Whisker Follicle Removal Affects Somatotopy and Innervation of Other Follicles in Adult Mice

Peter Melzer and Carolyn B. Smith

Laboratory of Cerebral Metabolism, National Institute of Mental Health, Bethesda, Maryland 20892

The present study shows that in the whisker-to-barrel pathway of adult mice surgical removal of three whisker follicles leads to the expansion of the functional cortical representation of the whiskers adjacent to the lesion into the deprived barrels within 8 months. Concomitant with this enlargement, there is an increase in follicular innervation of the corresponding whiskers. This reorganization of the peripheral innervation may be important for the observed reshaping of cortical somatotopy.

Electrophysiological recordings in the primate somatosensory cortex have shown that transection of the median nerve (Merzenich et al., 1983) or the amputation of finger digits (Merzenich et al., 1984) of adult monkeys results in the enlargement of the representation of intact skin neighboring the lesion into the deprived territory of the cortical somatotopic map of the hand. This plasticity is thought to be caused solely by adaptations in the neural circuitry of the central pathway. In the present study we investigated the effects of the removal of follicles of sinus hairs (whiskers) on the snout in the mature whisker-to-barrel pathway. This pathway connects the whisker follicles through the trigeminal nerve to a subdivision of the contralateral primary somatosensory cortex, called barrel cortex (Woolsey and Van der Loos, 1970). The pathway is unique in that a subset of trigeminal nerve fibers innervates whisker follicles exclusively, lending itself, therefore, superbly to the detection of changes in follicular innervation of the remaining whiskers after the removal of a few whisker follicles. Waite and Taylor (1982) have shown with electrophysiological recordings that deafferentation of whisker follicles leads to changes in their central somatotopic representations similar to those in primates, and in the present study such changes could be demonstrated with the deoxyglucose method. Moreover, we present evidence that after the removal of selected whisker follicles in adult mice, a novel innervation evolves in the neighboring whisker follicles; this must be considered to be a possible mechanism that contributes to the changes in their functional representations in barrel cortex found in the same animals.

Materials and Methods

Animals

All procedures were approved by the NIMH Animal Care and Use Committee. Male and female albino mice (International Charles Rivers) were used at 2 months of age. The follicles of left whiskers C1, C2, and C3, that is, the three caudal whiskers in the middle row, were removed by means of an incision in the skin between rows C and D under 1.5% halothane anesthesia (~1.5% in 70% N_2O/30% O_2). The wound was closed with one suture.

Measurement of Local Cerebral Glucose Utilization

Between 7 and 10 months after the lesion, metabolic activation in the whisker-to-barrel pathway was studied in eight mice with the autoradiographic [^{14}C]deoxyglucose method while six whiskers neighboring the lesion (whiskers B1-B3 and D1-D3), on which small metal pieces had been glued, were repeatedly deflected by exposure to a pulsing magnetic field (Melzer et al., 1985). All other whiskers were clipped. A paper collar around the animals' necks prevented them from removing the metal pieces. Two groups of age-matched mice underwent the measurement of glucose utilization as controls: (1) six mice with identical lesions, which had all whiskers clipped (unstimulated); and (2) five unoperated mice with identical stimulation.

In preparation for the deoxyglucose procedure, catheters were inserted in the left femoral artery and vein under halothane anesthesia (~1.5% in 70% N_2O/30% O_2), after which the mice were allowed to recover for 18–24 hr with food and water ad libitum. Then the mice were injected intravenously with 2-D-[1-^{14}C]deoxyglucose (specific activity, 50–55 μCi/mmol; Dupont-New England Nuclear, Wilmington, DE; dose, 120–150 mCi/kg) in ~40 μl saline, and timed 20 μl samples of arterial blood were collected to measure glucose and deoxyglucose concentrations in the plasma for the quantification of rates of glucose utilization (Sokoloff et al., 1977; Suda et al., 1990). During this procedure, the conscious animals were freely moving in a cage with or without whisker stimulation (see above) until they were killed at precisely recorded times between 45 and 55 min after the injection of the tracer. The brains were removed, and the hemispheres were divided and frozen at −55°C. Serial 20-μm-thick sections were cut tangentially through the barrel cortex in a cryostat at −22°C and exposed to x-ray film (Ektascan EMC-1, Eastman Kodak Co., Rochester, NY). After autoradiography, every second section was stained for cytochrome oxidase activity (Wong-Riley, 1979). Color-coded images of autoradiograms were compared by setting the lowest optical densities in barrel cortex blue and the highest optical densities red/white, while leaving the spectral width assigned to each color equal (IMAGE, NIMH, USPHS, Bethesda, MD). Rates of glucose utilization were quantified in barrels A1-A3, B1-B3, C1-C3, D1-D3, and E1-E3 by superimposing the outlines of the barrels, drawn from sections stained for cytochrome oxidase activity, on digitized images of autoradiograms with a computerized image analysis system (Imaging Research, St. Catharines, Ontario, Canada). The mean rates of glucose utilization in the selected areas were calculated from pixel-weighted averages for each animal.

Histology of the Whiskerpads

The whiskerpads of both sides were separated from the snout and stored in 10% phosphate-buffered (pH 7.4) formalin. Serial 20-μm-thick sections were cut parallel to the epidermis in a cryostat at −22°C and stained with a modified Liesegang method (Cruz et al., 1984). The sections were scrutinized under a microscope (Ortholux II, Leitz, Wetzlar, Germany) fitted with a 63 NA/oil fluorescence objective (Leitz, Wetzlar, Germany), and nerve fibers innervating whisker follicles adjacent to the lesion through the deep follicular nerves were counted. The counts were obtained from cross sections through the follicular nerves at the base of the follicles where the nerves rise perpendicularly to the plane of section.

Statistical Analysis

The mean local rates of glucose utilization in each area of barrel cortex were subjected to two-tailed Dunnett's and two-tailed Student's *t* tests. The nerve fiber counts were subjected to two-tailed Dunnett's *t* tests. The correlation between the metabolic rate in barrels C1-C3 and the follicular innervation density of deflected whiskers B1-B3 and D1-D3 was tested with linear regression analysis. The statistical analyses were performed with SAS (SAS Institute, 1988).

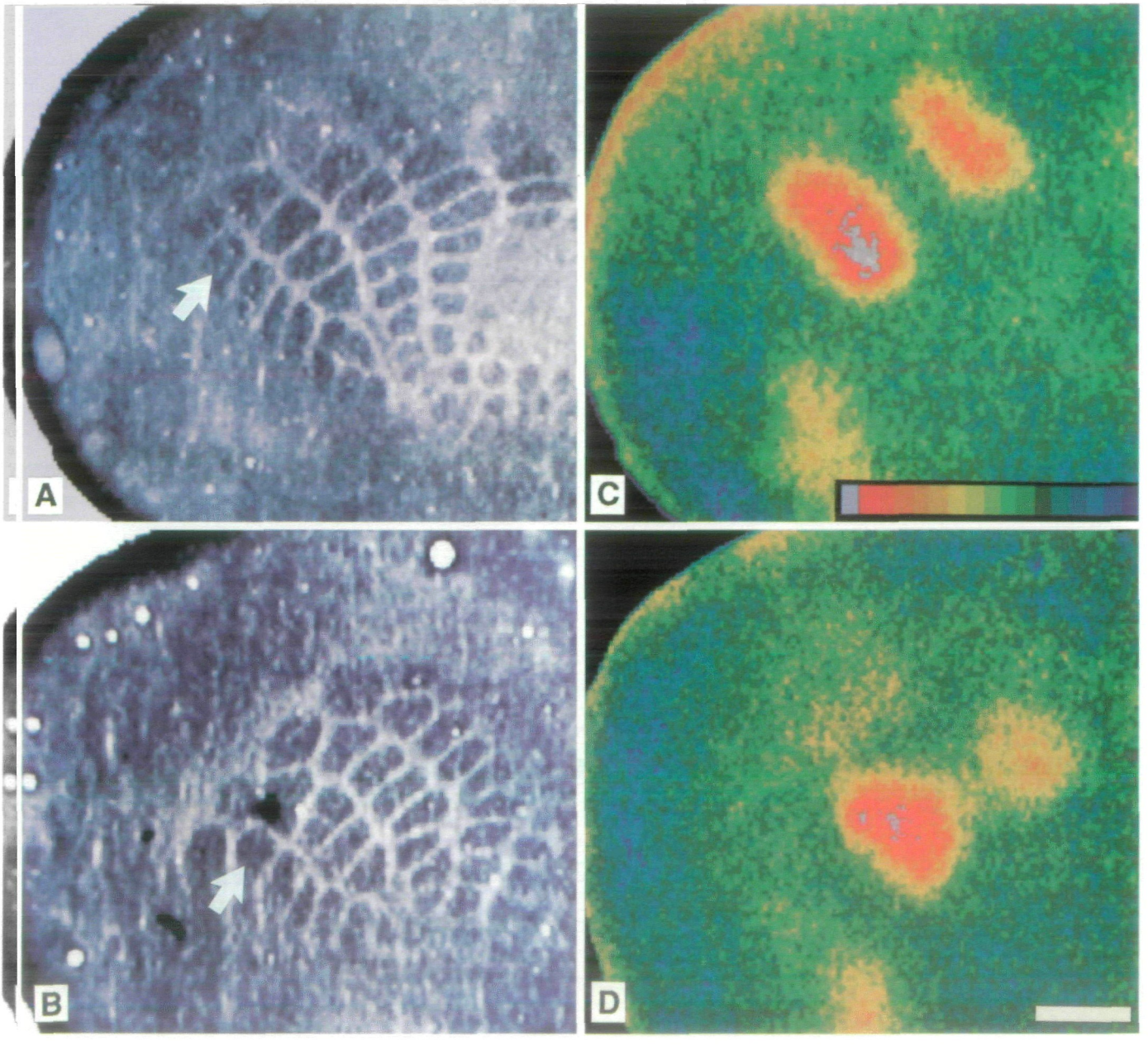

Figure 1. Morphology and metabolic activity in barrel cortex of stimulated mice: results of [^{14}C]deoxyglucose studies of an unoperated control (*top*) and a mouse with lesion (*bottom*). The latter was subjected to the experiment 9.5 months after the lesion. Shown are digitized monochrome images of sections stained for cytochrome oxidase activity (*A* and *B*) and pseudocolor-coded quantitative images of the corresponding autoradiograms (*C* and *D*; rostral is right, medial is up). Barrels are discrete cytoarchitectonic units in layer IV, composed of cell body–dense sides surrounding cell body–sparse hollows, and represent the whiskers on the snout topologically. The tall whiskers array in five rows, designated A (dorsal) to E (ventral), which are straddled by four whiskers, α to δ, at the caudal end. In each row, the whiskers are numbered beginning with 1 caudally (Woolsey and Van der Loos, 1970). In *A* and *B* barrel hollows appear as darkly stained patches of high enzyme activity revealing the representation of the whiskers (*arrows* point at the barrel of whisker A1). The most medial barrels represent the straddlers. Owing to the cutting angle, in row E only the barrels of rostral whiskers can be seen, and in *A* the barrels of whiskers γ, δ, and D1 stain faintly. In *C* and *D*, *white/red* indicates high, and *blue* low, metabolic activity (see *color bar*). In the two animals the stimulation of left whiskers B1–B3 and D1–D3 increased metabolic activity most in right barrels B1–B3 and D1–D3. In the control, metabolic activation in the neighboring barrels remained low. In the mouse with lesion, however, there is a marked increase in metabolic activity in deprived barrels C1–C3. Scale bar, 500 μm.

Results

Metabolic Activation in the Barrel Cortex

In unoperated mice deflections of whiskers B1-B3 and D1-D3 increased rates of glucose utilization to the greatest degree in the barrels representing these whiskers in the right hemisphere, that is, the appropriate barrels (Fig. 1*C*). Compared with the metabolic rates of the homeotopic barrels in the left hemisphere, the increase was on the average 46 and 29 μmol/100 gm/min in barrels B1-B3 and D1-D3, respectively. In contrast, the metabolic rates in inappropriate barrels were only 6 μmol/100 gm/min higher than those of the left hemisphere and remained uniform (Fig. 2*A*, top). The metabolic whisker map in normal mice, therefore, matched with the morphological whisker map. In stimulated mice with lesions, the metabolic activity of all assessed areas was higher than in unoperated mice. However, as in unoperated mice, the highest metabolic activity was found in the appropriate barrels (Fig.

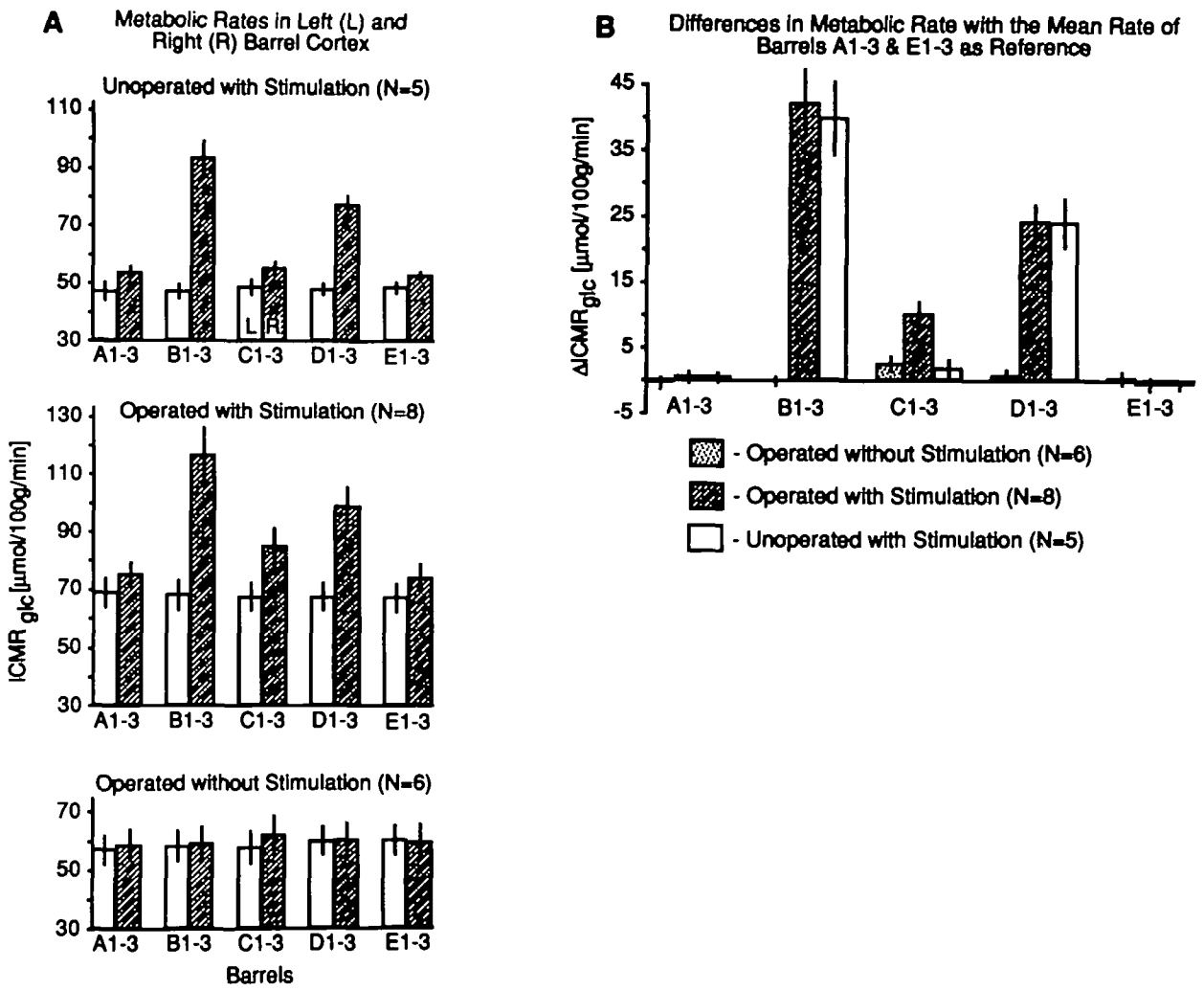

Figure 2. Rates of glucose utilization ($ICMR_{glc}$) in layer IV of barrel cortex of unoperated mice that had the follicles of left whiskers B1–B3 and D1–D3 stimulated, mice with lesions that underwent the same stimulation, and mice with lesions that had all whiskers clipped. $ICMR_{glc}$ was measured in the three caudal barrels of rows A–E in both hemispheres. The bars represent the group means ± SEM. In mice with lesions, the metabolic rate of right barrels C1–C3, that is, the barrels deprived by the lesion, was clearly affected by the stimulation of whiskers B1–B3 and D1–D3 (*A*). The increase above the rate of left barrels C1–C3 is statistically significantly higher than that in the stimulated unoperated controls (Dunnett's two-tailed *t* test, $P \leq 0.05$). In unstimulated controls with lesions the right-to-left differences in metabolic rate of all assessed areas were smaller than those in stimulated mice with lesions. Except in barrels A1–A3 with $P = 0.07$, the differences were statistically significant. Most strikingly, in the right hemisphere of stimulated mice with lesions the mean difference between $ICMR_{glc}$ of deprived barrels C1–C3 and the average $ICMR_{glc}$ of ipsilateral barrels A1–A3 and E1–E3 was 10.2 µmol/100 gm/min (*B*), whereas in stimulated unoperated controls this mean difference was only 2.0 µmol/100 gm/min. The disparity is statistically significant (two-tailed Student's *t* test, $P \leq 0.05$).

1*D*). When compared to the metabolic rates in the left hemisphere, the rates in barrels B1-B3 and D1-D3 were increased by 49 and 31 µmol/100 gm/min, respectively (Fig. 2*A*, middle). Whereas the rates in barrels A1-A3 and E1-E3 were increased by 7 µmol/100 gm/min, the metabolic rate in barrels C1-C3, that is, the barrels corresponding to the removed whisker follicles, was 17 µmol/100 gm/min higher than that of the left hemisphere. Therefore, while the metabolic rates in barrel cortex of mice with lesions were generally higher than those in unoperated controls, the local stimulus-related increases remained quite similar in all assessed areas except barrels C1-C3. In the latter the increase in rate was more than twice that of unoperated controls. This increase in metabolic activation appears even more striking when the metabolic rate in barrels C1-C3 is compared with the rates in ipsilateral barrels A1-A3 and E1-E3, that is, the barrels least affected by the lesion and the stimulation in the same hemisphere (Fig. 2*B*). The mean difference in metabolic rate between barrels C1-C3 and the average metabolic rate of A1-A3 and E1-E3 is about five times greater in stimulated mice with lesions than in stimulated unoperated mice. The difference is clearly expressed in the color-coded images of Figure 1. In unstimulated controls with lesions metabolic rates of all assessed areas in the two hemispheres were essentially equal. The mean metabolic rates of barrels A1-A3, C1-C3, and E1-E3 were slightly higher than those of unoperated stimulated mice. However, the right-to-left differences of the five areas were smaller than those in stimulated mice with lesions (Fig. 2*A*, bottom).

The Deep Innervation of Whisker Follicles

Figure 3*A* shows a typical example of the gross morphology of a whiskerpad from which the follicles of whiskers C1-C3 had been removed. Deep nerves penetrating the follicular wall at a dorsocaudal location, which is the normal site (Dörfl, 1985; Rice et al., 1986), can be seen at all follicles. Remarkably, an additional deep nerve enters the follicle of whisker B1 opposite to the normal site of entry. At the higher magnification of Figure 3*B* the myelinated fibers of the *anomalous* nerve are seen penetrating the follicular wall. Among the 17 whiskerpads with lesions anomalous nerves were noticed in

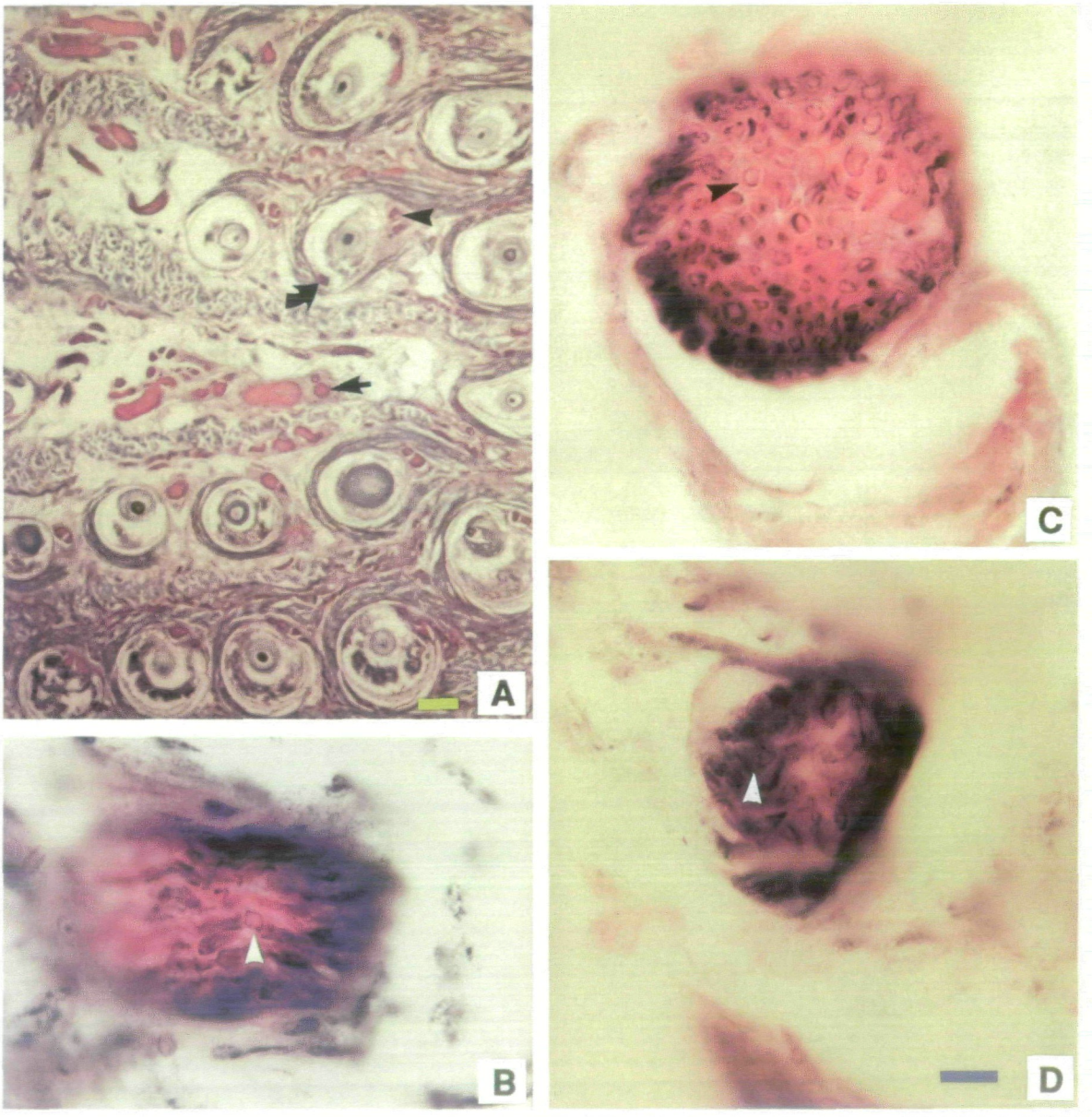

Figure 3. Micrographs from a 20-μm-thick section through the left whiskerpad of a mouse with lesion cut parallel to the epidermis and stained with a modified Liesegang method. The whiskerpad was taken from the animal, whose cortical activation pattern is shown in Figure 1. In all panels, whisker follicles of row A are at the top (dorsal) and rostral is on the left. *A*, Micrograph showing the caudal whisker follicles. The partially visible follicles on the right belong to the straddlers. Note the former site of the removed whisker follicles in row C between the follicles of whiskers γ and C4, in which stumps of regenerated deep follicular nerves can be seen (*arrow*). Normal deep follicular nerves are found near all follicles (*arrowhead at follicle B1*). They penetrate the follicular capsule wall from the dorsocaudal direction. However, at follicle B1 an anomalous deep nerve enters the follicle opposite to the normal site of entry (*curved arrow*). *B*, Micrograph showing nerve fibers of the anomalous nerve penetrating the follicular wall. Some are cut in cross section (*arrowhead*). *C* and *D*, Cross sections of the normal and the anomalous deep nerve of follicle B1, respectively. The micrographs were taken from sections at the base of the follicle where the nerve fibers are transected orthogonally to their axis. Myelin, wrapping the nerve fibers, and tubulin, as a thin, darkly stained inner rim, are distinguishable (see nerve fibers marked with *arrowheads*). Some fibers do not appear well differentiated because they are not in the plane of focus. The thickness of the myelin and the diameter of the nerve fibers varied considerably. Nerve fibers were counted in such cross sections. We counted 165 fibers innervating follicle B1, 39 of which are contributed by the anomalous nerve. Scale bars: *A*, 200 μm; *D*, 10 μm for *B–D*.

the follicles of whiskers B1 (three cases), β (once), γ (once), and B3 and β (once). They were never found in unoperated whiskerpads. Their origin could be traced to the division of the infraorbital branch of the trigeminal nerve into the row nerves. Three stimulated mice had anomalous nerves, of which one provided the observations shown in Figures 1 and 3.

Anomalous nerves were only detected because of the distinctive distance between their site of entry into the follicle and the normal site of entry of the deep nerve. Regenerated nerves may have entered the follicle unnoticed at the normal site. Moreover, regenerated nerve fibers may have joined ordinary follicular nerves proximal to their separation from the row nerves. Therefore, we counted the nerve fibers of the deep nerves innervating the follicles of whiskers B1–B3 and D1–D3. Only sections transecting the nerves orthogonally were used. Examples of a normal and an anomalous nerve are shown in Figure 3, *C* and *D*, respectively. Indeed, the deep innervation of whiskers B1–B3 and D1–D3 adjacent to the lesion was increased above that of unoperated whiskerpads not only in mice with anomalous nerves but also in those

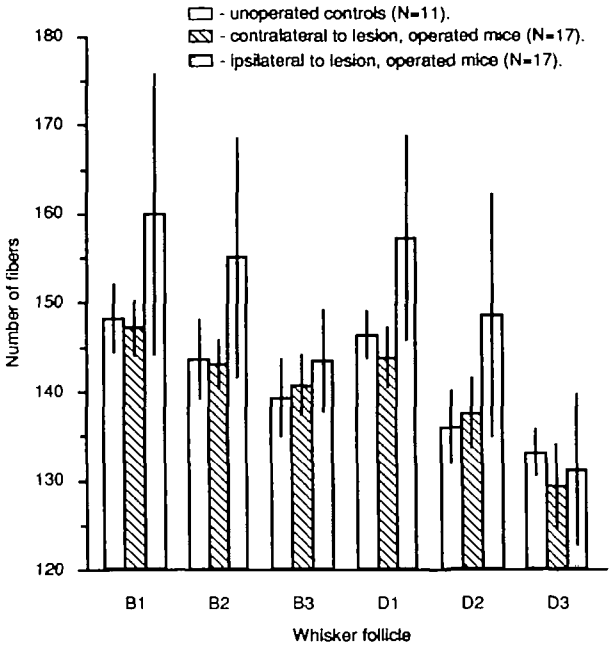

Figure 4. The number of nerve fibers in the deep follicular nerves innervating the follicles of whiskers B1–B3 and D1–D3, that is, the whiskers deflected in the stimulation experiments. The fibers were counted in sections stained with Liesegang's method, which transected the follicular nerves orthogonally to their axis (Fig. 3*C,D*). Counts were obtained from left and right whiskerpads of animals with lesions and unoperated controls. In the latter the counts from homeotopic whisker follicles of both whiskerpads were pooled. The bars represent the group means ± SD. In mice with lesions, the counts from the whiskerpads contralateral to the lesion were in good agreement with those of unoperated controls. However, the innervation in whiskerpads with lesions is enhanced. The side-to-side differences are statistically significant for the follicles of whiskers B1–B2 and D1–D2 (group comparison with Student's *t* test, $P \leq 0.05$).

animals in which such nerves were not found. Based on the counts from the 17 mice with lesions, the increase in innervation of the follicles of whiskers B1, B2, D1, and D2 reached statistical significance (Fig. 4). Linear regression analysis demonstrated that the metabolic rate in barrels C1-C3 of stimulated mice correlated significantly with the number of nerve fibers innervating the follicles of deflected whiskers B1-B3 and D1-D3 ($r^2 = 0.551$, $P = 0.006$).

Nerve stumps of deep follicular nerves, once innervating the removed whisker follicles, were common to all mice with lesions (Fig. 3*A*). Numerous regenerated nerve fibers emanated from the stumps and rose through the scar tissue toward the dermis, where they seemed to end. These fibers were not seen to enter whisker follicles.

Discussion

The findings of the present study show that the removal of whisker follicles from the snout of adult mice results in widespread as well as localized increases in metabolic responsivity to whisker stimulation in barrel cortex. The lesion alone may account for only a small portion of the increase. The widespread stimulus-related increase in metabolic activity across barrel cortex ipsi- and contralateral to stimulation may be mediated by divergent intracortical (Bernardo et al., 1990) and corticocortical connections (Welker et al., 1988). The only significant local increase occurred in deprived barrels C1-C3 and may reflect an increase in neural activity immediately associated with afferents from the thalamic ventrobasal complex, because their terminations normally maintain the one whisker/one barrel relationship (Jensen and Killackey, 1987). The increase in metabolic responsivity of deprived barrels C1-C3 to deflections of whiskers B1-B3 and D1-D3 indicates that the cortical representations of the intact whisker follicles had enlarged at the expense of the deprived territory in harmony with the findings in monkeys with irreversible lesions of the hand innervation.

If a monkey's hand nerve is repaired after transection, regenerating nerve fibers reinnervate the deafferented skin, and its original representation in somatosensory cortex can be, at least partially, restored (Wall et al., 1986). Extensive recovery of the original representation occurs after nerve crush and has been associated with the complete restoration of the original innervation in the deafferented skin by regenerating nerve fibers of the crushed nerve (Wall et al., 1983). In contrast, the occupation of the deprived territory in cortex by the representations of intact skin after nerve transection or digit amputation was persistent when the proximal nerve stumps had been ligated (Merzenich et al., 1983, 1984). The authors did not find outgrowth of regenerating nerve fibers from the nerve stumps and sought central mechanisms of adaptation to explain the persistent reorganization of cortical somatotopic maps. Similarly, the changes in whisker representation in barrel cortex we observed after the removal of whisker follicles were persistent and central mechanisms of reorganization seemed most plausible.

However, in mice with lesions we noticed separate deep nerves entering follicles neighboring the lesion at an anomalous site, and across all animals the number of deep nerve fibers innervating the follicles of whiskers B1-B2 and D1-D2 was increased statistically significantly above that of unoperated whiskerpads (Fig. 4). Our nerve fiber counts underrepresent the true number of fibers in the deep follicular nerves. We counted on the average 142 fibers in the deep nerve of whisker follicle C2 in unoperated whiskerpads, which is in good agreement with the 137 myelinated fibers reported by Lee and Woolsey (1975). Welker and Van der Loos (1986) counted 122 myelinated and 54 unmyelinated fibers in this nerve in electron microscopic preparations. Therefore, about 40% of the unmyelinated fibers were not stained by our method. The role of unmyelinated fibers in the whisker-to-barrel pathway is not well understood. Their postnatal destruction has been reported to enlarge the size of neuronal receptive fields in barrel cortex, but did not prevent the development of cytoarchitectonically normal barrels (Nussbaumer and Wall, 1985). In contrast, a certain number of myelinated fibers is essential for proper barrel development (Welker and Van der Loos, 1986). Because we were not able to detect all unmyelinated nerve fibers, the apparent increase in innervation could be the result of a mere shift in proportion between myelinated and unmyelinated nerve fibers, leaving their total number unchanged. The anomalous follicular nerves, however, provided additional myelinated nerve fibers adding to the total, which renders an increase in innervation density more likely. Hence, it seems that the removal of whisker follicles provokes outgrowth of nerve fibers, adding novel innervation to follicles in the immediate vicinity of the lesion. The novel innervation may contribute to the observed enlargement of the representation of these whiskers in barrel cortex. This contention is supported by the significant correlation between metabolic rate in barrels C1-C3 of stimulated animals and the deep follicular innervation of the stimulated whiskers. It is unlikely that fibers emanating from the nerve stumps were excited by the stimulus we applied. The transmission of force from the deflected whiskers into the surrounding tissue is minimal, because the hairs are anchored in blood sinus, the fluid of which absorbs most of the stimulus energy.

The rededication of the deprived barrels may not be triggered immediately by the removal of whisker follicles. Rather the changes may unfold according to the time course of the peripheral regeneration. The latter would support our hy-

pothesis that the observed persistent changes in central somatotopic representations may reflect the new innervation of peripheral somesthetic receptors. Experiments addressing this important point are under way. Whether the novel nerve fibers actually stem from ganglion cells originally innervating the removed whisker follicles remains to be demonstrated. Regardless of this uncertainty, the existence of new follicular innervation argues for a greater role of the reorganization of peripheral somesthetic innervation in the reshaping of central somatotopic maps than previously thought (Kaas, 1991).

Notes

We are indebted to L. Sokoloff for many helpful comments and his unrelenting support. J. D. Brown was of invaluable help with the preparation of Figure 3.

Correspondence should be addressed to C. B. Smith, Laboratory of Cerebral Metabolism, National Institute of Mental Health, Building 36, Room 1A05, 9000 Rockville Pike, Bethesda, MD 20892.

References

Bernardo KL, McCasland JS, Woolsey TA, Strominger RN (1990) Local intra- and interlaminar connections in mouse barrel cortex. J Comp Neurol 291:231-255

Cruz MC, Jeanmonod D, Meier K, Van der Loos H (1984) A silver and gold technique for axons and axon-bundles in formalin-fixed central and peripheral nervous tissue. J Neurosci Methods 10:1-8.

Dörfl J (1985) The innervation of the mystacial region of the white mouse. A topographic study. J Anat 142:173-184.

Jensen KF, Killackey HP (1987) Terminal arbors projecting to the somatosensory cortex of the adult rat. I. The normal morphology of specific thalamocortical afferents. J Neurosci 7:3529-3543

Kaas JH (1991) Plasticity of sensory and motor maps in adult mammals. Annu Rev Neurosci 14:137-167.

Lee KJ, Woolsey TA (1975) A proportional relationship between peripheral innervation density and cortical neuron number in the somatosensory system of the mouse. Brain Res 99:349-353.

Melzer P, Van der Loos H, Dörfl J, Welker E, Robert P, Emery D, Berrini J-C (1985) A magnetic device to stimulate selected whiskers of freely moving or restrained small rodents: its application in a deoxyglucose study. Brain Res 348:229-240.

Merzenich MM, Kaas JH, Wall J, Sur M, Felleman D (1983) Topographic reorganization of somatosensory cortical areas 3b and 1 in adult monkeys following restricted deafferentation. Neuroscience 8:33-55.

Merzenich MM, Nelson RJ, Stryker MP, Cynader MS, Schoppmann A, Zook JM (1984) Somatosensory cortical map changes following digit amputation in adult monkeys. J Comp Neurol 224:591-605.

Nussbaumer JC, Wall PD (1985) Expansion of receptive fields in the mouse cortical barrelfield after administration of capsaicin to neonates or local application on the infraorbital nerve in adults. Brain Res 360:1-9.

Rice FL, Mance A, Munger BL (1986) A comparative light microscopic analysis of the sensory innervation of the mystacial pad. I. Innervation of vibrissal follicle-sinus complexes. J Comp Neurol 252:154-174.

SAS Institute (1988) SAS/STAT user's guide, release 6.03, pp 549-640 Cary, NC: SAS Institute.

Sokoloff L, Reivich M, Kennedy C, Des Rosiers DH, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M (1977) The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure and normal values in the conscious and anesthetized rat. J Neurochem 28:897-916.

Suda S, Shinohara M, Miyaoka M, Lucignani G, Kennedy C, Sokoloff L (1990) The lumped constant of the deoxyglucose method in hypoglycemia: effects of moderate hypoglycemia on local cerebral glucose utilization in the rat. J Cereb Blood Flow Metab 10:499-509.

Waite PME, Taylor PK (1982) Removal of whiskers in young rats causes functional changes in cerebral cortex. Nature 274:600-602.

Wall JT, Felleman DJ, Kaas JH (1983) Recovery of normal topography in the somatosensory cortex of monkeys after nerve crush and regeneration. Science 221:771-773.

Wall JT, Kaas JH, Sur M, Nelson RJ, Felleman DJ, Merzenich MM (1986) Functional reorganization in somatosensory cortical areas 3b and 1 of adult monkeys after median nerve repair: possible relationships to sensory recovery in humans. J Neurosci 6:218-233.

Welker E, Van der Loos H (1986) Quantitative correlation between barrel-field size and the sensory innervation of the whiskerpad: a comparative study in six strains of mice bred for different patterns of mystacial vibrissae. J Neurosci 6:3355-3373.

Welker E, Hoogland PV, Van der Loos H (1988) Organization of feedback and feedforward projections of the barrel cortex: a PHA-L study in the mouse. Exp Brain Res 73:411-435.

Wong-Riley MTT (1979) Changes in the visual system of monocularly sutured or enucleated cats demonstrable with cytochrome oxidase histochemistry. Brain Res 171:11-28.

Woolsey TA, Van der Loos H (1970) The structural organization of layer IV in the somatosensory region (SMI) of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectonic units. Brain Res 17:205-242.