial multi-array recording system, we showed that SEP could be repeatedly assessed over different cortical areas in the same animal (Troncoso *et al.*, 2000). By applying this approach to mice, we report here that the time course of deficit and recovery produced by a focal cortical injury can be monitored over time and that this recovery process is correlated with corresponding changes in cortical metabolic activity and behavior. Application of this model to NCAM knockout mice reveals major deficits in the recovery process. This mouse model of functional recovery opens therefore new possibilities to investigate

the cellular, molecular and genetic determinants of cortical reorganization triggered by a focal lesion.

## Materials and Methods

### Animals

A total number of 38 male C57BL/6 mice aged 14–20 weeks and weighing 27–30 g were individually housed with water and food *ad libitum* at 12 h light/12 h dark cycle and removed from the housing environment only on experimental days. NCAM-deficient mice have been described previously (Cremer *et al.*, 1994). All analyses were performed on a C57BL/6J background (five backcrosses) in mice between 3 and 6 months of age. All experimental procedures were in accordance with Swiss laws, previously approved by the Office Vétérinaire Cantonal of Geneva.

### Lesions

Cortical injuries were performed under general anesthesia (pentobarbital 60 mg/kg, with an additional dose of 20 mg/kg if it was necessary) and sterile conditions. The skull was exposed and a small hole of 0.5 mm was made with a stereotaxic micromotor drill over the left hemisphere 1.5 mm posterior to the bregma and 2.3 mm lateral to the midline. These coordinates targeted the  $\delta$  barrel. Through the hole on the skull, a solid needle with 0.5 mm diameter was introduced in the cortex (1 mm deep from the surface) with a rotation rate of 20 000 r.p.m.; finally the skin was closed with surgical suture (Safil® 5/0). There was no evident morbidity associated with this injury. In the control (sham operated) group, injury was made outside the barrel cortex 2 mm posterior to the bregma and 2.0 mm lateral to the midline.

### Histological Control of Cortical Injury

The localization and extension of the lesion were histologically confirmed on cytochrome oxidase or Nissl-stained sections. Mice were killed 2 or 6 weeks after the injury with an overdose of pentobarbital and their brains were removed and fixed by chilled (4°C) 4% paraformaldehyde. Brains were kept in the same fixative for 24 h and then dipped in a 30% sucrose for another 24 h. For cytochrome oxidase reaction, cerebral hemispheres were frozen and serially sectioned at 40  $\mu$ m tangential to the pial surface. Sections were treated for reaction according to a protocol described by Wong-Riley (1979). Alternatively, 20  $\mu$ m thick coronal sections were cut with a cryostat and stained with cresyl violet. Injury of all mice was histologically verified.

### SEP Recordings

These experiments were conducted on two control groups of eight animals each (sham operated, and barrel cortex injury) and two groups of four NCAM mutant animals each (sham operated and

injured). SEP were recorded in both groups three times (before and 2 and 6 weeks after cortical injury). Epicranial SEP recordings were performed as described previously (Troncoso *et al.*, 2000). Briefly, the mouse head was placed in a stereotaxic frame under pentobarbital anesthesia (60 mg/kg i.p.) and loss of eye blink and withdrawal reflexes were observed for the assessment of anesthesia depth. Body temperature was maintained at  $\sim$ 37°C. An array of five stainless steel electrodes (0.45 mm in external diameter and 2 cm length), were positioned over the skull, in a row with the following coordinates related to bregma: AP +1/L 1.5, AP 0/L 2.0, AP -1/L 2.5, AP -2/L 3.0 and AP -3/L 3.5 (distances in millimetres). Series of 10 electromechanical stimuli driven by a computer-controlled signal were applied unilaterally at 10 min intervals to all whiskers at a distance of 10 mm from the face, with a vertical excursion of 300  $\mu$ m in the dorso-ventral direction and an inter-stimulus-interval of 3 s. Signals were amplified ( $\times$ 10 000) and filtered (high pass 4 Hz, low pass 300 Hz), then hooked up and digitally converted (16 bits, 2 kHz with triggered scan) and stored for post-hoc analysis. At the end of the recordings, the skull was carefully cleaned and the skin closed with surgical suture.

Data were then processed by statistical elimination of responses that fulfilled the rejection criteria (to be beyond 2 standard deviation from the original mean). The mean of three series obtained after signal processing was calculated for each experimental condition. The values obtained by the three series of stimulation were very constant. The peak positive and negative values between 10 and 30 ms post-stimulus were measured. Results are expressed as mean  $\pm$  SEM of voltage amplitude ( $\mu$ V) and latency (ms).

### Assessment of Metabolic Activity by 2 Deoxy-D-glucose (DG) Uptake

Three control groups of four animals each (before and 2 and 6 weeks survival after lesion) and three groups of NCAM knockout animals of four animals each (before and 2 and 6 weeks survival after lesion) were used for deoxy-D-glucose (DG) experiments. These animals were recorded before and 2 or 6 weeks after lesion. At the end of the last trial of SEP recording and before the recovery from anesthesia, all whiskers excepting  $\delta$ , E1 and E2 were bilaterally trimmed. Then animals were food-deprived overnight. On the next day, animals received a single injection of DG (167 nCi/kg i.p. of [ $^{14}$ C]deoxy-D-glucose MC355S; Moravek Biochem, Brea, CA), as described previously (Welker *et al.*, 1992) and bilateral  $\delta$ , E1 and E2 barrels were spontaneously activated during 45 min, by stimulation of the corresponding untrimmed whiskers. At the end of the experiment, animals received a lethal dose of pentobarbital (i.p.) and were perfused transcardially (cold formalin 3.3%, 0.1 M phosphate buffer, for 10 min). Then brains were removed and immediately frozen on dry ice. Serial 20  $\mu$ m thick sections were cut in a cryostat in a plane tangential to the pial surface overlying the barrel cortex. Design of experimental groups for SEP and DG uptake analysis is shown in Table 1.

**Table 1**

Design of experimental groups for SEP and DG uptake analysis

Group name	Experience	Before injury	2 weeks later	6 weeks later
Injured C57/Bl6	SEP recordings	Eight animals	Eight animals	Eight animals
Sham C57/Bl6	SEP recordings	Eight animals	Eight animals	Eight animals
Control C57/Bl6	DG uptake	Four animals		
2 weeks C57/Bl6	DG uptake		Four animals	
6 weeks C57/Bl6	DG uptake			Four animals
Injured NCAM knockout	SEP recordings	Four animals	Four animals	Four animals
Sham NCAM knockout	SEP recordings	Four animals	Four animals	Four animals
Control NCAM knockout	DG uptake	Four animals		
2 weeks NCAM knockout	DG uptake		Four animals	
6 weeks NCAM knockout	DG uptake			Four animals

### Data Analysis

To localize the site of lesion, the outlines of barrels in layer IV were drawn from Nissl-stained sections using a camera lucida. The corresponding autoradiograms were digitized with a digital video camera (Sony TRV-900) mounted on a microscope. The images obtained were aligned with the images of the drawings. Then, three consecutive Nissl-stained sections where the D or E row barrels were clearly identifiable, were considered as representative for layer IV, with two for layer II/III and two for layer V/VI (10 sections above and 10 sections under those from layer IV, respectively). The corresponding optical density of stimulated and inactive areas was measured with image analysis software (NIH Image). Digitized autoradiograms were calibrated using the co-exposed  $C^{14}$ -microscales (RPA504; Amersham, UK). At 200  $\mu\text{m}$  from the center of the lesion, an area situated over the stimulated barrels in the intact tissue was measured ( $400 \times 400 \mu\text{m}$ , black square in Fig. 4A). Another similar area over inactive barrels, 600  $\mu\text{m}$  away from the injury (gray square in Fig. 4A), was considered as a reference for background activity. The same procedure was applied to the contralateral non-injured cortex, where equivalent areas from stimulated (over E1 barrel) and inactive barrels (over C1) were measured. The ratio between stimulated/inactive areas was compared between injured and normal barrel cortex. The results are expressed as a percentage of activity related to uninjured barrel cortex.

### Statistical Analysis

Post-hoc analysis with unpaired *t*-test was used to compare the relative DG uptake between groups 2-w and 6-w of experiment III. One-way analysis of variance (ANOVA) followed by paired *t*-test corrected for multiple comparisons (Dunnett) was performed where the time course after cortical injury was assessed (Gct performance and SEP monitoring).

### Behavioral Assessment by the Gap-crossing Test (Gct)

The gap-crossing test was carried out as adapted to mice by Barnéoud *et al.* (1991) with the following modifications: mice were placed on a small platform ( $6 \times 6 \text{ cm}$ ) and trained to cross spontaneously to a transparent tube (54 mm in inner diameter, 12 cm length, built in transparent acrylic), without food reward; during the training period (2 weeks before the test), the distance from the platform to the tube was incremented by steps of 0.5 cm, from 3 to 6 cm. Whiskers were always trimmed to the skin surface on one side and mice were not blinded; vibrissa sensory function on the other side was sufficient to limit the performance. Trials were recorded with a video camera (Sony CCD-TRV69E) and the maximum distance crossed by each animal was measured. Video recordings allowed for precisely measuring the time spent to cross the gap and to observe the different strategies adopted.

Two groups of 10 mice each were tested. In the first group, five mice with all left whiskers trimmed, the innervation of right mystacial

pad was blocked with local anesthesia (bupivacaine 0.5%, 100  $\mu\text{l}$ , s.c.), while the five control mice received 100  $\mu\text{l}$ , NaCl (9%) s.c. In the second group of 10 mice, animals were trained for a period of 2 weeks and the maximum distance that mice could cross was assessed before and after injury. Trials with video recordings were carried out before and 1, 2 and 4 weeks after a cortical lesion similar to that described above. All animals were injured and in all mice all whiskers but three ( $\delta$ , D1 and E1), corresponding to those represented at the injured site, were trimmed. In the test group ( $n = 5$ ), the spared whiskers were contralateral to the lesion, while in the control group ( $n = 5$ ) the spared whiskers were ipsilateral to the injured cortex.

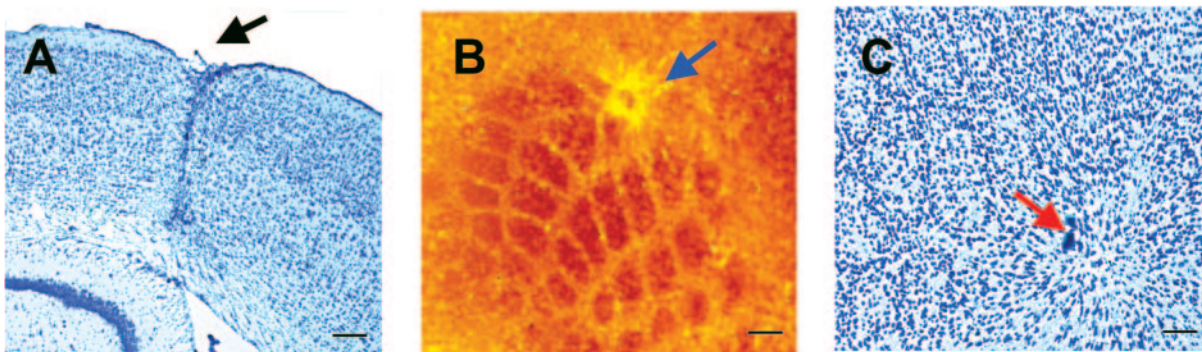
## Results

### Histological Verification of the Lesion

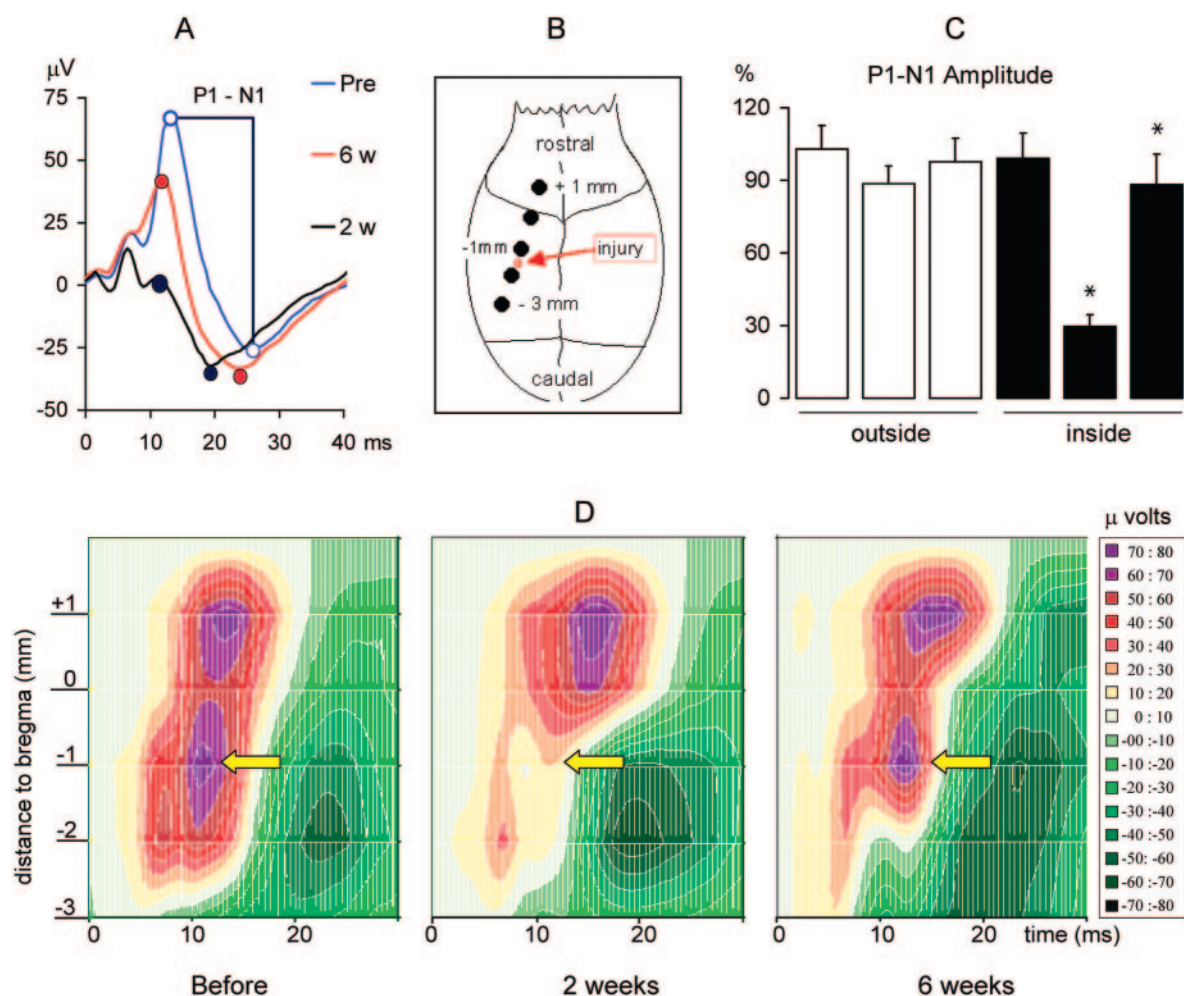
In order to produce a small localized lesion in the barrel cortex a focal lesion was performed with coordinates that targeted the  $\delta$  barrel in the left hemisphere. The injured area extended through the entire cortical thickness down to the subcortical white (Fig. 1A). Most of the lesions produced a superficial injury to the subcortical white matter. On tangential cytochrome oxidase stained sections, the lesion was centered on the  $\delta$  barrel and often extended into neighboring barrels (D1, E1, E2; Fig. 1B). The injured area shrank over the first 2 weeks after the lesion and by 6 weeks post-lesion, it corresponded to a discreet glial scar of  $\sim 200 \mu\text{m}$  (Fig. 1C). Although there was some variability in the extent and position of the lesion, no major difference in the measured physiological and behavioral data could be traced to apparent differences in lesion placement and size. The lesion did not result in any apparent changes in food intake and body weight and caused no mortality.

### Somatosensory Evoked Potential

To investigate whether and to what extent focal lesions affected the global activity of the barrel cortex, we applied multielectrode epicranial recordings. Sensory evoked potentials were elicited by stimulating all contralateral whiskers and recorded using an array of five electrodes placed in line at 1 mm distance from each other (Fig. 2B). The typical responses recorded over the barrel cortex before and 2 and 6 weeks after injury are shown in Figure 2A. Before lesion, the characteristic biphasic response was composed of a rapid positive peak (P1; mean delay of 13–14 ms) followed by a negative wave (N1;



**Figure 1.** Photomicrographs illustrating the lesion site in the barrel cortex 6 weeks after injury. (A) A cresyl violet stained coronal section showing a discreet glial scar (arrow) at the site of the injury. (B) A tangential 40  $\mu\text{m}$  section stained for cytochrome oxidase shows barrels and the lesion. (C) A cresyl violet stained tangential section showing the cytoarchitecture of injured barrels and the surround. Scale bars = 200  $\mu\text{m}$  (A, B); 100  $\mu\text{m}$  (C).



**Figure 2.** Epicranial SEP recording. (A) Representative barrel cortex responses contralateral to the stimulated whiskers in the same mouse before and 2 and 6 weeks after the injury. Each trace is the average of three trials of 10 consecutive responses recorded with an electrode placed at 1 mm posterior to bregma (see B). The responses recorded over the barrel cortex are characterized by biphasic primary responses composed of a rapid positive peak followed by a negative wave (N1). Note the changes in peak-to-peak amplitude (P1–N1) depicted by circles. (B) Schematic drawing displaying the relative position of recording sites (black circles) over the skull. The red open circle indicates the site of barrel cortex injury and the blue open circle shows the site of sham injury outside of the barrel field (stippled outline). (C) Quantitative analysis of PN1–N1 amplitude. The changes observed at 2 and 6 weeks after the injury of the barrel cortex (inside,  $n = 8$ ) is compared to an injury produced outside the cortical representation of whiskers (outside,  $n = 4$ ). While the responses decreased significantly 2 weeks after inside-injury and recover 4 weeks later, the injury outside the barrel cortex did not affect these responses significantly. Results represent the amplitude (mean  $\pm$  SEM) of the peak-to-peak (PN1–N1) responses, normalized to the SEP recorded over the contralateral uninjured cortex (100% =  $89.9 \pm 8 \mu\text{V}$ ). \*2w, Significantly different from responses recorded before the lesion. \*6w, Significantly different from values recorded 2 weeks after the lesion. Pre, before lesion; 2w, 2 weeks after injury; 6w, 6 weeks after injury. (D) Typical spatio-temporal representation of SEP multielectrode recordings, obtained in the same mouse before and 2 and 6 weeks after the injury in the barrel cortex. Horizontal axis represents time in milliseconds, vertical axis represents the distance in millimetres related to bregma and pseudo-color scale represents the amplitude in  $\mu\text{V}$  (red, positive; green, negative values). Arrows show the region (1 mm behind bregma, AP:  $-1 \text{ mm}$ ) where the most significant changes appear over the barrel cortex. Note that in the intact cortex (before lesion) the earlier positive responses are typically recorded above barrel cortex. Similar but delayed responses were consistently recorded with the electrode placed over the facial motor cortex (AP:  $+1 \text{ mm}$ ). The deficit observed 2 weeks after injury is characterized by a dramatic decrease in positive potentials in the barrel cortex. The positive response recorded over motor cortex seems to be unaffected. Six weeks after injury, there is the recovery of positive potentials at AP:  $-1 \text{ mm}$ .

mean delay of 25–27 ms). At  $\sim 8 \text{ ms}$  there is another smaller positive peak that was more clearly distinguishable from P1 after the lesion. These short latency responses concur with those recorded earlier in the rat somatosensory cortex (Di and Barth, 1991; Seo, 1992). An almost complete absence of P1 as well as a shorter N1 latency was typically observed 2 weeks after injury. However, a clear recovery was observed 6 weeks post lesion. A detailed quantitative analysis of the responses was performed using a group of 12 injured mice. To quantify data, we used as an index, the peak-to-peak amplitude (P1/N1), calculated as the difference between P1 and N1 values. SEP recorded in absence of injury showed that contralateral stimu-

lation evoked similar responses in the left (LH) and right (RH) hemispheres. Mean P1/N1 values were  $84.5 \pm 7.9$  and  $89.9 \pm 8.0 \mu\text{V}$  for LH and RH, respectively, while P1 and N1 latencies were  $13.6 \pm 0.4$  and  $26.7 \pm 0.9 \text{ ms}$  at LH and  $13.1 \pm 0.5$  and  $25.9 \pm 1.2 \text{ ms}$  at RH (Fig. 2C). Since there was no evident asymmetry in amplitude and latency, the right uninjured hemisphere was considered as control and all values measured in the left, injured cortex normalized to right values. Two weeks after cortical injury P1/N1 amplitude decreased significantly from  $104.7 \pm 15.6$  to  $29.7 \pm 4.9\%$  ( $n = 8$ ,  $P < 0.001$ ). Also, N1 latency decreased from  $98.5 \pm 1.8$  to  $79.1 \pm 5.4\%$  ( $P < 0.01$ ). However, P1 latency did not change significantly ( $99.7 \pm 1.3$  versus  $96.7$

$\pm 6.5\%$ ). Records performed 6 weeks after the injury showed a partial reversion of these changes: P1/N1 amplitude recovered to  $88.4 \pm 12.4\%$  and N1 latency to  $94.4 \pm 5.8\%$  ( $n = 8$ ,  $t < 0.01$ , for comparison between recordings performed 2 and 6 weeks after injury;  $t$ -test corrected for multiple comparisons). In another group of eight mice, a similar lesion was made outside the barrel cortex. In this group, P1/N1 amplitude and N1 latency values in the recordings performed before and 2 and 6 weeks after the cortical injury, showed no significant changes (Fig. 2C).

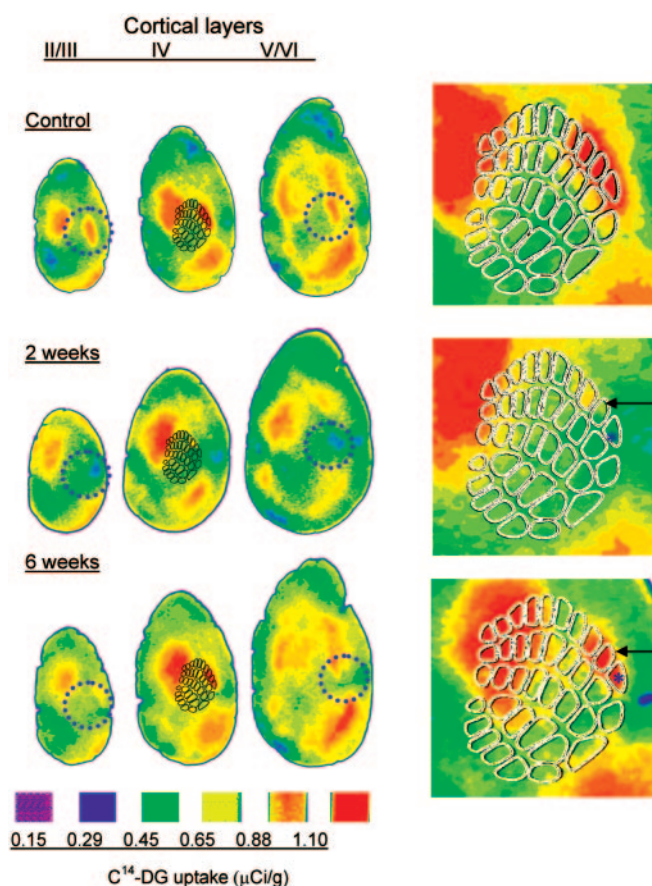
Figure 2D illustrates spatio-temporal maps obtained by analyzing responses before and after cortical injury. In these graphs, the horizontal scale represents time (ms), vertical scale the spatial topography (mm from bregma) and the color range is the amplitude ( $\mu\text{V}$ ) normalized to the maximum positive or negative peak responses (P1 and N1, respectively). As a rule, the main, earlier positive peak is recorded with the electrodes placed at  $-1$  and  $-2$  mm behind bregma ( $0.5$  mm from the site of injury), in proximity to barrel field. However, responses recorded over the motor cortex area did not show significant changes (data not shown). A clear recovery of P1, recorded by the electrode placed at  $-1$  mm behind bregma, was noticed 6 weeks after cortical injury. These results indicate that a focal lesion in the somatosensory barrel cortex induce deficit in global cortical activity that is followed by a partial recovery. These changes are specific for the barrel cortex.

#### [<sup>14</sup>C]2-DG Uptake

We investigated whether the recovery process demonstrated by SEP recording could be confirmed using quantitative [<sup>14</sup>C]2-deoxyglucose (DG) autoradiography. To do this, we measured DG uptake in response to whisker activation in injured mice 2 and 6 weeks after lesion. In these experiments, all whiskers except three ( $\delta$ , E1 and E2) were clipped on both sides, so as to make sure that activity was evoked only in the area of the lesion. Following DG injection, animals were allowed to explore a cage during 45 min to obtain spontaneous activation of intact whiskers (see Material and Methods). In tangential sections from control mice, the metabolic uptake occurred in areas that corresponded to the three stimulated barrels (Fig. 3). For the quantitative analyses, the metabolic activity of stimulated barrels was normalized to that of an inactive reference region (C1; Fig. 4A). In the right, uninjured hemisphere, this value, expressed as a percentage of a reference barrel, was very constant before and 2 weeks ( $33.8 \pm 3.4\%$ ,  $n = 3$ ) and 6 weeks ( $30.4 \pm 3.5\%$ ,  $n = 3$ ) after injury (Fig. 4B). Therefore we considered these values as control for comparisons with changes in relative DG uptake in the injured barrel cortex. The effect of injury on metabolic uptake was assessed in the intact barrels adjacent to the lesion. The quantitative analysis indicated that DG uptake was significantly ( $P < 0.05$ ) lower in the injured than in intact barrel cortex in all layers studied 2 weeks after lesion. This deficit in metabolic uptake partially recovered 6 weeks after lesion; the difference between the 2 and 6 weeks groups being significant ( $P < 0.05$ ).

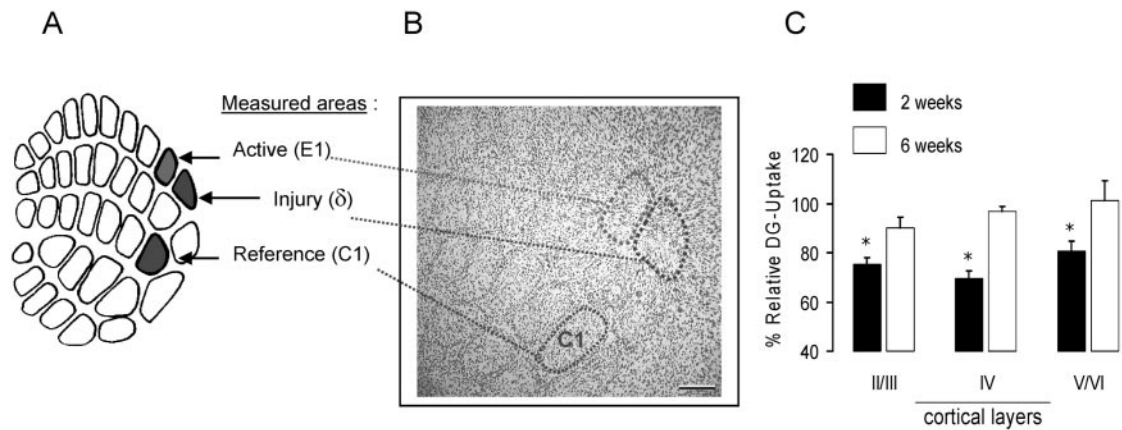
#### Behavioral Performances

An important question with regard to the recovery of electrical and metabolic activity in the barrel cortex concerned the possibility that this process could have functional consequences. To address this issue, we used a gap-crossing test to assess the



**Figure 3.** DG-uptake labeling. Representative maps are shown on tangential sections of the cortex taken from a control animal and from animals that survived 2 or 6 weeks following the injury. II/III, supragranular layers; IV, granular layer with schematic of the barrel field; V/VI, infragranular layers. In the control brain strong activation is seen over the three activated barrels ( $\delta$ , E1 and E2) and the corresponding supragranular and infragranular layers. Note the decrease in uptake on stimulated barrels after 2 weeks and the partial recovery 6 weeks after lesion. Circles represent 1 mm. At the right, magnified maps of the barrel cortex are shown illustrating the injured area centered on the  $\delta$  barrel (asterisk) and the nearest activated barrel (E1 depicted by the arrows) where the uptake measurements were performed.

functional importance of whisker-mediated information. In these experiments, whiskers were clipped on the left side and right vibrissae-dependent performance was analyzed by measuring the size of the gap that the mouse was capable of crossing using its whiskers. Infiltration of mystacial pad with local anesthetics (on one side and saline on the other; see Fig. 5) was used to test the reliability of gap-crossing task. Figure 5 shows that local anesthesia in the right mystacial pad produced a significant ( $P < 0.01$ ) decrease in the performance of animals compared to the control saline injected group. The control group with local anesthesia on the trimmed side did not show any decrease of the performance in the task. Injury in the left barrel cortex, however, resulted in a significant deficit in right vibrissae-dependent gap-crossing task 1 week after the injury ( $6.8 \pm 0.14$  versus  $6.0 \pm 0.25$  cm,  $P < 0.01$ ). Nevertheless, animals were always able to make the movements required to cross the gap. Mice made use of their nose tips or paws to gauge the distance to cross the gap in the dark. The performance then recovered and no significant deficit was detected when mice were tested 4 weeks after the injury. Clipping



**Figure 4.** Quantitative analysis of DG uptake. (A) Schema of barrel field indicating the position with respect to the lesion site of the activated and reference (whisker trimmed) barrels where DG uptake measurement was carried out. A rectangular area with a constant size of  $\sim 400 \times 400 \mu\text{m}$  was used to determine DG uptake in the different layers. The measured values over the activated barrels (E1 barrel) were standardized to DG uptake in reference areas (C1 barrel) in both intact and injured hemispheres. DG uptake in the injured barrel field is expressed as percentages of values calculated in the contralateral homotop area of the barrel cortex. (B) Photomicrograph of a cresyl violet stained tangential section illustrating the injured barrel ( $\delta$ ) and the surround. (C) Results of the quantitative analysis showing a significant difference between the DG uptake measured 2 weeks and 6 weeks after lesion ( $n = 4, P < 0.05$ ).

whiskers on the right side abolished the functional recovery (not shown), indicating that this phenomenon depended on the intact vibrissae–barrel cortex pathway. The control group, with spared whiskers ipsilateral to the lesion (unaffected cortical representation), did not show any deficit 1 week after the injury. In all these experiments, the sensory deficit, whether produced at the peripheral or cortical level, corresponded to a distance (0.7–0.8 cm) that is detected by whiskers.

#### Recovery in NCAM Knockout Mice

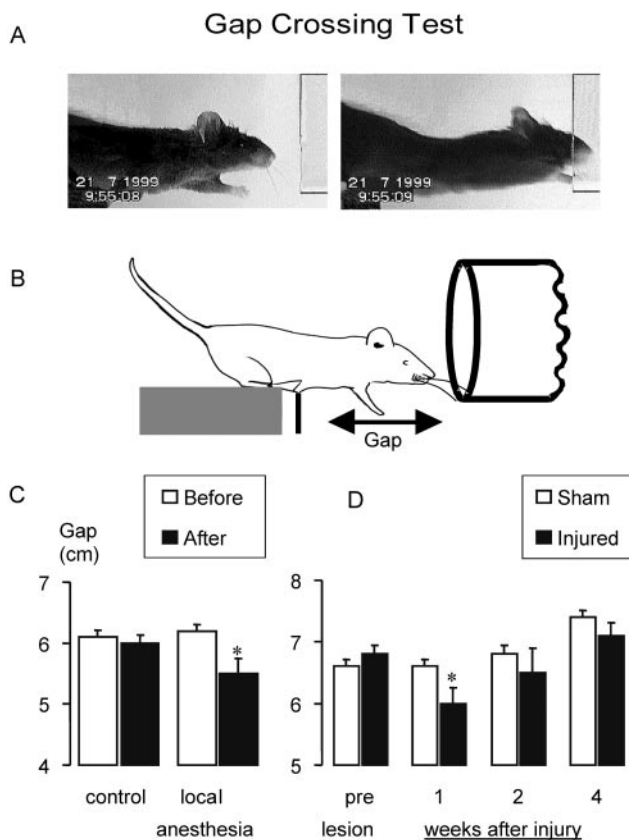
Since previous research had identified a key role for NCAM in different processes that could participate to functional recovery (see review in Kiss *et al.*, 2001), we decided to take advantage of NCAM knockout mice lacking all isoforms of NCAM to evaluate the recovery process using this model. As previously reported (Cremer *et al.*, 1994), NCAM knockout mice exhibit a reduced size of the olfactory bulb, but no other identified abnormalities in the size and organization of the central nervous system. On Nissl-stained sections, the rest of the brain displayed a normal cytoarchitectonics. The cerebral cortex had a typical six-layer organization and on tangential cytochrome oxidase-stained sections we observed a normal barrel pattern (not shown). Finally, the whisker pad in wild type and mutant animals showed no difference. The histological appearance of the lesion and the subsequent repair had the same characteristics in wild type and mutant animals. In a group of four animals, SEP responses were recorded before and 2 and 6 weeks after the lesion. As illustrated in Figure 6, SEP responses showed a similar deficit in control and mutant animals 2 weeks after the lesion. However, after 6 weeks, almost no recovery was perceptible in transgenic mice: the positive and negative peaks remained essentially unchanged. Overall, the peak-to-peak amplitude obtained by subtraction of the voltages measured at the times of the positive and negative peaks showed only  $\sim 10\%$  recovery, which is in marked contrast with what was observed in wild type animals (Fig. 6C). Similarly, measurement of DG uptake confirmed a reduced recovery of metabolic activity 6 weeks after the lesion. Figure 7 illustrates the level of uptake observed in an NCAM knockout

mouse before and 2 and 6 weeks after a lesion made in the region of the  $\delta$  barrel. While some activity is taken over by the adjacent barrels, the recovery at 6 weeks is significantly less than in control animals (compare with Fig. 4). A quantitative analysis (Fig. 7B) indicates that the level of metabolic activity recovered in the adjacent barrels is significantly lower in NCAM knockout mice than in control animals both 2 and 6 weeks after the lesion. These results thus clearly indicate that functional recovery is deficient in the NCAM mutant animal. Whether this defect also resulted in behavioral deficits could unfortunately not be tested, because, surprisingly, NCAM knockout mice used a different strategy than wild type animals in the gap-crossing test: they jumped over the gap rather than extending one leg. The reasons for this difference are unknown, but this prevented comparison between control and transgenic mice.

#### Discussion

The results of the present study provide evidence that the production of a small lesion in the somatosensory cortex of the mouse leads to a functional deficit that recovers over a period of 4–6 weeks. Both the deficit and the functional recovery could be monitored using repeated, multielectrode SEP recordings, analysis of metabolic activity and a behavior test, the gap-crossing test. Application of this model to NCAM knockout mice allowed us to detect a deficit in functional recovery that suggests an involvement of the NCAM molecule in the repair process. Together, these data indicate that this mouse model of lesion-induced recovery may represent an interesting tool for the analysis of the cellular and molecular mechanisms underlying brain injury and repair.

In these studies, activity in the barrel cortex was analyzed by recording potentials evoked by whisker deflection using an epicranial multielectrode recording system (Troncoso *et al.*, 2000). These surface potentials are thought to reflect the activation of apical dendrites of thousands of pyramidal cells sharing architectural and temporal coherence and generating post-synaptic activity. Intra-cortical laminar recordings of evoked field potentials in the rat barrel cortex have established



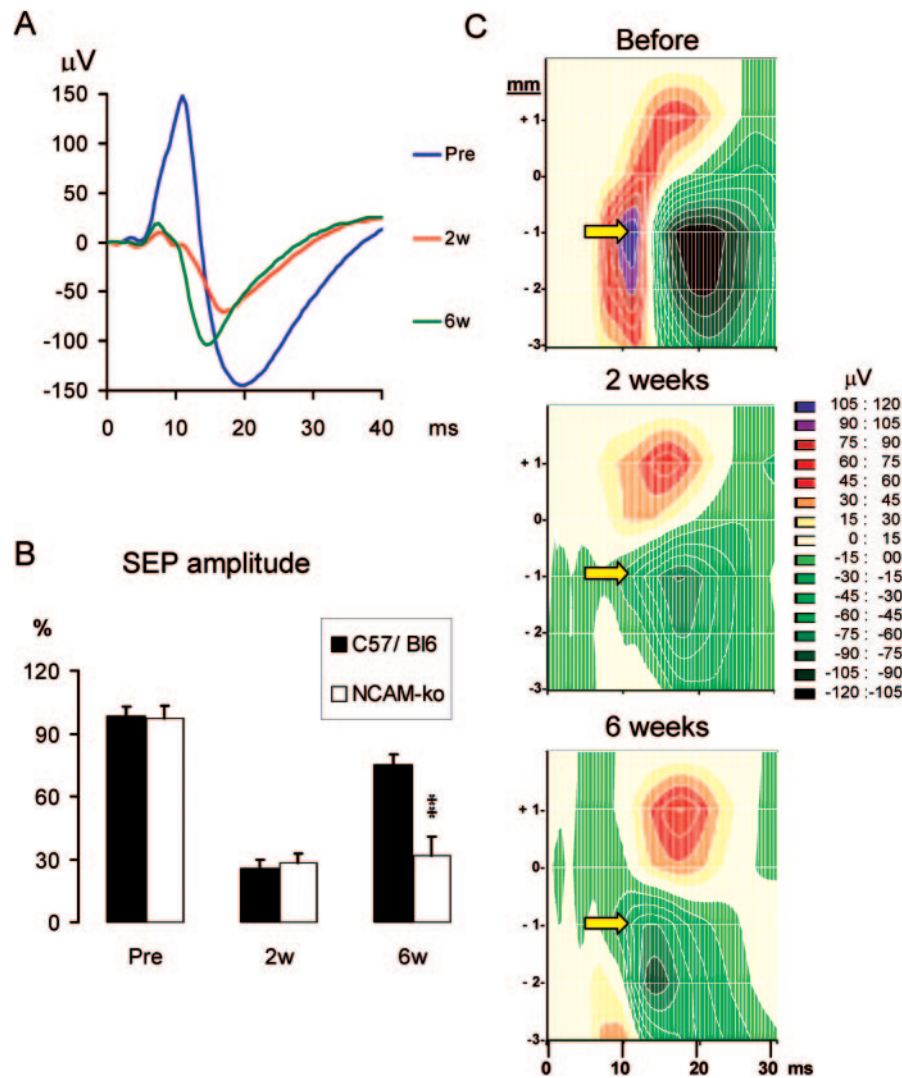
**Figure 5.** Gap-crossing test. (A) Video images showing a mouse crossing the gap between the platform and the tunnel made of transparent acrylic. Mice need to contact the opposite border with their whiskers before crossing. (B) Schematic drawing of the gap illustrating the distance measured in millimetres. (C) The effect of blocking the mystacial pad innervation with local anesthesia on the gap-crossing scores. In these experiments whiskers were clipped on the left side and the right vibrissae-dependent performance was tested with or without local anesthesia (see Materials and Methods). Values are mean  $\pm$  SEM,  $n = 5$ . \*Significantly different ( $P < 0.01$ ) from controls. (D) The effect of barrel field injury on the gap-crossing score. Injury was made in the left barrel field and all whiskers but three ( $\delta$ , D1 and E1) were trimmed. In the test group, the spared whiskers were contralateral to the lesion, while in the control (sham) group the spared whiskers were ipsilateral to the injured cortex. Values are mean  $\pm$  SEM,  $n = 5$ . \*Significantly different ( $P < 0.01$ ) from controls.

that the primary responses of the surface SEP complex are produced by synchronized activation of both supra- and infragranular pyramidal cells (Di *et al.*, 1990). In the current study, SEP recordings were performed after deflection of all contralateral whiskers in order to evaluate the impact of a small focal lesion on the global activity of the barrel cortex. These recordings, made 2 weeks after injury, disclosed a measurable deficit that involved mainly the (first) main positive peak of the SEP complex, occurring  $\sim 13$ – $14$  ms after the vibrissae were mechanically stimulated. This positive peak of the SEP complex most likely reflects intra-cortical integrative processes (Di and Barth, 1991). The present results indicate that a focal lesion of a few barrels markedly affected this integration, making it an interesting tool to monitor recovery. It is of interest that this peak is preceded by an earlier and lower amplitude positive response (occurring at a latency of  $\sim 8$  ms). This earliest positive signal is most likely due to direct thalamo-cortical activation and, as illustrated in Figure 3, was not significantly modified after the lesion upon stimulation of all contra-lateral whiskers. Importantly also, spatiotemporal anal-

yses indicated that the deficit and recovery observed 4 weeks later were restricted to the barrel cortex. No changes of activity were observed in the somatomotor cortex. Together, these results indicate therefore that a focal injury to the barrel cortex is associated with a loss of the cortical activity evoked by stimulation of all contra-lateral whiskers and a recovery process that take place within a few weeks.

In agreement with the results of previous studies (Glassman, 1971), the present data are consistent with and support the interpretation that functional recovery may take place due to a reorganization of cortical activity in the regions around the lesion. The lesion-induced SEP deficit was paralleled by a hypometabolism (28% decrease) detected by stimulus-induced DG uptake in the region surrounding the lesion. Similarly also, the recovery specifically occurred in the barrels adjacent to the lesion within the next 4 weeks. These observations are also consistent with previous reports showing dynamic changes in the functional representation of whiskers after focal lesions. Schiene *et al.* (1999) demonstrated in a rat model that photochemically induced ischemic lesions resulted in an enlargement of cortical vibrissa representation in the surrounding cortical area. They attributed the expansion of the activated area to a decreased GABA-ergic inhibition that facilitates a remapping of the cortical representation in neighboring brain areas. Similar changes in representation area of whiskers after kainate lesions were detected using repeated optical imaging (Nguyen *et al.*, 2000). These results indicate that dynamic changes in juxtalational areas could play an important role in remapping of cortical representation. In this regard, lesion studies are in agreement with earlier publications showing expansion or retraction of whisker cortical maps after activation (Polley *et al.*, 1999) or inactivation by peripheral deafferentation (Silva *et al.*, 1996; Bronchti *et al.*, 1999; Kossut and Juliano, 1999). In contrast to small focal lesions, however, larger injuries appear to produce persistently depressed metabolic activity in adjacent regions (Dunn-Meynell and Levin, 1995; Passineau *et al.*, 2000).

Interesting information added by this study is that the small deficit and recovery in cortical activity had functional consequences at the behavioral level. Using the gap-crossing test we could find a correlated deficit and recovery of performance. This test is sensitive and clearly depended upon a functionally intact vibrissae-somatosensory cortex pathway, as both anesthesia of the vibrissae and lesion of barrels resulted in behavioral deficit. Recovery of behavioral performance took place within 4 weeks, a time course very consistent with those found by previous studies using the gap-crossing test and diverse lesion models (Hutson and Masterton, 1986; Barnéoud *et al.*, 1991; Pazos *et al.*, 1995). Also, the time course of behavioral recovery closely corresponded to that of functional cortical activity (present study). A simple explanation for this observation could be a reorganization of whisker representations in the barrels adjacent to the lesion, although others factors, such as learning of new strategies to cross the gap, cannot be excluded. Similar conclusions were reached by other authors (Hutson and Masterton, 1986) who suggested that destruction of the barrel may impair a 'higher-order integration' necessary for a complex task such as sensory-guided movement and that the motor system involved in gap crossing might require sensory input from the somatosensory cortex. Studies in animal models and human data suggest that small lesions in the motorcortex or in the somatosensory cortex



**Figure 6.** SEP recording in NCAM knockout mice. (A) Representative SEP responses recorded over the barrel cortex of an NCAM knockout animal contralateral to the stimulated whiskers in the same mouse before and 2 and 6 weeks after the injury. Notice that the absence of positive response observed 2 weeks after the cortical injury remains pronounced 4 weeks later (6 weeks after injury). (B) Voltage values measured at a latency of 13 ms after stimulation. This latency corresponds to the mean positive peak values observed before cortical injury. Compared to control mice, 6 weeks after injury voltage at this latency was negative in all NCAM knockout mice ( $n = 5$  in both groups). (C) Typical spatio-temporal representation of SEP multielectrode recordings, obtained in the same NCAM knockout mouse before and 2 and 6 weeks after the injury in the barrel cortex. All three parameters (time, distance and voltage) are the same as described for Figure 3. Arrows indicates the loss of positivity and absence of recovery at AP: -1 mm. Responses over the motor cortex were not affected by the injury.

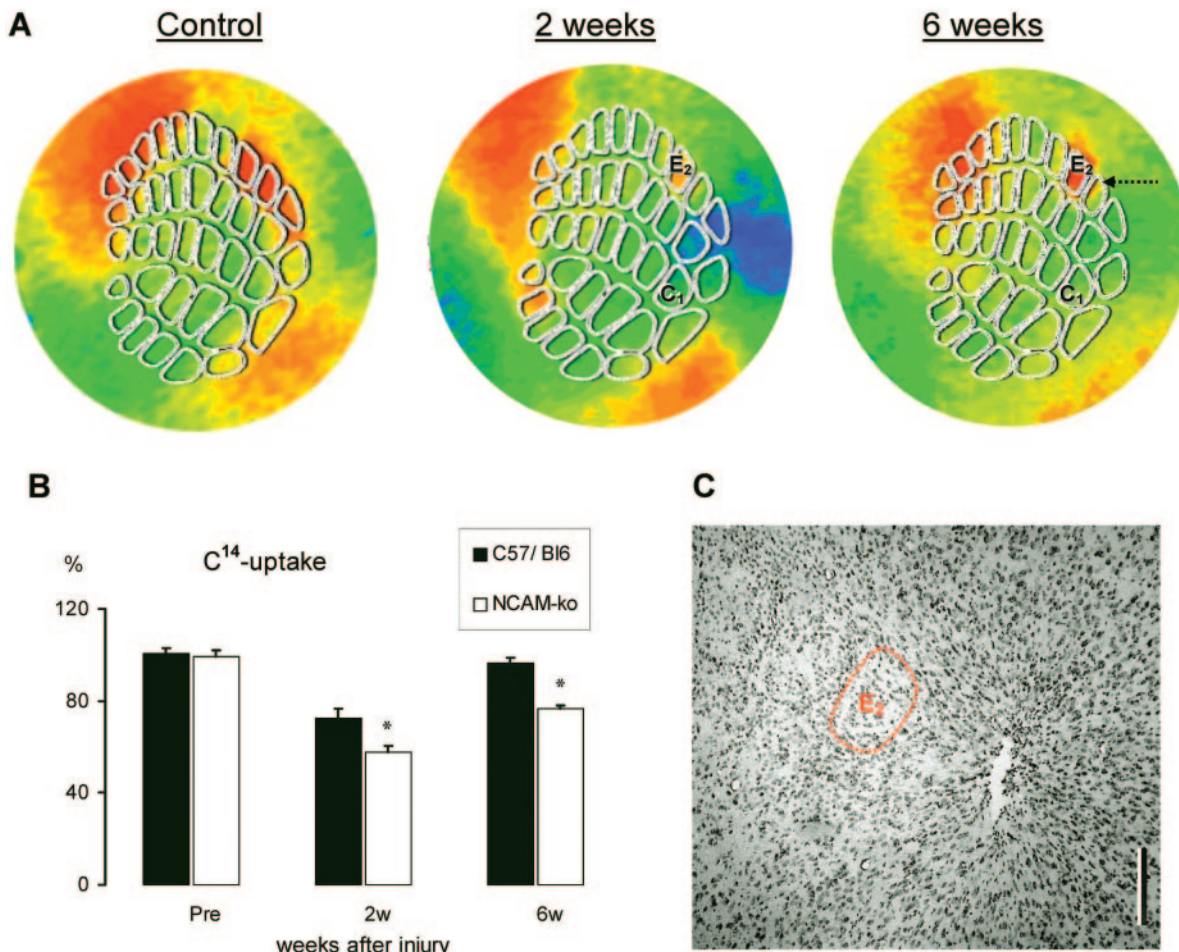
recover from the ipsilateral cortex adjacent to the lesion, whereas large lesions recover from the contralateral cortex. (Jenkins and Merzenich, 1987; Witte *et al.*, 2000). Human data also indicate that a new lesion in the surround of the lesioned cortex abolishes previous recovery (Jenkins and Merzenich, 1987; Witte *et al.*, 2000). It would be of particular interest to test in our model if lesions of the adjacent cortex 6 weeks after the first lesion would affect functional recovery.

The present model also opens the way to analyze the cellular and molecular mechanisms underlying the recovery. Several possibilities have been considered. One involves the resolution of acute pathological mechanisms such as edema, vascular perturbation and excitotoxicity. These events, however, are thought to be at least partially resolved by 2 weeks after the lesion. Another mechanism contributing to recovery could be a gradual reversal of diaschisis, a temporal suppression of surrounding and remote cortical tissues after focal lesions

(Witte, 1998). The effect of diaschisis has been substantiated by recent metabolic studies showing a persistent hypoactivity in perilesional areas (Schiene *et al.*, 1999; Passineau *et al.*, 2000). Finally, growth mechanisms and functional reorganization of cortical networks could also account for the take over of function (vicariation of function) of the damaged area by adjacent or remote areas (Witte, 1998). This process is likely to be complex and involve several cellular and molecular events, including a modulation of the expression of receptors (Schiene *et al.*, 1996), axonal sprouting and new synapse formation (Stroemer *et al.*, 1993). The use of transgenic models could reveal of interest to distinguish between all these possibilities.

The experiments with the NCAM knockout mice demonstrate the interest of this recovery model to identify, through the use of transgenic animals, the role of candidate molecules in functional recovery. While the functional organization of the whisker-barrel system in WT and NCAM mutant animals





**Figure 7.** DG- uptake labeling in NCAM knockout mice. (A) Representative DG maps of tangential barrel cortex sections taken from a NCAM knockout mice. Notice that the activated barrel (E1 depicted by the arrow) which recovers its activity after 6 weeks in wild type animals (see Fig. 3), displays low uptake compared to the reference barrel. (B) Quantitative analysis of  $C^{14}$ -DG uptake obtained in wild type animals (C57/Bl6) and NCAM knockouts. Values are mean  $\pm$  SEM ( $n = 4$  animals in each group) normalized to the contralateral homotop area of the barrel cortex (see legend for Fig. 4) and expressed as percentage of the value measured before the lesion (pre) (100%). \*Significantly different from the wild type group,  $P < 0.05$ .) (C) Representative cresyl violet stained tangential section showing the cortical injury in a NCAM knockout animal killed 2 weeks after the lesion. Red dashed line shows the position of E2 barrel. Scale bar = 200  $\mu$ m.

displayed no apparent differences, the functional recovery 6 weeks after the lesion was significantly reduced. This conclusion was reached through the use of epicranial SEP recordings and analyses of metabolic activity. The precise mechanism of the deficient recovery in the mutant mice remains unknown. It is of particular interest that NCAM/PSA-NCAM has been localized in synapses and that long-term synaptic potentiation (LTP) is impaired in CA1 synapses of the hippocampus in NCAM knockout animals (Muller *et al.*, 1996). It is thus possible that an altered synaptic plasticity in cortical circuits underlies the observed deficit in functional recovery. Alternatively, the absence of NCAM during development could result in a modified organization of cortical circuits that could lead to reduced plasticity and capacity to reorganize after a lesion. Clearly, further studies involving intra-cortical recordings and analyses of the synaptic organization of the barrel cortex will be required to verify these hypotheses.

### Conclusions

In this study we have assessed the electrical and metabolic activity of recovering circuits in the somatosensory barrel

cortex of the mouse. A time window has been identified between 2 and 6 weeks after injury, during which behaviorally relevant impairment and recovery was reliably measured in the surround of injury. This mouse model therefore opens new ways to investigate, at the molecular level, the mechanisms contributing to functional recovery.

### Notes

We are grateful to Cynthia Saadi and Nathalie Moulin for technical assistance, especially with the histological processing. This work was supported by the Swiss National Science foundation grants 31-64030.00 to J.Z.K. and PNR-38: 4038-0044151 to J.Z.K. and D.M.

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