ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1994, 54: 345-352

Short term changes of cortical body maps following partial vibrissectomy in adult mice

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Abstract. Vibrissae-to-barrels pathway is often used as a model for investigating CNS plasticity. We examined early changes in the cortical representation of row C of vibrissae in adult mice, following vibrissectomy removing all whiskers except row C. The changes of cortical representation of the spared row of vibrissae were mapped with 2-deoxyglucose autoradiography. We found that one day after lesion of vibrissal follicles the areal extent of cortical representation of row C is smaller than normally, but 7 days post lesion it increases significantly, by 60 to 90%, in all cortical layers. Additionally, seven days post-lesion the intensity of labelling was increased in cortical layer V. The result suggest that plasticity that can be observed with 2-deoxyglucose mapping in the barrel cortex is not due to unmasking of existing connection, but to reorganization of connectivity at many levels.

Key words: plasticity, barrel cortex, mice, vibrissectomy, 2-deoxyglucose

INTRODUCTION

Cortical body maps can undergo reorganization when peripheral sensory nerves are damaged (Wall and Egger 1971, Merzenich et al. 1983a, Kaas et al. 1984, for review see Wall 1988). The denervated region of cortex can then be driven by inputs that normally supply adjacent cortical areas. The process of reorganization of cortical maps has both fast components, occurring immediately after nerve transection or inactivation, and slower ones, that take a longer time - days, weeks, months - to develop (Merzenich et al. 1983b, Kaas et al. 1984, Cusick et al. 1990).

We have previously reported several instances of cortical map changes in the barrel cortex of mice and rats (Kossut et al.1988, Kossut 1992, Kossut et al. 1993). In this part of somatosensory cortex cortical representations of vibrissae can be easily identified because of a special cytoarchitectonic structure of layer IV, where neurones aggregate to form ring-like structures, each being a centre of cortical representation of a particular vibrissa (Woolsey and Van der Loos 1970). The arrangement of barrels mimics the arrangement of vibrissae on the snout. The barrel cortex displays two kinds of plastic changes - morphological plasticity, present only in neonatal animals, when destruction of vibrissal nerves induces changes in morphological structure and dimensions of the barrels (Van der Loos and Woolsey 1973), and functional plasticity, present also in adult animals, where manipulations of sensory input change the volume of cortex activated by the involved vibrissae, while not altering the dimensions of the barrels (Kossut 1992). The vibrissae-to--barrels sensory pathway is an increasingly popular model for studying neuronal mechanisms and biochemical correlates of neocortical plasticity in adult rodents (Dietrich et al. 1981, Welker et al. 1989, Dunn-Meynell et al.1992, Skangiel-Kramska et al. 1994). To provide a better physiological basis for the biochemical studies, more information was needed about the dynamics of cortical maps changes in the barrel field induced by a lesion of selected rows of vibrissal follicles in adult animals (this is the paradigm most frequently used in these studies). In the present communication we report the effects of vibrissectomy sparing one row of whiskers upon cortical representation of the spared row, examined at two post-lesion times.

METHODS

The experiments were performed on 8 young adult (5 weeks old) Swiss albino mice. The mystacial pad of one side of the muzzle was anaesthetized locally with 0.25% Xylocaine. All vibrissae except row C were removed mechanically with fine forceps through incisions in the skin of the mystacial pad between rows A and B and D and E.

One day (4 mice) or seven days (4 mice) after surgery the 2DG mapping experiment was performed. The unanaesthetized mice were taped to a padded box by their trunks. On the side of muzzle opposite to the lesion all vibrissae except row C were clipped close to the skin. 2DG (Amersham, s.a. 55.4 Ci/mmol, 5 μCi/mouse) was injected i.m. and the spared row C on the lesioned side and the control row C on the other side of the muzzle were stroked with a mechanical stimulator of 3 Hz frequency for 45 min. The strokes were in the direction along the row of whiskers. After 45 min of isotope incorporation the mice received an overdose of Nembutal and were perfused with 3.3% formalin (Hand 1980). The brains were rapidly frozen in isopentane at -60°C and sectioned on a cryostat at -18°C into serial 20 μm thick sections, in a plane tangential to the barrel field. We cut 50 - 60 serial sections from each hemisphere. The sections together with [^{14}C] standards (American Radiochemical) were apposed to X-ray film in X-ray cassettes for 30 days. After developing, the sections were counterstained with cresyl violet for identification of barrels and cortical layers the autoradiograms were filmed with CCD camera and analyzed with computer controlled image analyzer (Visionetics).

The width of the labelled region and its labelling intensity (in grey level units, calibrated with respect to [^{14}C] standards exposed together with the sections) were measured on serial sections through the barrel cortex. The software allowed us to display on

a computer screen the image of a stained section from which the autoradiogram was obtained, and to mark the barrel outlines, which were superimposed on the autoradiogram so that the relations of the labelled regions to the morphological barrels could be accurately determined. The barrels of row C (C1 to C4) were measured on all layer IV sections (6-9) on which they were well visible. The dimensions of barrels were carefully examined to correct for possible variations of the plane of sections. The labelled representation of row C whiskers in layer IV was measured in exactly the same locations. The labelled representation was also identified and measured on all sections through layers II/III and V (in layer VI the labelling was very faint and diffuse and no reliable measurements were possible), where the barrels are not present. The disappearance of barrels and the presence of pyramidal neurones differentiated the border of layers IV and III. Values for layers II/III were calculated together as they could not be reliably distinguished on the Nissl stained sections. The disappearance of barrels and the appearance of large pyramids marked the border of layer IV and V. The border of layer V and VI was characterized by an increase in cellular density.

We determined the width of labelling of row C representation using the previously established criterion (Kossut et al. 1988) which considered as activated the regions with level of 2DG labelling 15% higher than in the surrounding cortex. We have found previously (Chmielowska et al. 1986) that the 15% difference on the average corresponds to two standard deviations above the mean value of labelling in the surrounding cortex. To obtain the width measurement, an optical density scann was taken across the labelled region (for example of such scann see Fig. 3), the density values of the surrounding cortex were found on the graph and the readings of width of labelling were taken according to the described above criterion. The values obtained in histological and autoradiographic measurements were averaged to give the mean value for width of the barrels and the width of labelling of the entire row C. Statistical significance of the results was assessed with Mann Whitney U test.

RESULTS

We have previously ascertained that stimulation of vibrissae produces strictly unilateral effect upon 2DG uptake (Chmielowska et al. 1986, Kossut et al 1988). In control groups of animals used previously in different experiments, where corresponding rows of whiskers were stimulated on both sides of the snout, the difference in width of labelling beetween the two hemispheares in the same animal did not exceed 10%. Therefore in the present experiment we could directly compare the results obtained in the two hemispheres of the same animal, one contralateral to the lesioned mystacial pad and one to the control side of the muzzle.

Comparing the labelling in the two hemispheres of the same animal gave results that were free from unspecific effect affecting glucose metabolism such as stress and also controlled for differences in labelling intensity frequently observed between animals. Stroking of row C of whiskers produced a band of labelling extending throughout the depth of the cortex. The densest labelling was observed in cortical layer IV. As described before, the labelling was centred upon the appropriate row of barrels (Kossut and Hand 1984, Chmielowska et al. 1986, Kossut et al. 1988), but the activated area extended into the septa and edges of adjacent rows of barrels. The extent of labelling did not differ significantly between cortical layers.

After one day

One day after vibrissectomy sparing row C, cortical representation of the spared row did not expand (Figs. 1, 3 and 4A). On the contrary, in layers II/III the width of the labelled region was 25% less than on the control side (440 μm ±35 and 585 μm ±62, respectively, $P<0.05$). The intensity of label over row C representation was decreased in all cortical layers (Fig. 5A). The intensity of labelling over the neighboring, unstimulated row B was also significantly decreased in all layers except V, as compared to the control hemisphere (Fig. 5B).

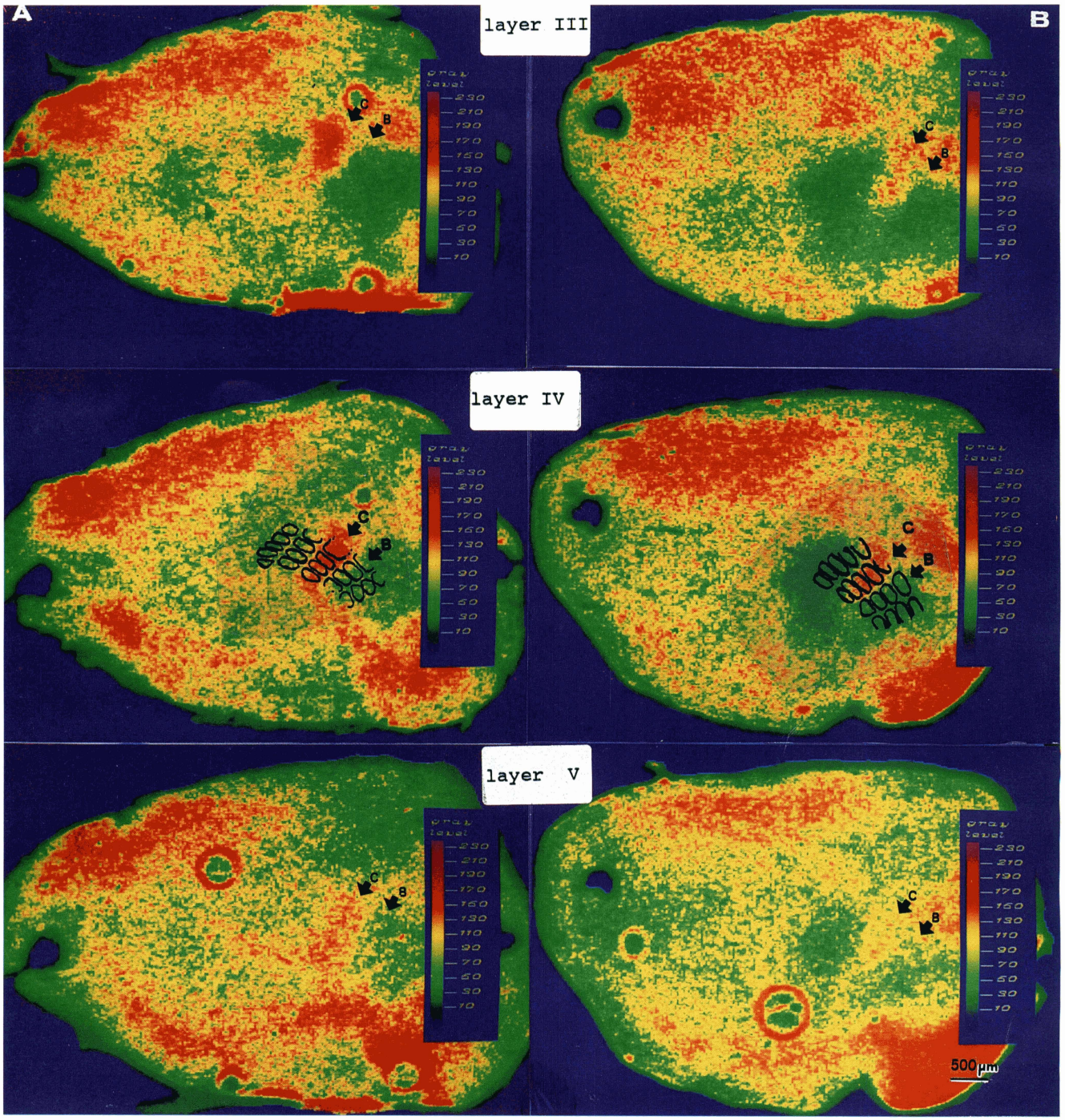

Fig. 1. Cortical representation of row C of vibrissae labelled with [^{14}C] 2DG one day post-lesion. Pseudocolor digitized image of autoradiograms of sections cut tangentially to the barrel fieled, at the level of different cortical layers. Arrows point to row C and row B. At the level of layer IV barrel outlines taken from counterstained section from which autoradiograms were obtained are superimposed upon pseudocolored image. A, control side; B, experimental side. The section labelled as layer III was taken 40 µm above the first section from layer IV.

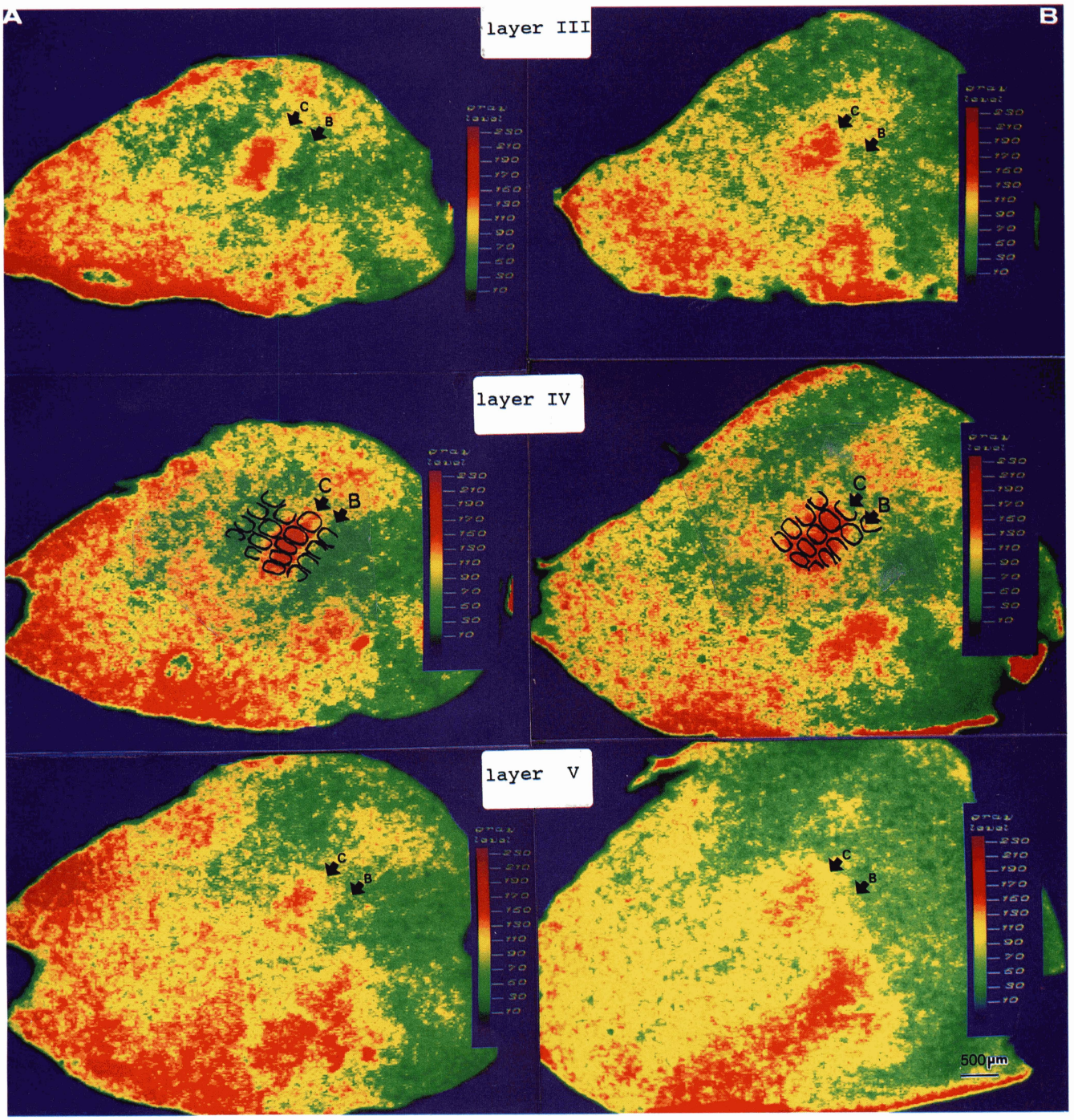

Fig. 2. Cortical representation of row C of vibrissae 7 days post-lesion, descriptions as in Fig. 1.

After seven days

Seven days after vibrissectomy, the spared row C representation was significantly ($P<0.05$) enlarged in all cortical layers (Figs. 2,3, and 4B). The increase amounted to 370 µm ±47 in layer II/III, 470 µm ±52 in layer IV and 620 µm ±69 in layer V. The greatest expansion of the spared column representation occurred in cortical layer V (92%) (Fig. 3). Characteristic fragmentation of the borders

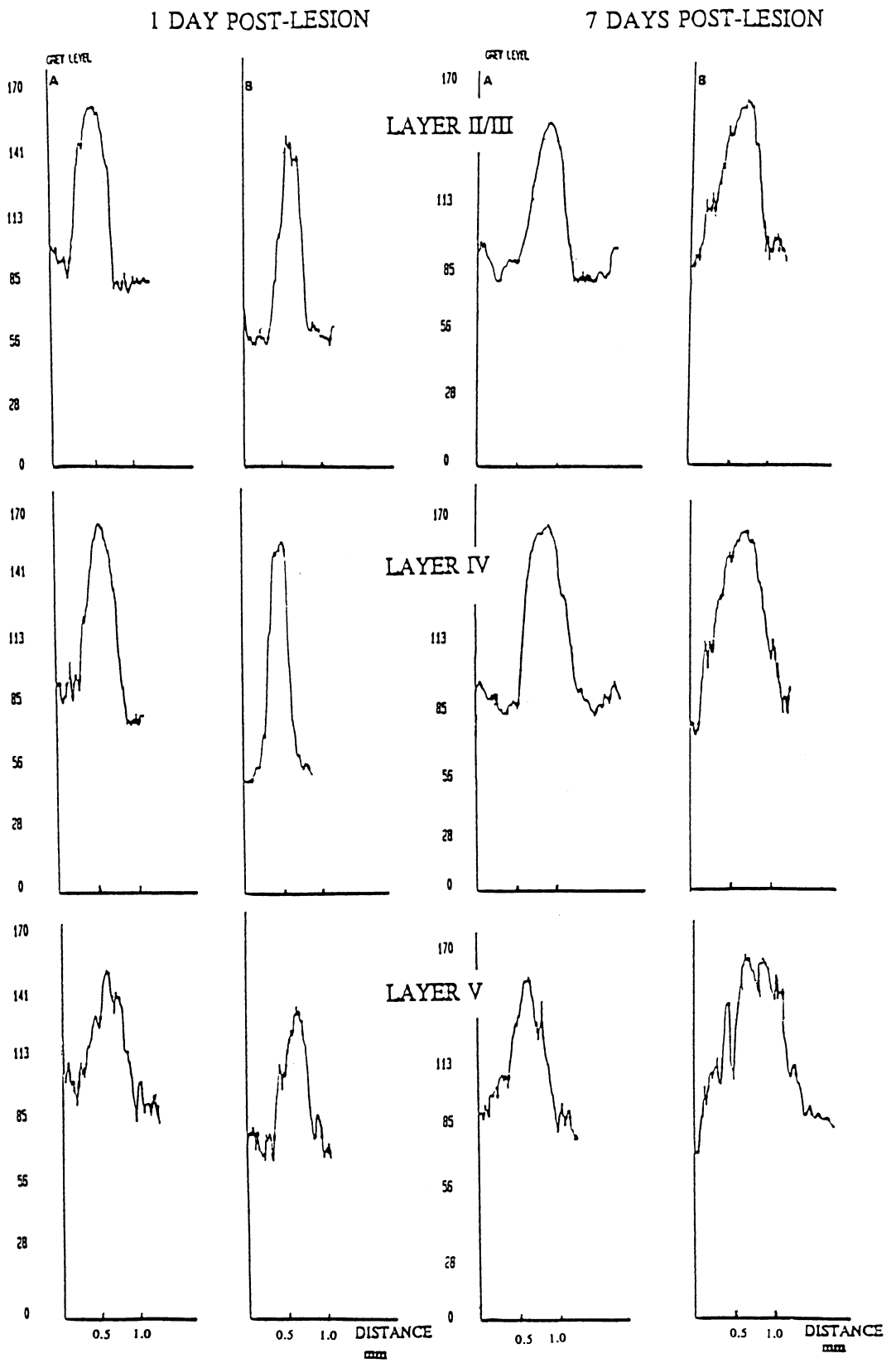

Fig. 3. Optical density scans across the cortical representation of row C labelled with 2DG. The width of the scanning window was 300 μm. Grey level in arbitrary units. A, control side; B, experimental side.

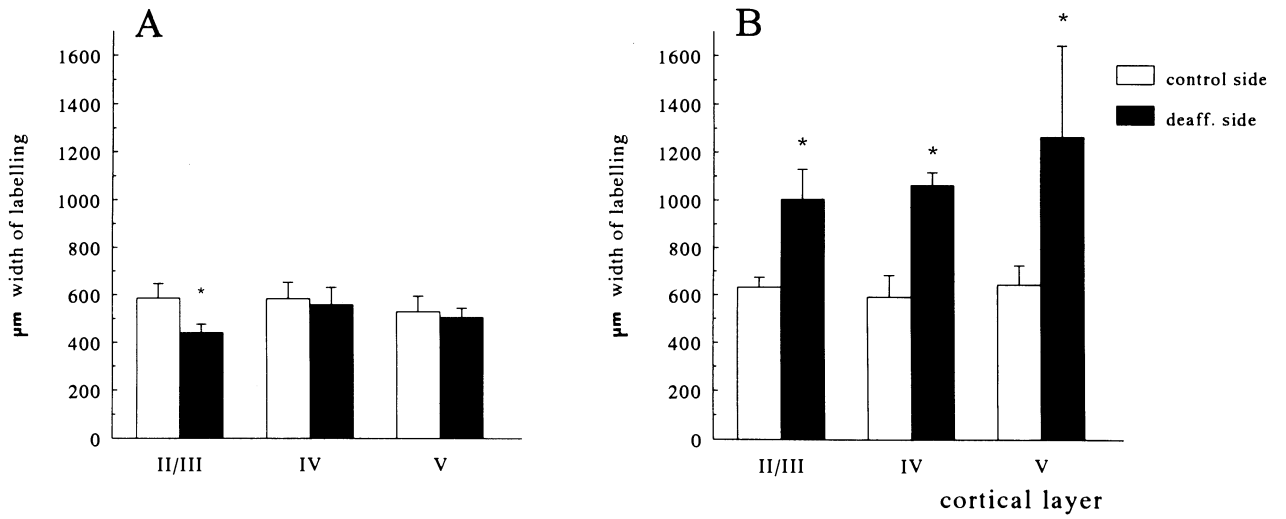

Fig. 4. Changes of width of labelling of cortical representation of row C in different layers after vibissecomy sparing row C. A, one day post-lesion; B, 7 days post-lesion. The average values ±SD were obtained from measurements taken from all serial sections in a given layer. *P<0.05.

of the labelled area with appearance of many radiating processes was observed (Fig. 2). The intensity of 2DG uptake did not differ from the values obtained in the control hemisphere in layers II/III and IV, but in cortical layer V the uptake was significantly (P<0.05) higher (Fig. 5A). This increase in labelling intensity amounted on the average to 25% ±18 %. The labelling over the unstimulated row B was significantly (P<0.05) higher in all cortical layers (Fig. 5B) indicating expansion of row C of vibrissae rep-

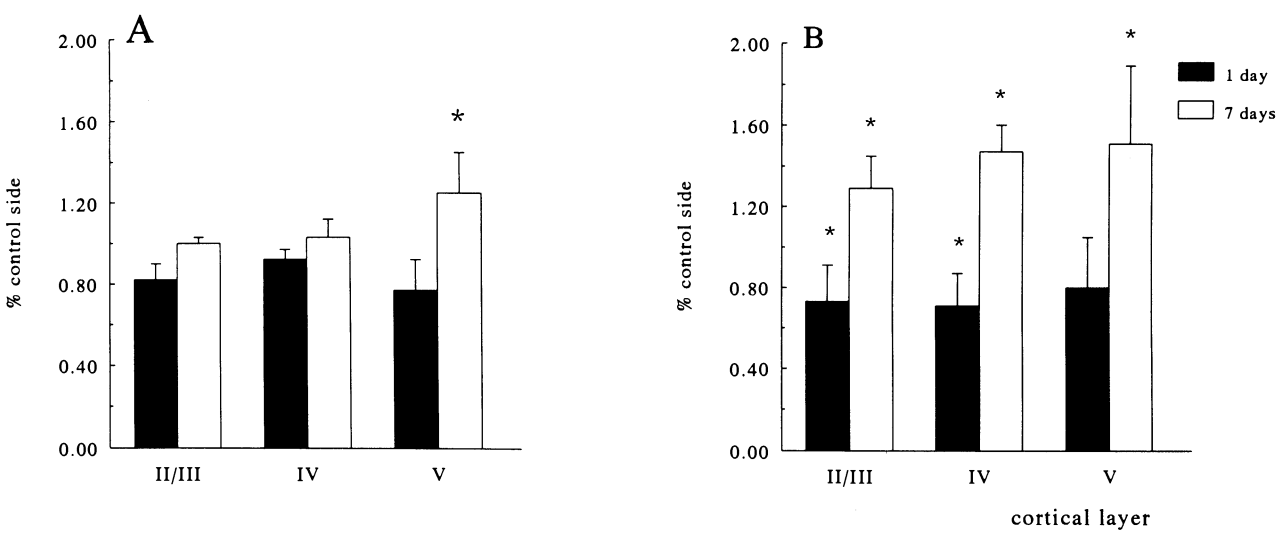

Fig. 5. Changes of labelling intensity expressed as % of labelling in the contralateral hemisphere after vibrissectomy sparing row C. A, measured over row C of barrels and over- and underlying regions of layers II/III and V; B, measures over row B of barrels and over- and underlying regions of layers II/III and V. The average values ±SD were obtained from measurements taken from all serial sections in a given layer. *P<0.05.

resentation over cortical territory normally occupied by row B representation.

DISCUSSION

In adult mice, extensive changes of cortical representation of a spared row of vibrissae can be seen 7 days after partial vibrissectomy. These changes involve an expansion of cortical representation of the spared row, observed in all cortical layers and an increase of energy metabolism in cortical layer V. Such alterations were not observed one day after vibrissectomy. At this post-lesion time we observed primarily a decrease of extent in labelling in cortical layers II/III and decrease of intensity of labelling in supra- and infragranular layers. This effect are most likely due to the immediate effects of surgery. Lowering of metabolism in the barrel field was reported after cutting or plucking off of the whiskers, either immediately or a day before the 2DG mapping (Durham and Woolsey 1978, Chmielowska et al. 1986). The surgical removal of vibrissal follicles could have made it more pronounced. Additionally, the effects of trauma in the vicinity of the activated mechanoreceptors could alter their responsiveness.

The early appearance of plastic changes in neuronal reactivity in the barrel cortex are in agreement with the electrophysiological data of Diamond et al. (1993), who found that 3 days of whisker pairing produced profound changes in the responsiveness of the barrel neurones. Our data on mice barrel cortex confirm the results of 2DG mapping studies on spared single whisker preparation of the adult rat (Levin and Dunn-Meynell 1988). We also have previously documented, using 2-deoxyglucose (2DG) mapping of functional activity, a rapid enlargement of vibrissal cortical column C3 in the SI cortex of rats and mice, following seven days of sensory deprivation of all whiskers except C3 (Kossut et al. 1993). In these experiments deprivation was accomplished by plucking out the whiskers. Thus a few days of either denervation or deprivation are sufficient for remodelling the sensory pathway so that the spared receptors activation involves significantly larger cortical area.

The possible mechanisms that could underlie cortical plasticity in adult animals include activation of silent synapses, reorientation of dendritic fields or sprouting of thalamo-cortical and cortico-cortical axon (Wall and Egger 1970, Wall 1988). The activation of silent synapses and previously ineffective, suppressed connections is thought to underlie the immediate of very fast changes observed in cortical representations (Calford and Tweedale 1991). In the present experiment, using the 2DG mapping to visualize plastic changes, we observed no immediate expansion of row C representation. It can either be due to the method of surgery we employed, or the sensitivity of 2DG mapping, or, possibly to the peculiar structure of the vibrissae to barrels pathway, where little divergence of thalamo-cortical input is observed (Bernardo and Woolsey 1987, Jensen and Killackey 1987).

Recent evidence stressed the importance of strengthening and sprouting of cortico-cortical projections. Darian-Smith and Gilbert (1994) demonstrated an elongation of cortico-cortical connections in supragranular layers of the visual cortex of adult cats, when plasticity of the cortex was induced by retinal lesions. Fox (1992) argued that in adult rat vibrissae to barrels pathway plasticity is only observed in supragranular cortical layers. Interestingly, Armstrong-James et al. (1993) and Diamond et al. (1993b) have reported that, in a whisker pairing paradigm, first plastic changes seem to be mediated *via* supragranular layers, but later a thalamo-cortical input change becomes visible. In our results row C representation was expanded in all cortical layers, but we can not say whether involvement of layer IV was primary, due to changes of thalamocortical input, or secondary, reflection activation of this layer *via* intracortical projections. Particularly large increases of diffuse activation were found in layer V. Also, an elevation of glucose uptake was noted in this layer - a phenomenon that was previously observed when plastic changes were induced in adult rat barrel cortex (Hand 1982, Kossut et al. 1988). Layer V receives no direct input from the ventrobasal nucleus of the thalamus, except for the region bordering layer VI

(Bernardo and Woolsey 1987, Jensen and Killackey 1987), so synaptic activity in this layer reflects primarily activity of intracortical inputs (Bolz et al. 1989). Although 2DG mapping visualizes primarily the regions of high synaptic activity (Schwartz et al. 1979), restoration of membrane potential after intensive spike generation also consumes energy and can contribute to the density of labelling (Sokoloff 1993). Thus both augmentation of intracortical and thalamo-cortical (*via* the input to layer V) transmission and strengthening of links with the superior colliculus can be among cellular mechanisms underlying plastic changes in the adult cerebral cortex.

ACKNOWLEDGEMENT

This work was supported by a statutable grant to the Nencki Institute from The State Committee for Scientific Research and by The Foundation for Polish Science grant Brain 1/94 to M.K.

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Received 3 October 1994,accepted 17 October 1994