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# Overlap of sensory representations in rat barrel cortex after neonatal vibrissectomy

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**Abstract**. Cortical representation of the common fur of mystacial pad is situated outside postero-medial barrel subfield (PMBSF) in rat primary somatosensory cortex. Following neonatal vibrissectomy, stimulation of the common fur activates the neurones in PMBSF. We examined if sparing of one mystacial vibrissa from the neonatal ablation, which results in a very extensive increase of its cortical representation, would prevent the invasion of the common fur inputs into the PMBSF. The cortical representations were mapped with 2-deoxyglucose (2DG). It was found that six weeks after neonatal vibrissectomy sparing C3 vibrissa and common fur inputs were represented in the PMBSF. Their representation shifted from its normal location into the barrel field. This effect was observed in cortical layers II/III, IV and V.

**Key words:** barrel field, 2DG, fur, vibrissectomy, plasticity, rat, somatosensory cortex

## INTRODUCTION

Sensory tactile information from the face region of rodents is transmitted to mechanoreceptors in the skin by the short pelage hair and by the vibrissae. The vibrissae growing on the mystacial pad activate the neurones of the large barrels in the somatosensory cortex (Welker 1971). These barrels are arranged into five rows in cortical layer IV, mimicking the distribution of vibrissal follicles on the snout (Woolsey and Van der Loos 1970). The part of the barrel field containing the large barrels was named postero-medial barrel subfield (Woolsey and Van der Loos 1970). Short pelage hairs form the common fur that grows on the mystacial pad between the rows of vibrissae (Fig.1). Despite the fact that in the periphery rows of vibrissae are interspaced with rows of the common fur, the cortical representation of the common fur is situated outside the PMBSF (Pidoux et al. 1980, Chmielowska 1985, Nussbaumer and Van der Loos 1985, Sharp et al. 1988, Siucińska and Kossut 1994). This break in somatotopy is most likely caused by the necessity of tight integration of the input from the vibrissal sensory scanning apparatus.

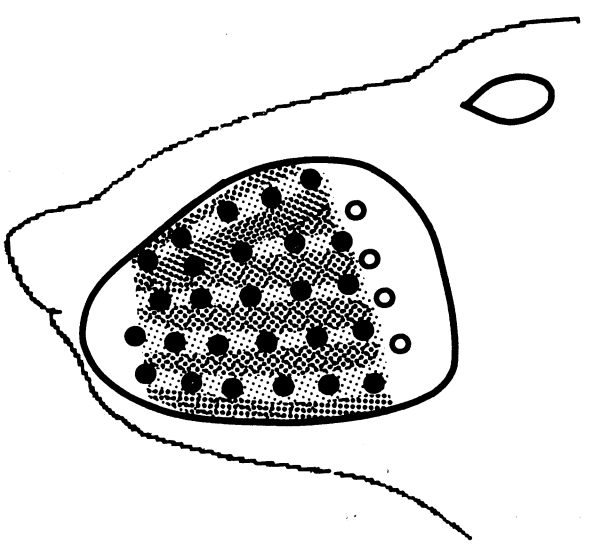

Fig. 1. Schematic drawing of the rat muzzle with mystacial pad outlined. Black circles represent mystacial vibrissae; white circles represent stradlers vibrissae; shading represents mystacial common fur growing between the rows of vibrissae and around vibrissae, that was stimulated during 2DG experiments.

The developing barrels compete for cortical space, and neonatal injury of vibrissal receptors or their sensory nerve results in atrophy of the denervated barrels and expansion of the spared inputs in the cortex (Van der Loos and Woolsey 1973, Killackey et al. 1978, Kossut and Hand 1984, Kossut et al. 1988). There are also competitive interactions between the vibrissal and non-vibrissal inputs to the cortex. Waite and Taylor (1978) found that after neonatal destruction of all vibrissal follicles, the cells in the barrel field responded to stimulation of surrounding areas of the face: the nose, the line of small hairs along the lip (F line hairs), and the common fur of the mystacial pad. Similar, although more restricted plastic changes were observed in mouse barrel field (Pidoux et al. 1979).

Waite and Taylor (1978) also found that stunted vibrissae that have regrown after electrocautery of vibrissal follicles performed neonatally, excite the barrel cortex otherwise dominated by the fur input. These stunted vibrissae are represented at their normal positions in the cortex, but often at reduced area, presumably because of their reduced innervation. Pidoux et al. (1979, 1980) also found that following partial removal of vibrissae, the common fur invades only the denervated cortical regions. We have previously described, that neonatal vibrissectomy sparing the C3 whisker, results in a great expansion of the cortical representation of the only remaining vibrissa (Kossut and Hand 1984, Kossut et al. 1988). This effect was described using 2DG mapping of cerebral activity (Sokoloff et al. 1977). Two months after such vibrissal lesion most of the barrel field could be activated by stimulation of the spared vibrissa (Kossut et al. 1988).

It seemed conceivable that the spared whisker, which could drive neurones in most of the PMBSF region, could protect the barrel field from being activated by the common fur input. This paper examines if the expansion of the spared whisker representation would prevent the invasion of the common fur inputs into the barrel field territory.

## METHODS

The experiments were done on 6 Sprague-Dawley rats. On the second postnatal day the pups were anaesthetized by cooling and unilateral vibrissectomy sparing C3 whisker was performed. The skin over rows A and B and D and E was incised and the vibrissal follicles were mechanically removed with fine forceps. The skin was then cut close to row C and follicles of vibrissae C1, C2, C4, C5 and C6 and the straddlers were removed. This procedure left relatively little scar on the mystacial pad and the common fur was growing vigorously on it after a week. The skin with the involved mystacial pad was examined on frozen, unstained sections for the completeness of the follicle ablation.

Six weeks after the surgery the 2DG mapping was performed on restrained, unanaesthetized rats. Prior to 2DG mapping, 4 rats were lightly anaesthetized with ether and all the mystacial vibrissae, including the spared C3 were plucked out. Twenty minutes later the rats were restrained on a padded block by their trunks and a single dose of 25 Ci [1-$^{14}$C ]-D-glucose (Amersham, spec.act.48 mCi/mmol) was injected intraperitoneally. During isotope incorporation the mystacial fur on both sides of the muzzle was stimulated with hand held soft brushes. Great care was taken to avoid touching of the skin or large deflections of hairs. In two rats representation of the spared C3 vibrissa and the control C3 on the intact side of the snout were mapped. In this animal the spared C3 whisker and the control C3 were stimulated during the 2DG experiment, while all other vibrissae on the intact mystacial pad were clipped close to the skin just before isotope injection. The stimulation lasted for 45 min, the rats were then sacrificed with an overdose of Nembutal, quickly perfused through the heart with 3.3% formalin in phosphate buffer, the brains were dissected out, the cortex separated from the thalamus, spread on a slide and frozen at -70°C. The sections (20 μm thick) were cut on a cryostat at -18°C in a plane tangential to the barrel field. The sections were picked up on coverslips, rapidly dried on a hot plate and glued to cardboard sheets. They were exposed to X-ray mammography film and developed in a conventional way. After obtaining the autoradiograms the sections were stained with cresyl violet to identify the barrel field and the cortical laminae.

The autoradiograms were analysed with Imaging Research image analysing system. The areal extent of labelling was estimated using the previously established criterion (Kossut et al. 1988) which considered as activated the regions with levels of 2DG labelling 15% higher than in the surrounding cortex.

## RESULTS

When the spared C3 vibrissa was stimulated during 2DG incorporation, its cortical representation, visualized by an increased uptake of 2DG, was expanded as compared to the control hemisphere (Fig. 2) and occupied most of the PMBSF area (Table I), in agreement with the previous report (Kossut et al. 1988). Thus the spared whisker was capable of driv-

TABLE I

Extent of 2DG labelling of PMBSF evoked by stimulation of inputs spared by neonatal vibrissectomy

| | PMBSF area mm$^2$ | Labelling area mm$^2$ | % of PMBSF |
|---|---|---|---|
| Spared C3 | | | |
| # 1 | 3.06 | 2.08 | 68 |
| # 2 | 2.88 | 1.47 | 51 |
| *# 3 | 3.31 | 1.85 | 56 |
| *# 4 | 3.34 | 2.09 | 61 |
| mean | 3.11 | 1.87 | 59 |
| Common fur | | | |
| # 1 | 3.51 | 2.49 | 71 |
| # 2 | 3.01 | 2.34 | 79 |
| # 3 | 2.81 | 1.46 | 52 |
| # 4 | 3.12 | 2.18 | 70 |
| mean | 3.17 | 2.11 | 68 |

*values from rats described previously (Kossut et al. 1988).

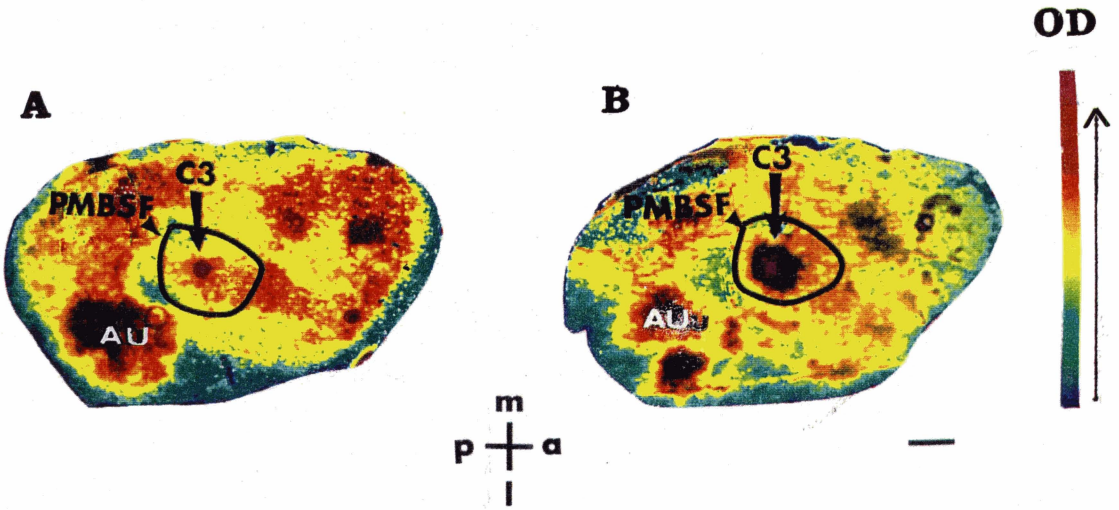


Fig. 2. Pseudocolor reconstruction of autoradiograms of layer IV sections showing a crossection of control C3 (A) and spared C3 (B) vibrissal column. Black line outlines the borders of PMBSF, arrowhead point it. Arrow points to C3 column. Au, auditory cortex. Scale bar, 1 mm; OD, optical density; a, p, m, l, anterior, medial, lateral, respectively.

ing, with the stimulation applied, up to 68% of the barrel field area.

In rats where the fur representation was mapped, the autoradiograms from the hemisphere contralateral to the intact mystacial pad, revealed two areas of labelling adjacent to the barrel field; one medial to the delta straddler and row E of barrels, and another along row A of barrels. These small zones of labelling were visible also in supra- and infragranular layers in register with labelling seen in layer IV. The labelling of the mystacial fur representation had much lower intensity than that of the spared or intact vibrissae representations (Fig. 3A).

Stimulation of the common fur on the lesioned mystacial pad resulted in increased 2DG labelling situated within the barrel field (Fig. 3B). The area

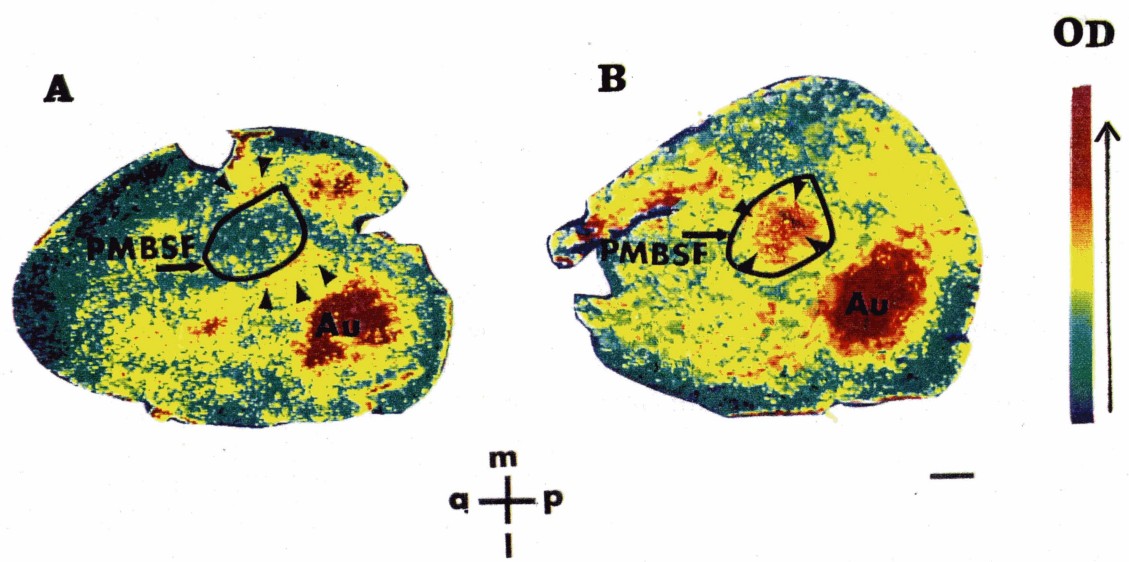


Fig. 3. Pseudocolor reconstruction of layer IV autoradiograms showing labelling of cortical representation of mystacial common fur. A, control side; B, vibrissectomized side. Arrowheads point to common fur representation. Black line outlines PMBSF, arrow points PMBSF. Au, auditory cortex. Scale bar, 1 mm; OD, optical density; a, p, m, l, anterior, medial, lateral, respectively.

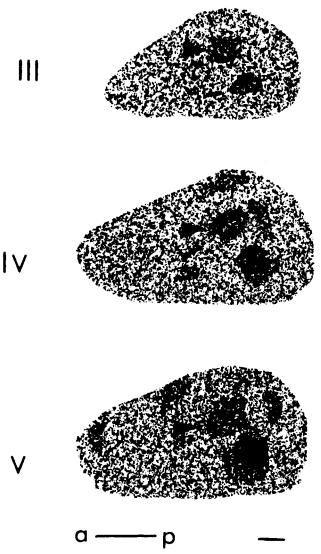


Fig. 4. Printouts of digitized, contrast enhanced, averaged autoradiograms representing labelling in different cortical layers of vibrissectomized barrel cortex (layer III, layer IV, layer V). Six autoradiograms were averaged for each level. Arrowhead points to labelling of common fur representation. Scale bar, 1 mm; a, anterior; p, posterior.

of this labelling was measured on serial sections together with the area of histologically defined PMBSF (Table I). On the average, the representation of the mystacial fur occupied 68% of the PMBSF area.

In the supra-and infragranular layers, when the barrels can not be observed, this labelling was found in the region above and below the barrel field of layer IV (Fig. 4).

The intensity of labelling within the PMBSF was not homogenous, but no pattern could be discerned in the patches of more and less dense label. The labelling in most cases did not fill the entire PMBSF, but it always overplayed the C3 barrel-like territory. The regions around the barrel field, normally occupied by the common fur input, did not show any increased labelling that could differentiate them from the adjacent, unlabelled cortex.

## DISCUSSION

The results of this study indicate the possibility of coexistence of the "original" vibrissal input and the "new" common fur input in the barrel cortex. In the case of a spared vibrissa preparation, the only whisker remaining on the snout after neonatal removal of all vibrissal follicles could activate most of the barrel field. On the other hand, if the spared whisker is plucked out just before the 2DG experiment, and only the common fur is stimulated, the fur also activates most of the barrel field. Previous electrophysiological experiments (Pidoux et al. 1980) did not reveal this phenomenon, finding that the presence of residual whisker representation prevented surrounding regions of the face from taking over the whole whisker area. These experiments, however, were done under deep barbiturate anaesthesia, when a considerable part of activity, and especially of intracortical activity is suppressed (Armstrong-James and George 1988). In this study no anaesthesia was used during the 2DG mapping. On the other hand, the spared whisker was removed just before the isotope injection and the onset of fur stimulation, so it could be argued that the removal unmasked the fur inputs that were not visible in electrophysiological experiments because of the presence of the "original" (and presumably stronger) vibrissal input. However, the experiments on acute rat preparation by Taylor and Waite (1978) found that there are no signs of fur input to the barrel field for up to 4 h after infraorbital nerve sectioning, indicating that the presence of a suppressed input is unlikely. Our experiments on adult animals support this finding. We find that this coexistence of inputs from the spared vibrissa and the common fur is a result of a plastic change induced by a removal of vibrissal follicles.

This study provides evidence of extensive overlap of cortical representations of skin mechanoreceptors, following plastic changes evoked by neonatal vibrissectomy. In normal control rats and on the SI cortex contralateral to the intact side of the snout, the overlap of common fur and mystacial vibrissae projection can be detected with 2DG autoradiography (Siucińska and Kossut 1994), but it is very small. The localization of 2DG labelling of the mystacial fur representation corresponds well with its localization found in electrophysiological experiments, as described by Nussbaumer and Van der Loos (1985). We found that after neonatal vi-

brissectomy sparing C3 vibrissa, the PMBSF can be activated by the spared whisker as well as by the common fur of the mystacial pad. The cortical representation of the spared vibrissa occupies about 85% of the PMBSF area. The region of PMBSF labelled after stimulation of the common fur varied between 70 and 90%. The experimental design does not reveal if the extent of PMBSF labelling by each input would be the same if they were activated simultaneously. Nonetheless, the pattern of 2DG functional labelling is strikingly different from that observed in control animals.

The response to common fur inputs in the barrel cortex following neonatal removal of all vibrissal follicles were reported by several authors (Killackey et al. 1978, Waite and Taylor 1978, Pidoux et al. 1980). The results of partial vibrissectomy were reported by Pidoux et al. (1980) and Simons (1985). While Pidoux et al. (1980) found that responses to common fur stimulation can be found in the regions of barrel field nor drivable by the spared vibrissae, Simons (1985) did not observe any responses to common fur in the deafferented barrel field. We expected that the expanding input from the spared vibrissa would be strong enough to prevent the nonvibrissal inputs from activating the barrel field neurones. However, we found this not to be the case. The common fur activated the barrel field quite strongly, and in each examined case it activated the C3 barrel itself.

Another observation concerning the neonatally lesioned animals is that the common fur representation shifted into the barrel field, "vacating" its normal sites. Such phenomenon was not observed in adult rats. Similar observation concerning the supraorbital vibrissae was reported by Hand et al. (1990). It is known from morphological studies (Waite and Cragg 1979, Savy et al. 1986) that severe degeneration follows the neonatal vibrissectomy and that the subcortical trigeminal centres are smaller than on the control side of the brain and their somatotopy is disorganized (Verley and Onnen 1981, Waite 1984, Nicolelis et al. 1991). These considerable anatomical changes may contribute to abnormal formation of cortical maps.

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*This paper is dedicated to Professor Stella Niemierko on the occasion of her 90th birthday, with esteem and admiration*