Pergamon

PII: S0306-4522(97)00332-1

Neuroscience Vol. 83, No. 1, pp. 27–41, 1998

0306-4522/98 $19.00+0.00

PLASTICITY OF CEREBRAL METABOLIC WHISKER MAPS IN ADULT MICE AFTER WHISKER FOLLICLE REMOVAL—I. MODIFICATIONS IN BARREL CORTEX COINCIDE WITH REORGANIZATION OF FOLLICULAR INNERVATION

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Abstract—We investigated alterations of the metabolic whisker map of barrel cortex after the removal of the follicles of left whiskers C1, C2 and C3 in adult albino mice. The quantitative autoradiographic [^{14}C]deoxyglucose method was used to measure local cerebral metabolic rates for glucose in barrel cortex of mice two, four, eight, 64, 160 and 250 days after the lesion. Metabolic rates were measured in three groups of animals: (i) mice with lesions that had all whiskers clipped; (ii) mice with lesions that had left whiskers B1–3 and D1–3 stimulated; and (iii) unoperated mice that had left whiskers B1–3 and D1–3 stimulated. Compared with the metabolic rates in barrels C1–3 of stimulated unoperated mice, barrels C1–3 of stimulated mice with lesions showed the first discernible increase in metabolic rate four days after the lesion. The increase became distinct at 64 days, but attained statistical significance only ~160 days after the lesion. The lesion *per se*, i.e. without whisker stimulation, caused only a small increase in metabolic rate in barrels C1–3 accounting for not more than one fourth of the increase in metabolic rate measured after whisker deflection. The removal of whisker follicles C1–3 led, therefore, to an enlargement of the metabolic representations of the adjacent whiskers into the barrels deprived by the lesion. The gradual consolidation of the alterations of the metabolic whisker map coincided with the regeneration of follicular nerves in the whiskerpad. We detected anomalous deep nerves innervating follicles surrounding the lesion at ~64 days, and the number of myelinated nerve fibres in the deep nerves of these follicles was increasing with increasing time after the lesion.

The coincidence of peripheral and central change suggests that the reorganization of the innervation of the sensory periphery plays an important role in the persistent alterations of the cortical somatotopy in adults following a lesion in the sensory periphery. © 1997 IBRO. Published by Elsevier Science Ltd.

Key words: regeneration, vibrissa, barrel, deoxyglucose, somatosensory cortex.

Restricted lesions in the peripheral somatosensory receptor sheet of adult primates have been shown to alter the cortical somatotopic representation of the deprived territory as well as adjacent areas. The transection of a sensory hand nerve[29] or the amputation of digits of the hand[30] resulted within three months in the expansion of the representation of adjacent intact skin in primary somatosensory cortex into the territory which previously represented the removed or denervated soma. In a previous investigation[26] on plasticity of barrel cortex we found that the removal of three whisker follicles on the snout of adult mice produced alterations of their cortical representation similar to those in somatosensory cortex of primates with amputated digits. The barrel cortex is a subdivision of the primary somatosensory cortex containing cytoarchitectonic units in layer IV, named barrels, which are composed of cell body-dense sides surrounding cell body-sparse hollows and represent the whiskers topologically.[44] We observed that stimulation of whisker follicles adjacent to the removed follicles metabolically activated the deprived barrels eight months after the lesion and that the deep innervation of the stimulated follicles was increased. In a number of mice, we detected distinctly anomalous deep nerves. The present report describes the progression of the changes in the metabolic whisker map of barrel cortex after whisker follicle removal in relationship with the degeneration of the deprived nerve fibres and their subsequent regeneration.

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Abbreviation: lCMR$_{glc}$, local cerebral metabolic rate for glucose.

EXPERIMENTAL PROCEDURES

Animals

All procedures were in accordance with the National Institutes of Health Guide for the Care and Use of

Laboratory Animals and were approved by the NIMH Animal Care and Use Committee. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to *in vivo* techniques. Male and female Swiss albino mice of International Charles Rivers origin (Harlan Sprague–Dawley, Indianapolis, IN, U.S.A.), >eight weeks-of-age, were used. The tall whiskers on the snout of the mouse are arrayed in five rows. Woolsey and Van der Loos[44] designated the rows A (dorsal) to E (ventral) and numbered each whisker in a row beginning caudally with 1. Four single whiskers, α to γ, straddle the rows at the caudal end. The follicles of the left whiskers C1, C2 and C3 were removed through an incision in the skin between rows C and D under halothane anaesthesia (~1.5% in 70% N_2O/30% O_2). The wound was closed with one suture.

Measurement of rates of glucose utilization in barrel cortex

Preparation of animals. Measurements of local cerebral metabolic rate for glucose ($lCMR_{glc}$) were carried out two (2^d), four (4^d), eight (8^d), 64 (64^d), 160 ± 3 days (160^d) and 250 ± 45 days (250^d) after follicle removal. One hundred and ten mice were studied. Ten were unoperated controls with whisker stimulation (see below), five studied at six to 16 weeks-of-age and five studied at 40–56 weeks-of-age. Measurements of local cerebral metabolic rate for glucose ($lCMR_{glc}$) were completed in five to nine operated animals under each condition of stimulation.

On the day prior to the deoxyglucose study, the mice were anaesthetized with halothane (~1.5% in 70% N_2O/30% O_2), and a femoral artery and vein were catheterized. The catheters were routed to the nape of the neck and coiled up under the skin. Stimulated mice had pieces of Ni/Fe wire (0.2 mm in diameter and 2.5 mm long) glued on left whiskers B1–3 and D1–3 ~5 mm above the skin, and the portion of the whiskers distal to the metal piece was trimmed off. All other whiskers were clipped close to the skin. Unstimulated mice had all whiskers on both sides clipped above the skin. Paper collars were placed around the animals' necks to prevent the animals from removing the metal pieces. The mice were allowed to recover for 16–24 h and provided with food and water *ad libitum*. Then they were anaesthetized with halothane (~2.0% in 70% N_2O/30% O_2) for less than 5 min, the catheters were exposed, and the mice were placed in a small cage inside an electromagnetic coil. They were allowed to rest for ~30 min, after which 2-deoxy-D-[1-^{14}C]glucose (DuPont-NEN, Wilmington, DE, U.S.A.; specific activity 50–55 mCi/mmol, dose 120–150 μCi/kg) in ~40 μl heparinized saline was injected intravenously, and simultaneously a pulsing magnetic field was initiated in the coil. The magnetic field bursts repetitively deflected the whiskers with the metal pieces.[28] Timed 20 μl arterial blood samples were collected. Arterial blood pressure and haematocrit were checked immediately before and ~40 min after the tracer injection. The blood pressure was measured with an air-damped mercury manometer. At ~40 min an additional arterial blood sample was drawn for the determination of pH, pO_2 and pCO_2 with a Corning 158 pH/Blood Gas Analyser (Corning Ltd, Halstead, Essex, U.K.). The arterial plasma glucose concentration was measured in various samples with a Beckman Glucose Analyser 2 (Beckman Instruments, Fullerton, CA, U.S.A.). The means and the standard deviations of the averaged physiological variables of all mice studied were as follows: haematocrit, 42 ± 4%; blood pressure, 90 ± 12 mmHg; pH, 7.27 ± 0.09; pO_2, 83.9 ± 15.8 torr; pCO_2, 41.5 ± 7.2 torr; and glucose concentration, 7.3 ± 1.9 mM. The mice were killed at a precisely recorded time 45–50 min after tracer injection, and the brains were removed and divided at the border between the superior and inferior colliculi. The rostral portion of the brain was split into the cortical hemispheres, and all tissue blocks were frozen in isopentane at −55°C. The cortical hemispheres were cut tangentially to the pia in alternate series of 20 μm-thick sections in a cryostat at −22°C. One section series was dried on a hotplate at 60°C, autoradiographed along with calibrated [^{14}C]methyl methacrylate standards on X-ray film (Ektascan® EMC-1, Eastman Kodak Co., Rochester, NY, U.S.A.) at 5°C and stained for cytochrome oxidase activity.[42] The other series was air-dried and stained with thionin for Nissl substance.

Densitometry. Local rates of glucose utilization were determined from the autoradiograms in the barrels representing whiskers A1–3, B1–3, C1–3, D1–3 and E1–3 in both hemispheres with a video camera-based image analysis system (Imaging Research, St Catharines, Ontario, Canada) and the time courses of the arterial plasma specific activities. The 10 areas of interest were outlined on digitized images of sections stained for cytochrome oxidase or Nissl substance on the basis of the circumferences of the barrels. The outlines were superimposed on the corresponding autoradiograms, and the concentration of ^{14}C in each delineated area was determined from the optical density in the autoradiogram and the calibration curve derived from the methyl methacrylate standards. Local rates of glucose utilization were calculated by means of the operational equation of the deoxyglucose method[36] with corrections of the lumped constant for hyper-[34] and hypoglycemia.[37] Pixel-weighted averages of $lCMR_{glc}$ were determined from autoradiograms of all sections through layer IV of a given animal. The mean of the pixel-weighted averages of each assessed area was taken to represent the area's $lCMR_{glc}$ of that animal. Mean differences in metabolic rate between homeotopic barrels contra- (right side) and ipsilateral (left side) to lesion and/or stimulation were used as measures of stimulus-related metabolic activation in the statistical analyses to reduce the effect of interindividual variability and account for a decrease in $lCMR_{glc}$ with increasing age.

Imaging. Colour-coded digitized images of autoradiograms were prepared to illustrate the patterns of metabolic activity in barrel cortex (IMAGE, W. Rasband, NIMH, USPHS, Bethesda, MD, U.S.A.).

The whiskerpad

Histology. The whiskerpads were removed from the skull, fixed in 10% phosphate-buffered (pH 7.4) formalin, immersed in 40% sucrose, frozen at −22°C in a cryostat and cut in serial 20 μm-thick sections parallel to the surface of the skin. The sections were stained for axons with a modified Liesegang method.[9]

Sensory innervation of the lesion and surrounding follicles. The site of the lesion and the adjacent whisker follicles in the left whiskerpads were examined in the Liesegang-stained preparations with a microscope (Ortholux II, Leitz, Wetzlar, FRG) fitted with a x63/oil fluorescence objective (Leitz, Wetzlar, FRG). We noted follicular remnants and conuses at the site of the lesion, de- and regenerating nerve fibres, and anomalous deep follicular nerves. The myelinated nerve fibres in the deep nerves of the follicles of whiskers B1, B2, B3, C4, γ, D1, D2 and D3 as well as in nerve stumps and follicular remnants at the site of the lesion were counted. Data were obtained from 13 to 17 whiskerpads per time interval after the lesion and 17 unoperated whiskerpads. In the latter, nerve fibres were counted also in the deep nerves of the follicles of whiskers C1, C2 and C3. The data include all mice in which cerebral metabolic activity could be determined.

Statistical analyses

The mean left-to-right differences in $lCMR_{glc}$ of stimulated mice with lesions were compared with those of the

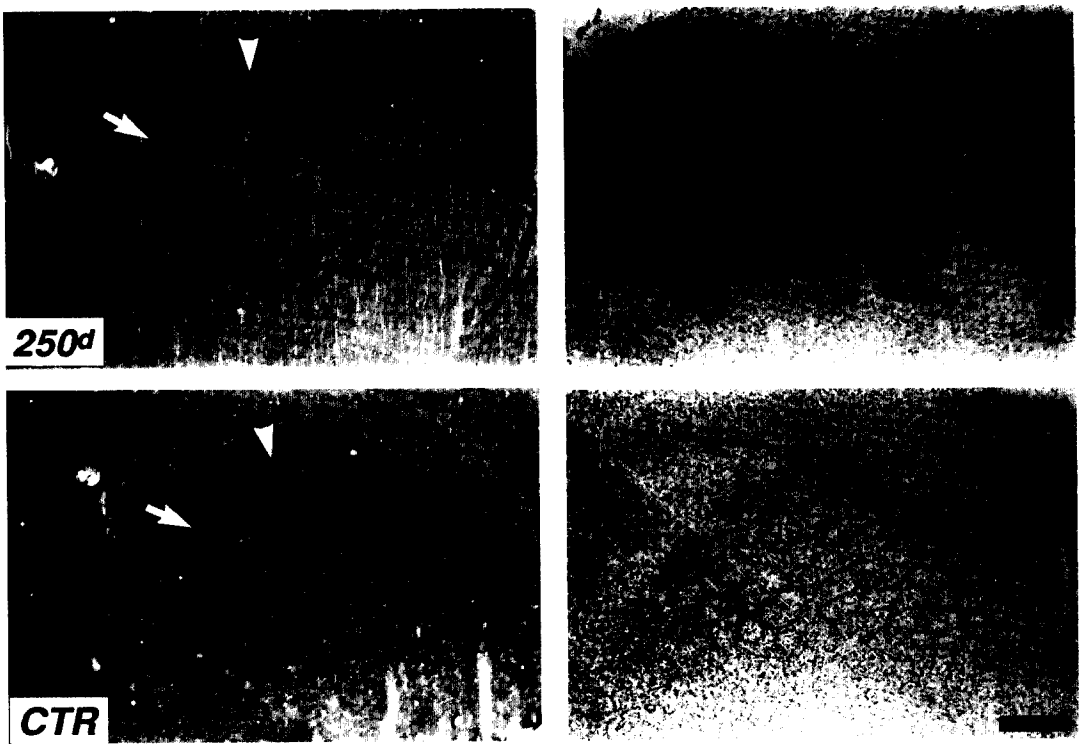

Fig. 1. Cytoarchitectonics and cytochrome oxidase activity in barrel cortex. 20 μm-thick tangential sections through the right hemisphere stained for cytochrome oxidase activity (left column) or for Nissl substance (right column). Preparations are shown from mice killed nine months after the removal of the follicles of left whisker C1, C2 and C3 (250^d) and from age-matched unoperated controls (CTR). In the Nissl preparations, barrels are visible in layer IV as cell body-dense rings, termed "sides", surrounding cell body-sparse centres, termed "hollows". The barrels are separated by cell body-sparse "septa". In the sections stained for cytochrome oxidase, the hollows stain darkly. Though the somatotopic representation of the five rows of tall caudal whiskers is not fully contained in one section, the layout of the whisker map is apparent. Whisker row A, dorsal on the snout, is represented caudally and laterally, and whisker row E, ventral on the snout, is represented rostrally and medially. The arrow points to barrel B1, the arrowhead to barrel D1. The straddlers are represented medially, the rostral, small whiskers laterally. Note that the lesions did not affect either cytoarchitecture or cytochrome oxidase activity (medial is up, rostral is on the right; scale bar=500 μm).

unstimulated mice with lesions at the same time interval after the lesion and those of unoperated stimulated controls with two-tailed Dunnett's *t*-tests (SAS®; SAS Institute, Cary, NC, U.S.A.).

The deep nerve fibre counts obtained from whisker follicles at each time interval after the lesion were compared with those from the homeotopic whisker follicles of unoperated whiskerpads with a two-tailed Dunnett's *t*-test (SAS®; SAS Institute, Cary, NC, U.S.A.).

Finally, the correlation between the metabolic rate of barrels C1–3 contralateral to the lesion and/or stimulation and the total number of deep nerve fibres innervating the follicles of deflected whiskers B1–3 and D1–3 was tested by linear regression with a fit weighted for unequal variances.[4]

RESULTS

The barrel cortex

Morphology. The removal of three whisker follicles had no apparent impact on the cytoarchitecture of barrel cortex. On tangential sections stained for Nissl substance, neither deprived barrels C1–3 nor their neighbours differed in size or shape from unoperated controls even at the longest time intervals after the lesion (Fig. 1). Sides and septa remained intact. Also the pattern of cytochrome oxidase activity remained unaltered. The activity of the enzyme in the deprived barrels was not different from that of their neighbours (Fig. 1).

Metabolic activity. The most comprehensive change in metabolic activity occurred two days after whisker follicle removal. Stimulated as well as unstimulated mice showed a generalized decrease in $lCMR_{glc}$. Since the metabolic activity in the five areas of each hemisphere was almost equally affected, this change is not visible in colour-coded images (Fig. 2). In stimulated mice, the average decreases in $lCMR_{glc}$ of the five areas were 48 and 36% contra- and ipsilateral to stimulation (Fig. 3C), respectively, in comparison with age-matched unoperated controls. In unstimulated mice with lesions, the $lCMR_{glc}$ in both hemispheres were on the average 27% lower than in the left hemisphere of the controls (Fig. 3B). Four days after the lesion (Fig. 3D, E), $lCMR_{glc}$ had returned to the level of the controls (Fig. 3A).

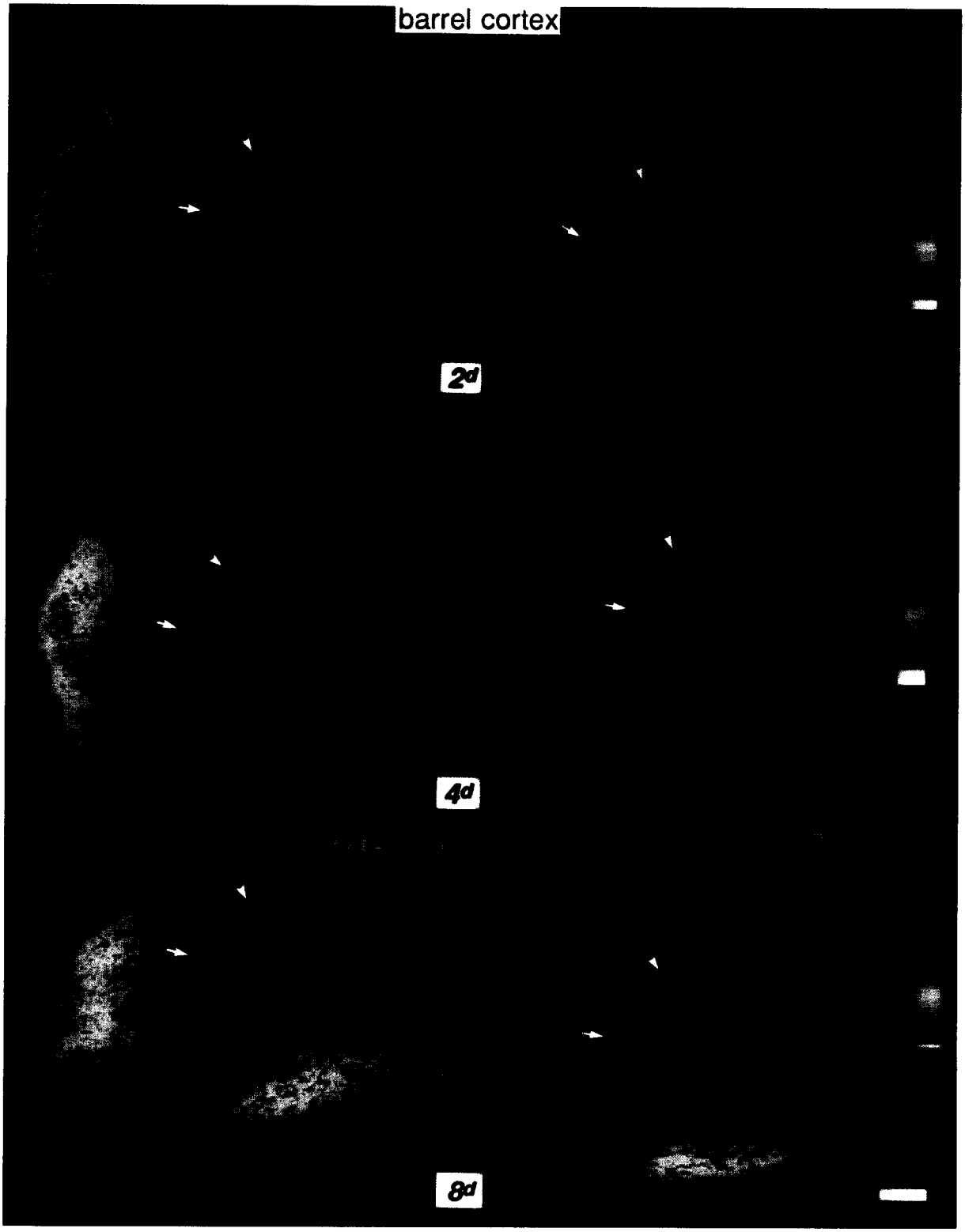

Fig. 2 (*caption on p.* 32).

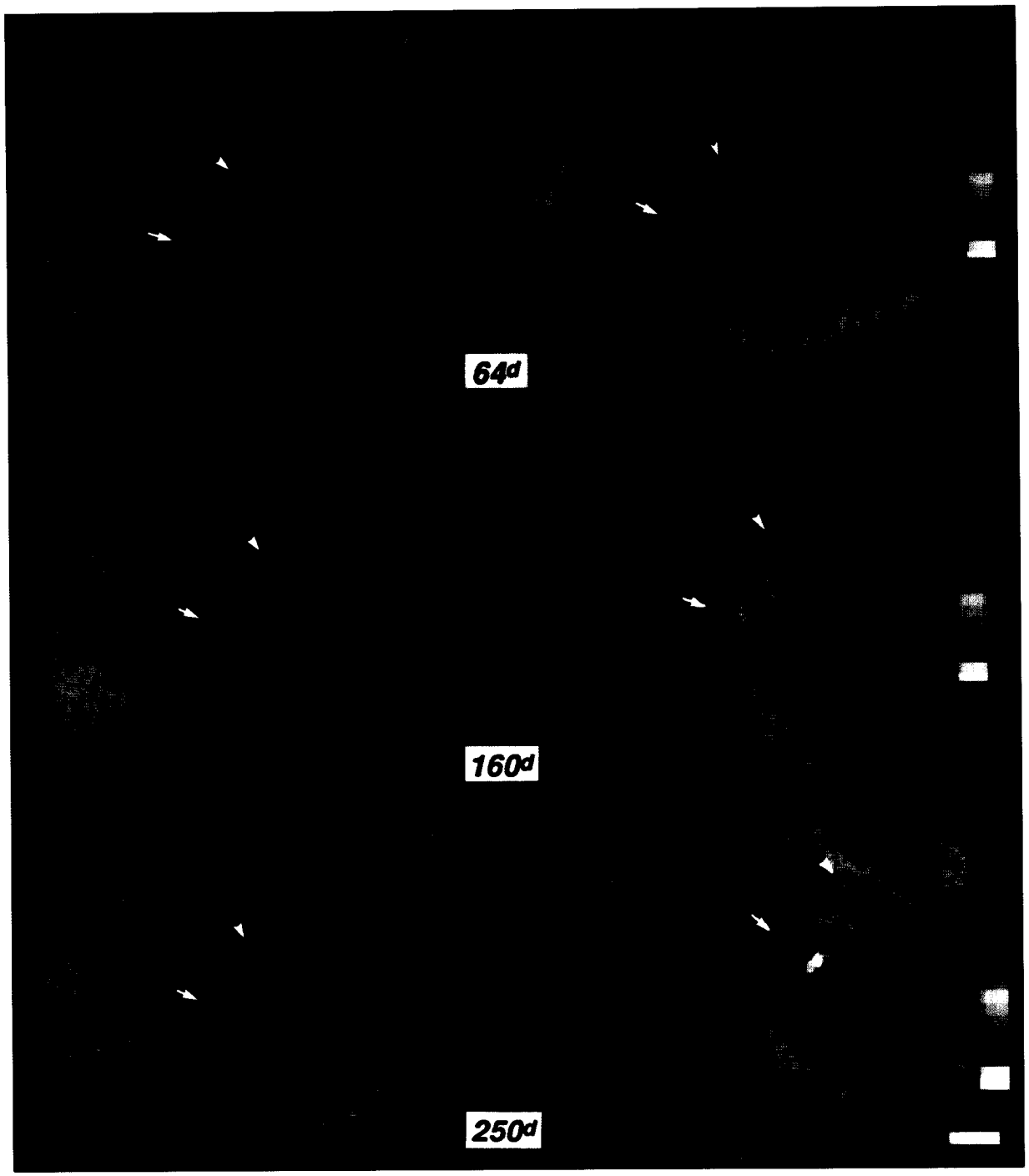

Fig. 2 (*caption overleaf*).

The deflection of whiskers B1–3 and D1–3 produced striking effects on metabolic activity in contralateral barrel cortex (Figs 2, 3), but no effect on the ipsilateral side (Fig. 3). In stimulated mice, with or without lesions, the greatest metabolic activation was found in the barrels representing the deflected whiskers (Fig. 2). The increase in $lCMR_{glc}$ was always greater in barrels B1–3 than in barrels D1–3 (Fig. 3A, C, E, G, I, K and M). The side-to-side differences were on the average 65 and 46%, respectively. Up to eight days after follicle removal, the metabolic activation remained restricted to barrels B1–3 and D1–3 (Fig. 2, 2^d–8^d) and did not differ in appearance from unoperated controls (Fig. 2CTR). In contrast, $lCMR_{glc}$ in deprived barrels C1–3 was slightly increased four (Fig. 3E) and eight days after

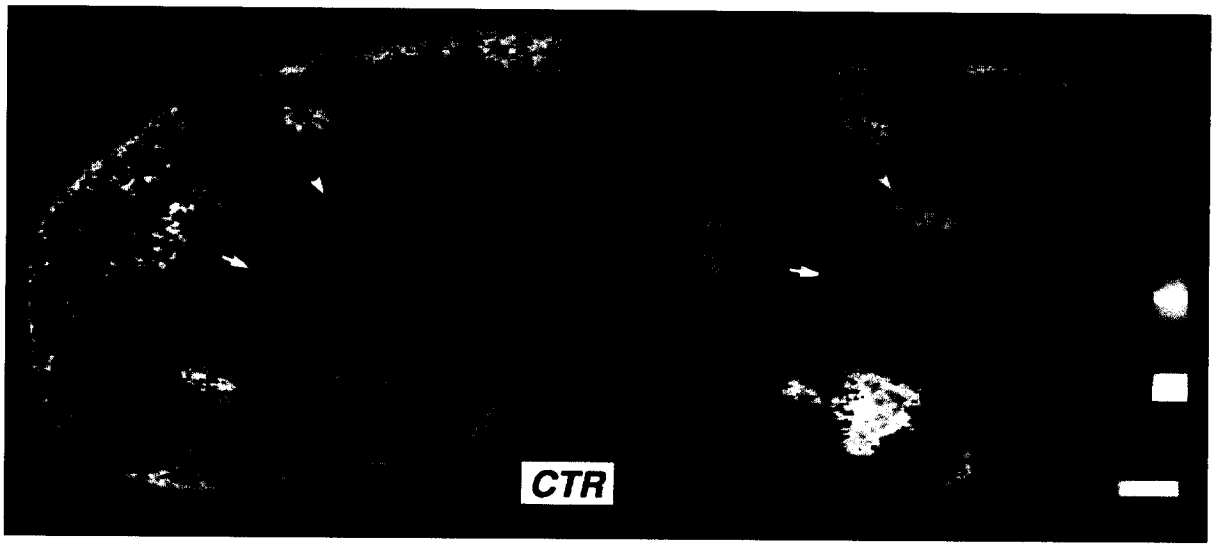

Fig. 2. Metabolic activation in the right barrel cortex of mice with lesions. Colour-coded images of autoradiograms from tangential sections. The metabolic activation is expressed by the colours in the bar on the right of each each pair of images (white/red is high, blue is low). The outlines of the barrels drawn from sections stained for cytochrome oxidase or Nissl substance have been superimposed on the autoradiograms. The arrows point to barrel B1, the arrowheads to barrel D1. The mice had the follicles of left whiskers C1, C2 and C3 removed in adulthood and were subjected to a deoxyglucose study at increasing time intervals between two and ~250 days after the lesion. The intervals are indicated for each row of panels. On the two facing pages results from mice that had all whiskers clipped prior to the deoxyglucose study are shown in the left columns and results from mice that had whiskers B1–3 and D1–3 deflected during the deoxyglucose study are shown in the right columns. Images from the left (left panel) and the right (right panel) barrel cortex of unoperated mice stimulated in the same fashion are displayed on the overleaf page (CTR). The image from the left barrel cortex was reversed about the caudorostral axis. In mice with lesions as well as in unoperated controls, the deflection of whiskers B1–3 and D1–3 increased metabolic activity to the highest degree in the corresponding barrels. However, in deprived barrels C1–3 metabolic activity was noticeably increased 64 days after the lesion (64^d) and this increase became more pronounced at longer time intervals (160^d and 250^d). In unstimulated mice with lesions, metabolic activity was as uniform as that in the left barrel cortex of controls, except an occasional slight increase in barrels C1–3 (apart from the reversal of left barrel cortex in CTR, medial is up, rostral is on the right; Scale bars in panels of 8^d, 160^d and CTR=500 μm).

the lesion (Fig. 3G), reaching a side-to-side difference of 18% compared to 12% in unoperated controls (Fig. 3A). This difference surpassed 25% 64 days after the lesion (Fig. 3I), becoming distinctive in colour-coded images (Fig. 2, 64^d–250^d), and it was statistically significantly greater than the side-to-side difference in barrels C1–3 in the controls from 160 days onwards (Dunnett's t-test; $P \leq 0.05$). $lCMR_{glc}$ in barrels A1–3 and E1–3 of all stimulated mice with and without lesions remained low. The side-to-side differences were on the average <12% and did not attain statistical significance.

In unstimulated mice with lesions side-to-side differences in $lCMR_{glc}$ remained small (~8%). Occasionally, metabolic activity in deprived barrels C1–3 was slightly increased above that of adjacent barrels. Two-hundred and fifty days after follicle removal, the deprived barrels had the highest $lCMR_{glc}$ compared with adjacent barrels (Fig. 3L), and a focus of metabolic activation was clearly discernible in colour-coded images (Fig. 2, 250^d). However, the 6% increase in side-to-side difference was only about one fourth of that found in stimulated mice with lesions at the same time interval (Fig. 3M) and, consistently, the side-to-side difference in $lCMR_{glc}$ of deprived barrels C1–3 in stimulated mice with lesions was statistically significantly greater than that of unstimulated mice with lesions from four days after follicle removal onwards (Dunnett's t-test; $P \leq 0.05$).

In summary, $lCMR_{glc}$ was highest in the appropriate barrels of stimulated operated and unoperated mice. Two days after the lesion metabolic rates in the two hemispheres of stimulated and unstimulated mice were markedly depressed, but four days after the lesion, the rates had returned to normal levels. In stimulated mice, the metabolic activation of deprived barrels C1–3 increased with time after the lesion and reached the highest magnitude ~160 days after follicle removal. Because metabolic activation of the deprived barrels of unstimulated mice remained less than one fourth of that of stimulated mice, most of the increase in metabolic activation of the deprived barrels was associated with the stimulation of the whiskers adjacent to the lesion.

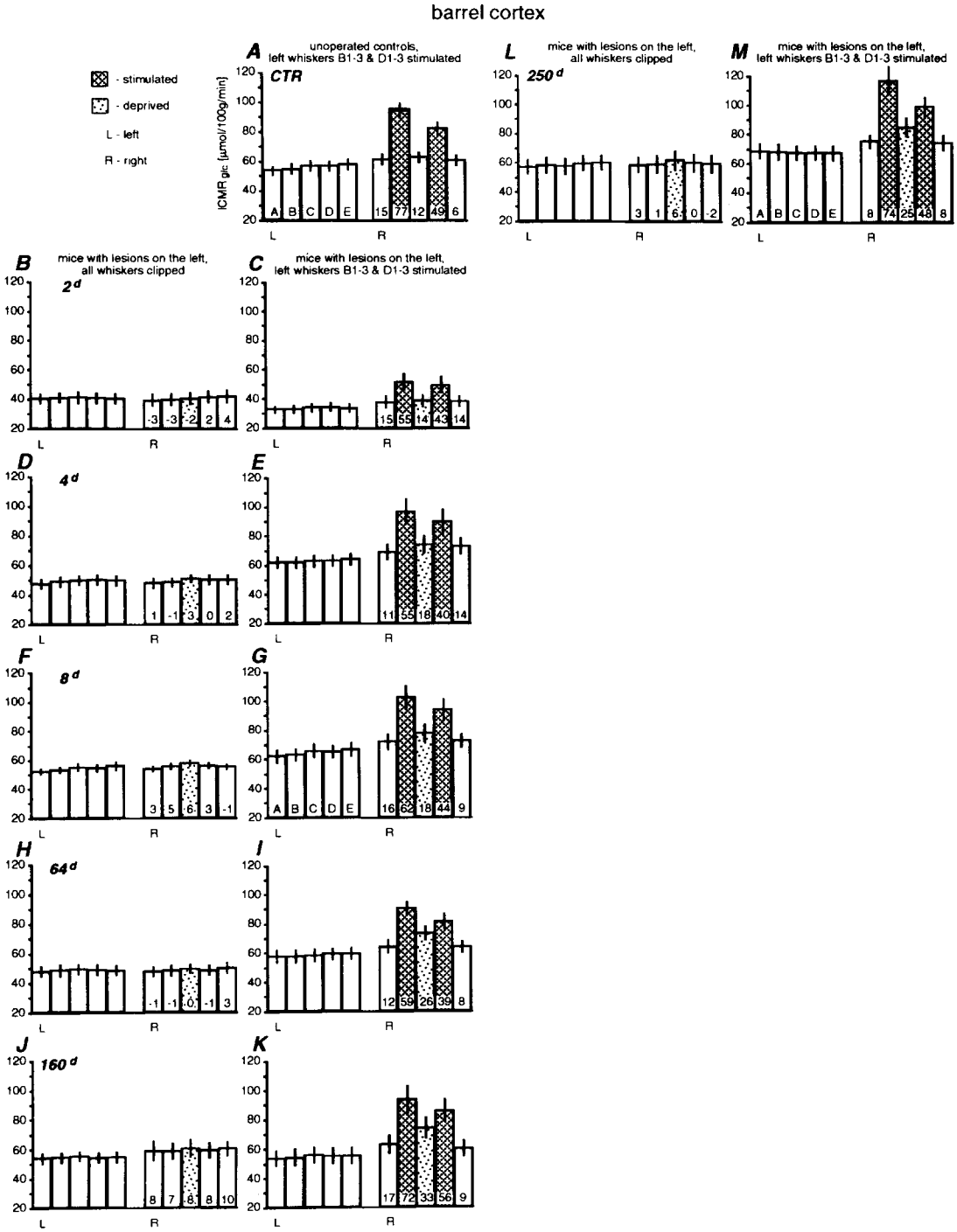

Fig. 3. Local cerebral metabolic rates for glucose ($lCMR_{glc}$) in barrel cortex. The bar graphs, labelled A to N, represent mean $lCMR_{glc} \pm$ S.E.M. of the sides ipsi- (left) and contralateral (right) to lesion/stimulation obtained from five to 10 mice for each area. The numbers inserted in the bars for the right hemisphere express the right-to-left percent difference in $lCMR_{glc}$. $lCMR_{glc}$ of the unoperated stimulated controls are shown in A. B–M show $lCMR_{glc}$ measured two (2^d), four (4^d), eight (8^d), 64 (64^d), 160 (160^d) and 250 days (250^d) after the removal of the follicles of left whiskers C1, C2 and C3. Left column: Unstimulated mice with lesions that had all whiskers clipped. Right column: Mice with lesions that had left whiskers B1–3 and D1–3 deflected. The greatest $lCMR_{glc}$ was found in the barrels representing deflected whiskers B1–3 and D1–3 whereas $lCMR_{glc}$ remained low in barrels A1–3 and E1–3. In deprived barrels C1–3, whisker stimulation slightly increased $lCMR_{glc}$ four days after follicle removal (E), but became statistically significant only $=160^d$ (K).

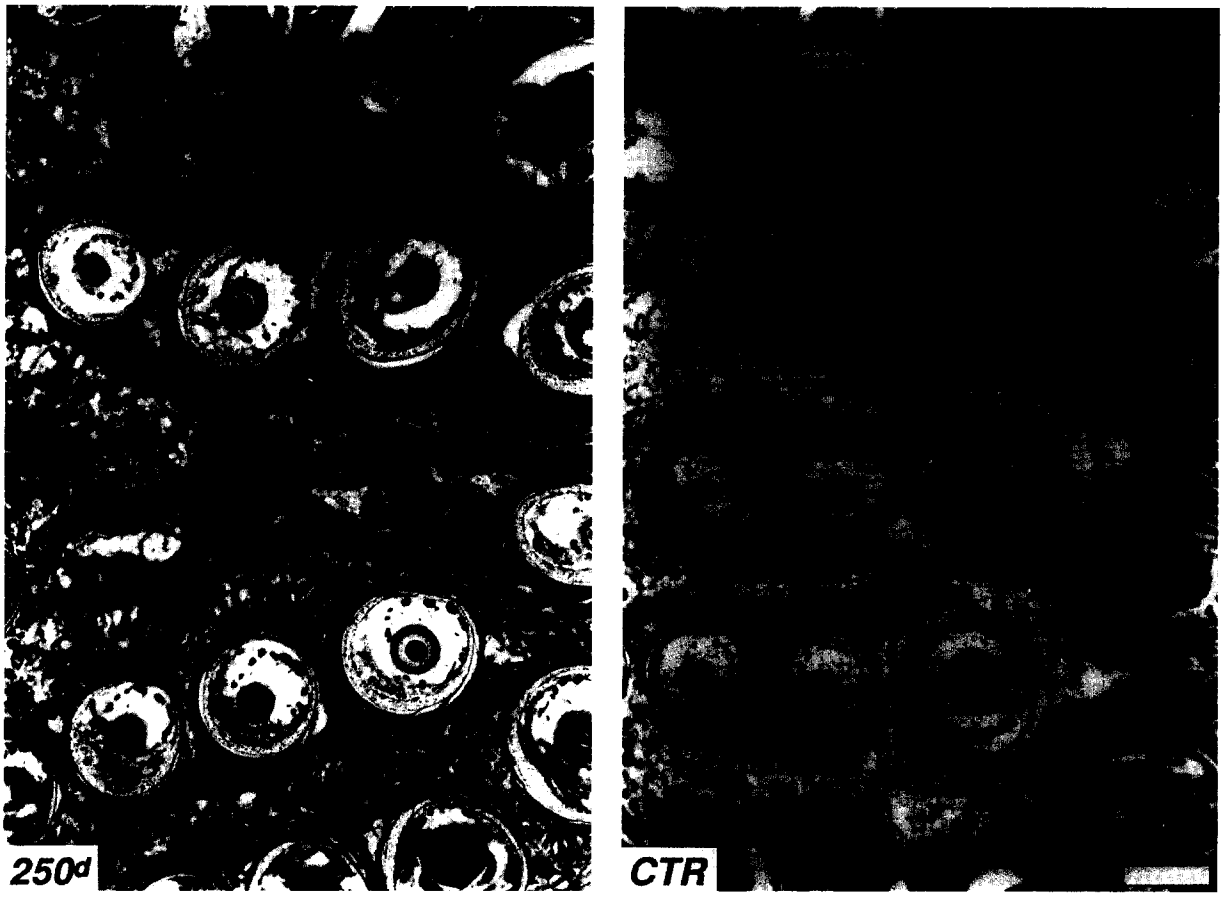

Fig. 4. The caudal middle portion of the left whiskerpad from a mouse ~250 days after the removal of the follicles of whiskers C1, C2 and C3 (250^d) and an unoperated control (CTR). Micrographs from 20-μm-thick serial sections cut parallel to the surface of the whiskerpad and stained with a modified Liesegang's method. The tall whisker follicles on the snout of the mouse are arrayed in five rows, named A (dorsal) to E (ventral). In each row the whiskers are numbered beginning with one caudally. The follicles of the three most caudal whiskers in these rows can be seen. The follicles of row A (top) and row E (bottom) are partially cut. Four whiskers, named α to γ, straddle the rows at their caudal end. Their follicles are partially visible on the right. Note that in the mouse with lesion the follicles of whiskers C1–3 are entirely missing (scale bar at lower right=200 μm).

The whiskerpad

Histology of the lesion. The morphology of the murine whisker follicle, its receptors and their innervation have been described elsewhere.[13,33] In ~60 percent of the cases we achieved a complete excision of the follicles of left whiskers C1, C2 and C3 (Fig. 4). However, in the other ~40% we found hairless follicular remnants and conuses at the site of the lesion. Their presence and that of stumps of follicular nerves at the site of the lesion as well as the presence of nerve fibres in these structures are recorded for each time interval after the lesion in Table 1. In particular, we observed the following:

Two days after whisker follicle removal myelinated fibres did not appear different from those in deep follicular nerves of unoperated whiskerpads (Fig. 5 CTR). Degenerating nerve fibres were detected first at the site of the lesion at four days. Many fibres innervating follicular remnants and the nerve stump, that was found in the dermis of one mouse, were swollen and their myelin stained faintly (Fig. 5, 4^d). Eight days after the lesion most myelinated fibres in the nerve stumps were degenerating progressively. Dispersed clumps of myelin had formed. Some fibres stained darkly and were gravely hypertrophic. Others stained so faintly that they appeared translucent (Fig. 5, 8^d).

Regenerated myelinated nerve fibres were detected 64 days after follicle removal and became commonplace at longer time intervals after the lesion (Fig. 5, 250^d). As normal nerve fibres, they consisted of rings of myelin and tubulin. However, they appeared plied, less numerous and smaller in diameter. Regenerated nerve fibres were present in nerve stumps, follicular remnants and conuses at the site of the lesion. The fibres emanating from the nerve stumps were grouped in major bundles wrapped by spiralling nerve fibres that were surrounded by loosely scattered individual fibres (Fig. 5, 250^d, left). The bundles

Table 1. Innervation with myelinated nerve fibres of structures at and adjacent to the site of the lesion

Group	Innervation of the whiskerpads							
	Whiskerpads (number analysed)	Nerve stumps	Follicular remnants		Follicular conuses		Follicles with anomalous deep nerves	Follicular deep innervation of whiskers (B1–3 and D1–3)† (number of myelinated nerve fibres counted)
			Bare	Innervated	Bare	Innervated		
CTR	17							842 ± 12
2^d	12	3	7	3		3		840 ± 17
4^d	16	1	3	2	3			840 ± 24
8^d	15	3	11	4	6	2		851 ± 20
64^d	17	7	2	6	2		5	865 ± 28
160^d	16	2	1	8	2	6	7	856 ± 41
250^d	17	17		4			7	896 ± 40
Sum (2^d–8^d)	43	7	21	9	9	5	0	
Sum (64^d–250^d)	50	26	3	18	4	6	19	

†Means of sums ± S.Ds.

did not end in encapsulated neuromas, but penetrated the dermis to fan out in the epidermis (Fig. 5, 250^d, right). Their nerve fibres were not seen to innervate follicular remnants, conuses or the intact follicles neighbouring the lesion. However, at 64 days we detected anomalies of the deep innervation of whisker follicles surrounding the lesion that were not seen either at shorter time intervals or in unoperated whiskerpads. Three mice had an additional deep nerve entering whisker follicle β at a novel location. Furthermore, anomalous nerves were detected in one mouse at whisker follicle B1 and in another at B2. 160 days after the lesion, anomalous deep follicular nerves were found innervating whisker follicle β (one mouse), B1 (one mouse) and γ (three mice) as well as whisker follicles β and B1 (one mouse). 250 days after the lesion, one mouse had an anomalous deep follicular nerve at whisker follicle β, one mouse at γ, three mice at B1 and one mouse at whisker follicles β and B3. Figure 6 provides an example of such anomalous innervation.

In summary, nerve stumps and innervated follicular remnants and conuses were commonplace at the site of the lesion after the onset of regeneration of nerve fibres (Table 1). It is interesting to note that not all remnants were innervated. In some cases the follicular wall was approached but not penetrated by fibres. Concomitant with the regeneration of nerve fibres at the site of the lesion, anomalous deep follicular nerves appeared at intact whisker follicles surrounding the lesion. They were detected at 19 whisker follicles in 17 of the 50 mice ~64 days after follicle removal.

Innervation density of whisker follicles. Loss of nerve fibres in the deep nerve of the whisker follicles surrounding the lesion was found in nine of the 93 mice with lesions. Except for one animal, which had lost half the deep innervation of whisker follicle B2, no losses were observed in the follicles of whiskers B1–3 and D1–3. These whisker follicles received a markedly greater than normal number of nerve fibres at the longest time interval after the lesion (Table 1). In particular, we observed an increase in the innervation density of the most caudal whisker follicles with increasing time after the lesion (Fig. 7). In comparison with the follicular innervation in unoperated whiskerpads, the increase in deep innervation at whisker follicle γ was statistically significant ~64 days after the lesion (Dunnett's *t*-test; $P \leq 0.05$). The increase in whisker follicle D1 reached statistical significance ~160 days after the lesion, whereas increases in the innervation of whisker follicles B1, B2 and D2 became significant at 250 days. However, the total number of additional nerve fibres was only ~15% of the number of nerve fibres innervating whisker follicles C1–3 in unoperated whiskerpads. In cases, in which nerve stumps were found at the site of the lesion, the number of fibres in the stumps did not reach more than one third of the number of fibres innervating the follicles of whiskers C1–3. Therefore, only up to one half of the nerve fibres which were deprived by the lesion had eventually regenerated. In spite of the reduced rate of restoration, linear regression analysis yielded a positive, statistically significant correlation between $lCMR_{glc}$ of barrels C1–3 contralateral to lesion/stimulation and the total number of deep nerve fibres innervating stimulated whisker follicles B1–3 and D1–3 (n=43; r=0.45; P=0.0026).

DISCUSSION

The removal of three whisker follicles in adult mice led to an enlargement of the metabolic representations of whiskers adjacent to the lesion into the deprived territory of barrel cortex.[26] Remarkably, the follicles of these whiskers received novel, anomalous innervation. The present study provided two major additional findings: (i) the enlargement was

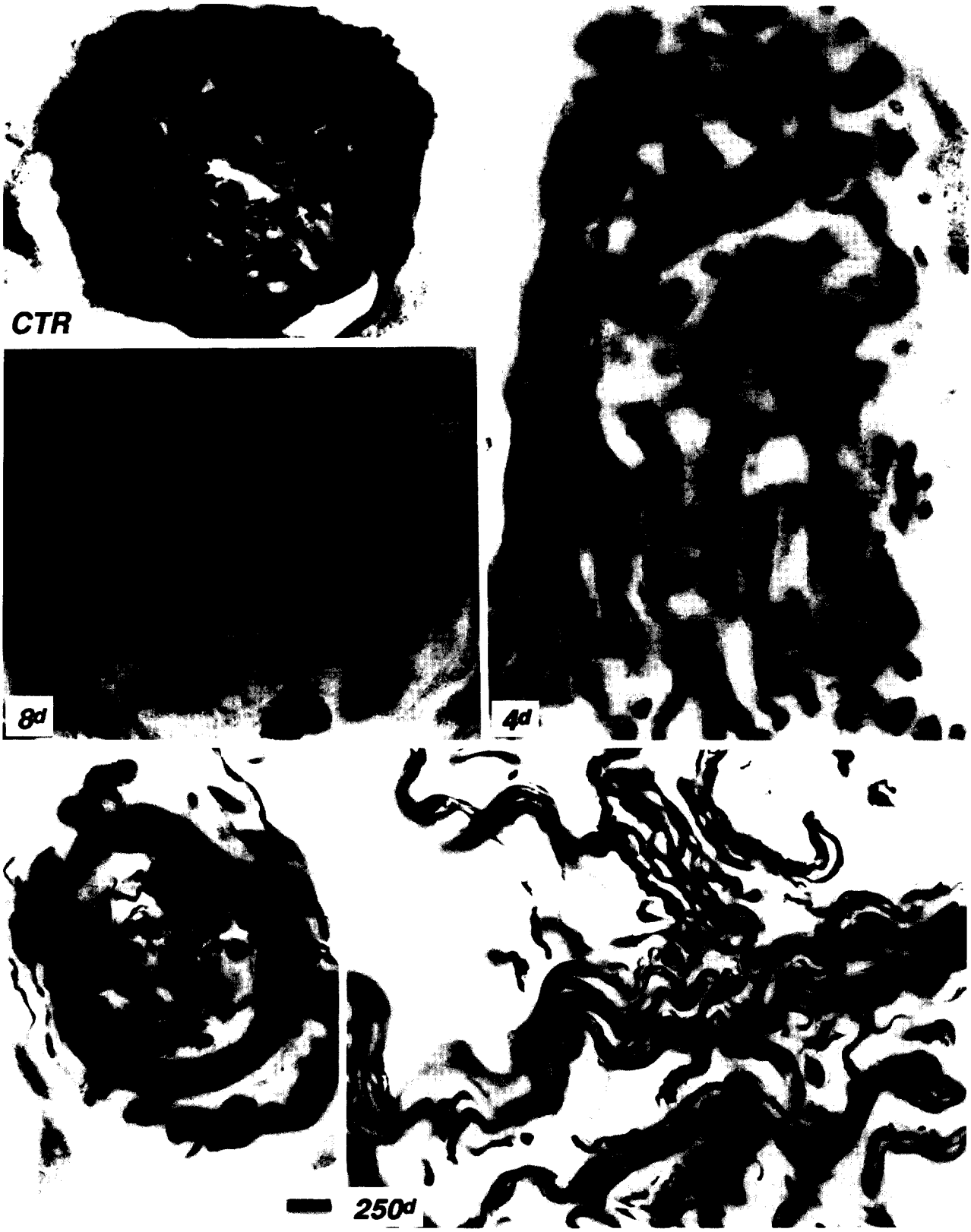

Fig. 5. Degeneration and regeneration of trigeminal nerve fibres in the whiskerpad after the removal of the follicles of whiskers C1, C2 and C3. The histological method is the same as that in Fig. 4. The panels show cross-sections of the deep nerve at the base of follicle C3 in an unoperated control (CTR), and of nerve stumps at the site of the lesion four (4^d), eight (8^d) and 250 days (250^d) after the lesion. The arrowheads point to individual myelinated nerve fibres consisting of a lightly-stained outer rim, the myelin sheeth, and a darkly-stained inner rim, probably tubulin (rostral is up and dorsal is right; scale bar=10 μm).

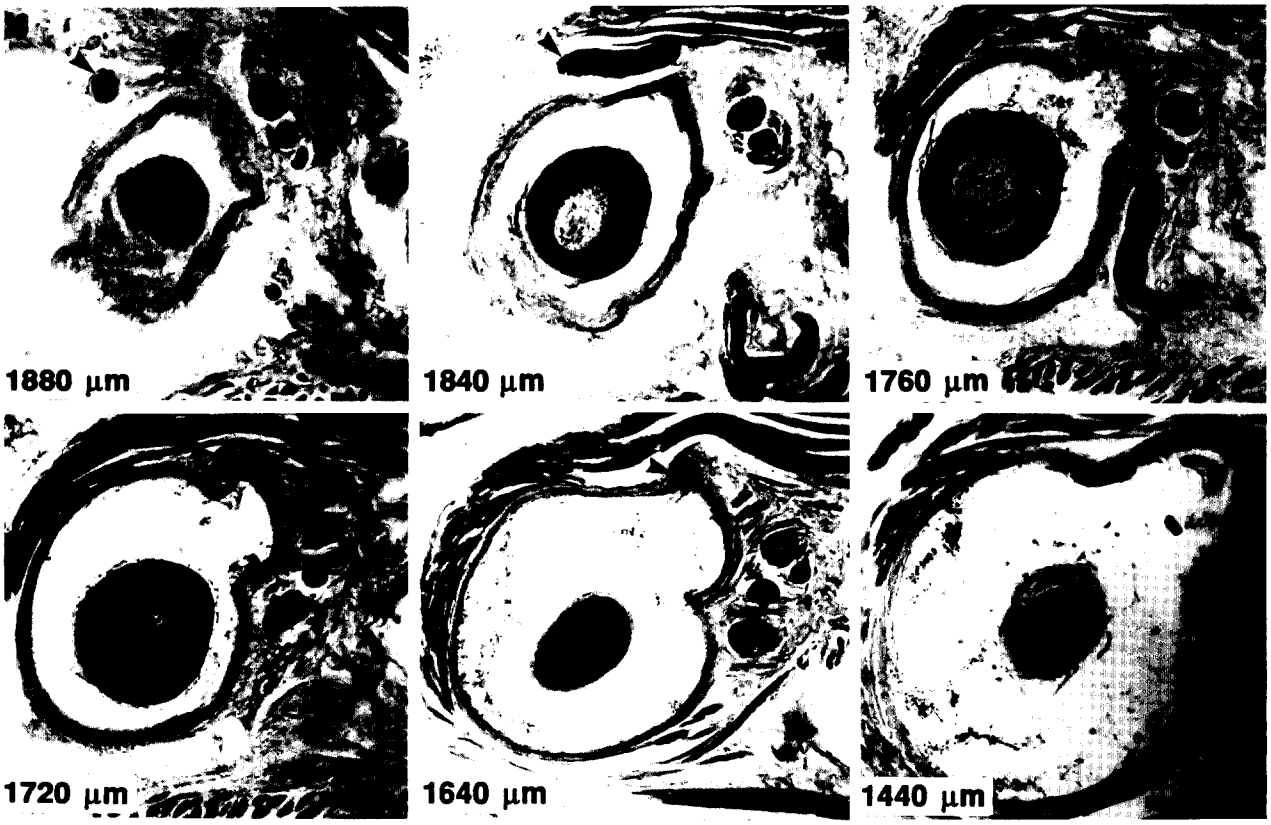

Fig. 6. A deep nerve entering a whisker follicle neighbouring the lesion at an anomalous site eight months after the removal of the follicles of left whiskers C1, C2 and C3. The histological method is the same as that in Fig. 4. The micrographs show cross-sections of the follicle of whisker B1, a whisker adjacent to the lesion, and its deep follicular nerves at the base of the follicle and, in progressive sequence, toward the epidermis. The depth below the epidermis, at which the sections were taken, is given at the lower left of each panel. Myelinated nerve fibres stain darkly. A group of three nerve rami can be seen approaching the follicle at a dorsal and caudal location (open arrow). They are eventually joined by a fourth ramus and penetrate the follicular wall through an invagination. This is the normal site of entry of deep follicular nerves. An additional nerve (arrowheads) approaches the follicle from a rostral and dorsal direction, extends caudally and enters the follicle at a location distinctly separate from the normal site of entry. Such anomalous deep nerves have only been observed in mice with lesions. They were most frequently seen at the most caudal whisker follicles (rostral is right and dorsal is up; scale bar=50 μm).

distinguishable on autoradiograms two months after the lesion, and the change in lCMRglc in the deprived territory progressed to attain statistical significance; and (ii) the enlargement evolved concomitantly with the reorganization of the follicular innervation in the sensory periphery. These findings suggest that the observed changes in representation of the sensory receptor sheet in primary somatosensory cortex may primarily reflect reorganization of the peripheral innervation.

Morphology of barrel cortex

Removing three whisker follicles did not have any effect on barrel cytoarchitecture or cytochrome oxidase activity in adult mice. This finding is not surprising. The critical period for altering barrel cytoarchitecture ends when the development of the barrels is completed, i.e. six days after birth.[19,32] In agreement with our findings, Wong-Riley and Welt[43] did not observe alterations of barrel size after the cauterization of whisker follicles in adult mice. In contrast to our findings, they observed a decrease in cytochrome oxidase activity in the hollows of the corresponding barrels. One major difference between the present study and that of Wong-Riley and Welt[43] is that these investigators cauterized all whisker follicles of row C whereas we removed only three. Therefore, diminution in cytochrome oxidase activity in a deprived barrel may depend on the number of deprived barrels in its vicinity. Interestingly, this dependency emphasizes that the loss of a whisker follicle may not influence only its appropriate barrel, but also surrounding barrels as well, and that this interaction may be synergistic.

Metabolic activity in barrel cortex

The observed changes in stimulus- and lesion-related metabolic activity in the deprived barrels

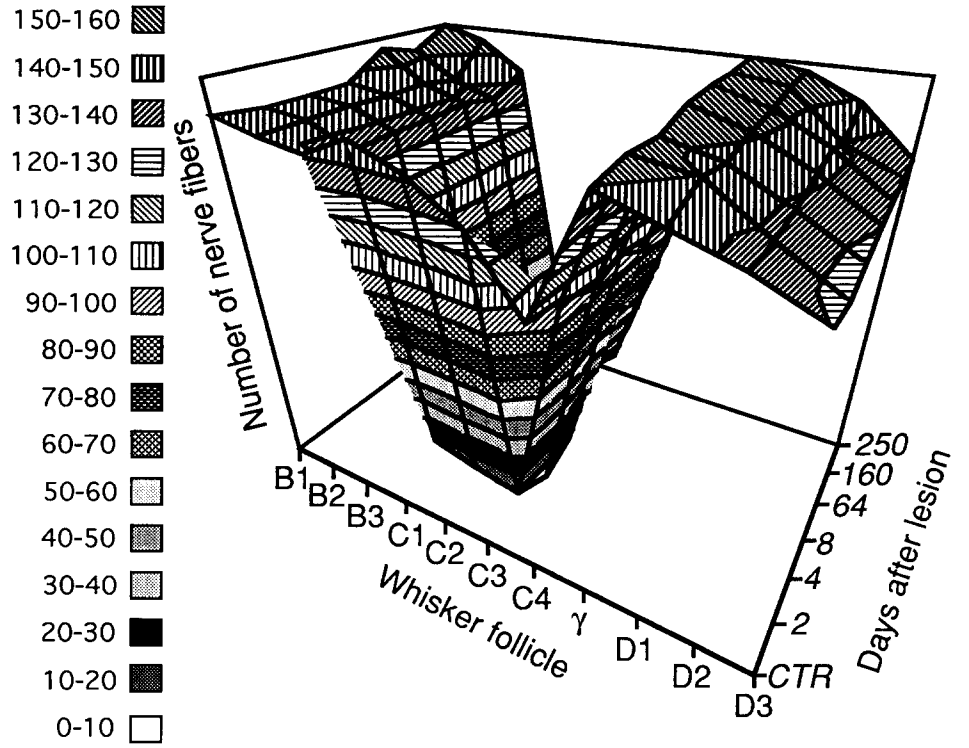

Fig. 7. Regeneration of nerve fibres in the deep nerves of whisker follicles surrounding the lesion. The mean number of deep nerve fibres innervating the follicles listed on the ordinate are plotted against the days after the removal of the follicles of whiskers C1–3 (abscissa). The sections of the whiskerpads were processed as in Fig. 4. The nerve fibres were counted in cross-sections transecting the deep nerves perpendicularly at the base of the follicles. Fibre counts were obtained from 13–17 mice at each interval of time after the lesion (most mice were subjected to deoxyglucose studies). Counts from 17 unoperated whiskerpads served as controls (CTR). In each row of whisker follicles, innervation declines from caudal to rostral. Note the increase in follicular innervation with increasing time beginning 64 days after the lesion (64^{d}). This trend is greatest in the most caudal whisker follicles. Coincidentally, in these follicles, anomalous nerves were found most frequently.

reflect short- (<8 days) as well as long-term effects of the lesion.

Short-term effects. Two days after follicle removal, we observed a remarkable depression in metabolic activity of the five examined areas of barrel cortex in both hemispheres. Even the stimulus-related metabolic activation was reduced. The barrel cortex recovered from the depression within the following two days. In harmony with our findings, Dietrich *et al.*[14] observed a depression of metabolic rate in layer IV of contralateral barrel cortex one day after the transection of the infraorbital nerve in adult rats. Thirty days after nerve transection the metabolic rate was normal. Depression of metabolic rate followed by recovery was observed also in the visual system of unilaterally enucleated rats,[7] and therefore such fluctuation of metabolic activity may be a common response to a lesion in the sensory periphery. The reduction of metabolic activity in barrel cortex ipsi- and contralateral to the lesion regardless of whisker deflection may reflect a general down-regulation of neural activity in the somatosensory system in response to the lesion.

Long-term effects. The pattern of metabolic activity in barrel cortex ipsilateral to lesion/stimulation remained essentially unaltered by either the deflection of whiskers or the lesion. Removal of whisker follicles by itself had only a minor influence on the metabolic activity in barrel cortex. In contrast, striking changes in metabolic activity were observed in mice with lesions after whisker stimulation. Deprived barrels C1–3 showed a consistent and statistically significant increase in metabolic response to the stimulation of whiskers adjacent to the lesion. In view of the results from unstimulated mice with lesions, three quarters of this increase can be attributed to whisker stimulation. Apparently, the representations of the stimulated whiskers enlarged into the barrels deprived by the lesion. Similarly, the removal of all whisker follicles but one in adult rats led to the omnidirectional enlargement of the metabolic column in barrel cortex evoked by the deflection of the remaining whisker.[17,25] In accord with this observation and our findings, digit amputation in monkeys resulted in the enlargement of the areas in somatosensory cortex that were metabolically activated by the somatic stimulation of intact digits

adjacent to the lesion.[6] Remarkably, the observed change in whisker representation, though distinct two months after follicle removal, consolidated to attain statistical significance only at longer time intervals.

Mechanisms underlying whisker map plasticity

Central mechanisms of plasticity. The enlargement of cortical somatic representations in somatosensory cortex has been associated with a variety of intracortical neurotransmitter-related mechanisms that may involve rearrangement of connections.[10] Inhibitory GABAergic interneurons limit the size of neuronal receptive fields[16] and the area of metabolic activation.[21] After whisker follicle removal, the intensity of the immunohistochemical staining for glutamic acid decarboxylase,[41] and the total binding of muscimol to $GABA_A$ receptors[35] decrease transiently in the deprived barrels indicating that pre- as well as postsynaptic elements of inhibitory circuitry are affected by the deprivation of input. *N*-methyl-D-aspartate receptor-modulated excitatory amino acid synapses also play a crucial role in the shaping of neuronal centre-surround responses[2] that change after chronic stimulation of selected whiskers and deprivation of the surrounding whiskers[11,12] and may "unmask" originally silent connections.[40] In addition, facilitatory cholinergic inputs to somatosensory cortex reportedly affect the lesion-induced enlargement of metabolic representations of intact soma.[20,22] However, in the present study the increase in metabolic activation in the deprived barrels consolidated only after the reorganization of peripheral innervation had begun and, therefore, possible alterations of intracortical circuitry remain subject to the reorganization of peripheral input.

Peripheral mechanisms of plasticity. The progression of the degeneration of myelinated deep nerve fibres in the present study is roughly in accord with those described in rat,[31] guinea-pig[18] and rabbit[23] after infraorbital nerve transection. In the three studies, the first regenerating deep nerve fibres were observed entering whisker follicles 30 days after transection. In accord, in the present study 64 days after follicle removal anomalous deep nerves were found at whisker follicles neighbouring the lesion and the number of deep nerve fibres innervating these whisker follicles had increased. That the caudal follicles received the greatest supernumerary innervation is not surprising because they were the first to be encountered by axons regrowing from the infraorbital nerve.

The increase in deep innervation became most prominent 250 days after the lesion, which suggests that regeneration of nerve fibres continued up to that time. In harmony, Kadanoff,[23] Jałowy[18] and Renehan and Munger[31] observed signs of regeneration up to the longest time intervals after infraorbital nerve transection investigated, i.e. 120–150 days. In these studies, regenerated deep nerve fibres innervated receptors that had not received such innervation originally. Renehan and Munger[31] found lanceolate receptors with numerous unmyelinated axons whereas this receptor type normally accommodates only one axon. Kadanoff[23] reported an increase in intraepithelial innervation. Jałowy[18] observed that Merkel cells decreased in number initially after the lesion, but regenerated or newly developed in even greater number on contact with the endings of regenerated nerve fibres. In the present study, the novel follicular innervation of the whiskers neighbouring the lesion may, therefore, be capable of creating its own receptor endings.

Though we detected innervated follicular remnants* and conuses† at the site of the lesion, it is unlikely that their nerve fibres were excited by the stimulation of intact whisker follicles because the whiskers are anchored in the follicular blood sinuses[33] that dampen the propagation of mechanical forces into the surrounding tissue during whisker deflection. Therefore, the supernumerary innervation of whisker follicles neighbouring the lesion remains the most likely factor to bring about the change in the metabolic whisker map of barrel cortex. This interpretation is supported by the statistically significant correlation between the metabolic activity of barrels C1–3 evoked by the deflection of whiskers B1–3 and D1–3 and the density of the deep follicular innervation of these whiskers.

Plasticity in barrel cortex with other types of lesion

Similar to the findings of the present study, the removal of whisker follicles C1–3 in neonates resulted

*We found reinnervated follicular remnants at the site of the lesion in some cases. In spite of their presence, regenerating axons could evidently accept a nearby intact follicle as target. Because anomalous nerves were more frequent in mice that did not have any follicular remnants, the acceptance of a new target may have been augmented by the completeness of the follicle removal. Consistently, deafferented whisker follicles were reinnervated by regenerating deep nerve fibres after the transection of their row nerve, and there was no increase in deep innervation of the whisker follicles adjacent to the deafferented row.[8] Therefore, as long as the original follicle is available as target to regenerating trigeminal axons they seem to innervate this follicle preferentially. Since some follicular remnants were reinnervated, but others were not, the condition of the whisker follicle governing reinnervation remains to be elucidated.

†In addition to the deep nerve, the whisker follicle is innervated by a superficial nerve,[33] also termed "conus nerve",[13] that terminates in the inner conical body. In contrast to the crucial role of the deep innervation in the transmission of sensory responses, that of the superficial innervation is controversial.[3,39] Although we did not assess the superficial innervation quantitatively, we noticed that the proportion of innervated conuses at the site of the lesion increased with the appearance of regenerated nerve fibres in the stumps of the deep nerves 64 days after follicle removal. However, we could not trace any of these fibres to a conus.

in an enlargement of the metabolic representation of whiskers B1–3 and D1–3 into the deprived territory, though the follicles of these whiskers did not receive supernumerary deep innervation.[27] The deep innervation was even diminished in some cases. Yet, in agreement with the findings of others,[15,24,38] the neonatal lesions drastically changed the morphology of barrel cortex. The barrels destined to represent whiskers C1–3 did not develop, and the neighbouring barrels enlarged into the vacant territory. The profound change in the morphological whisker representation in barrel cortex and the absence of novel follicular innervation after neonatal lesions point rather to a reorganization of cortical afferents than to a reorganization of the peripheral innervation. We hypothesize that these drastic changes in the central pathway followed neonatal lesions, because the deafferented Gasserian ganglion cells did not survive and could not repair their peripheral connections. Indeed, 60% of the ganglion cells die within two months after the transection of the infraorbital nerve in neonatal rats[5] whereas there is only a 14% loss after the same type of lesion in adults.[1] The findings of the present study suggest that lesions in adults result in notable reorganization of peripheral innervation leading to more subtle changes in the connectivity of the central stations of the pathway which do not manifest themselves in gross alterations of barrel morphology.

CONCLUSION

It seems a peculiar feature of the plasticity in barrel cortex that, in spite of the differences in peripheral organization found in mice subjected to the removal of whisker follicles as neonates or as adults, the resulting changes in metabolic whisker representation of barrel cortex share a common theme, i.e. the intact whisker follicles surrounding the lesion attain the capacity to metabolically activate the barrels deprived by the lesion, but not other barrels. This similarity raises the possibility that there may be one common central mechanism limiting plasticity in somatosensory cortex in development and in maturity. If it can be confirmed that regenerated nerve fibres induce the redevelopment of their own receptors in the sensory periphery as Jałowy[18] reported, this central mechanism may even influence the reorganization of the peripheral reinnervation.

Acknowledgements—We are indepted to L. Sokoloff for many helpful comments and his support. J. D. Brown, J. Jehle, J. Kang,, and K. Schmidt were of invaluable help during various phases of the study.

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(*Accepted* 16 *June* 1997)