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Dendritic pattern in mouse barrel field after a neonatal vibrissal follicles removal: MAP-2 immunohistochemistry

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Abstract. A distribution of dendrites was studied in mouse barrel field after a neonatal unilateral partial lesion of vibrissal follicles using anti-MAP-2 immunohistochemistry. The effect of a neonatal vibrissal follicles removal was studied in adult mice: barrels corresponding to intact follicles were enlarged whereas those representing removed follicles had not developed. MAP-2 immunopositive profiles were considered to be dendritic clusters and their packing density (a number per unit area) was calculated in an enlarged barrel and compared to a control barrel in a contralateral hemisphere. A decrease in the packing density of large dendritic clusters (area over 10 μm^2), presumably arising from layer V, was observed in an enlarged barrel in comparison to its control counterpart. This result may indicate a rearrangement of a dendritic pattern in mouse barrel field after a selective neonatal lesion of vibrissal follicles.

Short communication

Key words: barrel field, cortical plasticity, dendrites, MAP-2 immunohistochemistry, mouse

Barrel field is a part of rodent somatosensory cortex containing a representation of contralateral whiskers including large facial vibrissae (Woolsey and Van der Loos 1970). An individual vibrissa representation appears as an aggregate of neurones (a barrel) in layer IV which in mice form a cylindrical structure with cell-dense sides surrounding a hollow of lower cell density. Barrels are separated from each other by narrow, relatively cell-free septa, and a septum together with the sides of two neighbouring barrels form a wall. A barrel is an anatomical representation in layer IV of a functional column running across the depth of cortex (Armstrong-James 1975). Barrels in the barrel field are arranged in five rows (A-E) corresponding to five rows of facial vibrissae. The morphology of the barrel field can be altered as a result of a neonatal lesion of vibrissal follicles (Van der Loos and Woolsey 1973, Killackey and Belford 1979, Jeanmonod et al. 1981, Durham and Woolsey 1984). Barrels corresponding to lesioned follicles develop poorly whereas those representing intact follicles are enlarged. It was found (using a Golgi-Cox method) that a neonatal vibrissal follicles damage caused a reorientation of dendritic fields of layer IV stellate cells away from a denervated and toward an active zone (Harris and Woolsey 1981). It was also reported from the studies on striate cortex in visually deprived cats that dendritic fields of layer IV stellate cells, but not layer V pyramidal cells, are affected by changes of afferent input (Kossel et al. 1995). Clusters of apical dendrites arising from layer V pyramidal cells have been postulated to be the centers of cortical columns (Peters and Kara 1987, Peters and Sethares 1991, Peters and Yilmaz 1993, White and Peters 1993) and they are also the main output from a column to adjacent columns (Armstrong-James 1995).

MAP-2 is a neurone specific cytoskeletal protein which occurs almost exclusively in cell bodies and dendrites (Matus et al. 1981). It has been postulated to play a role in plasticity of the central nervous system mainly through its contribution to cytoskeletal changes, and to dendritic growth, arborization and synaptic plasticity (for review see Johnson and Jope 1992, Avila et al. 1994). Because MAP-2 is commonly considered a dendritic marker and anti-MAP-2 immunohistochemistry has been successfully used to visualize dendrites in adult mouse barrel field (White and Peters 1993) we decided to use this method to examine the effect of a neonatal unilateral partial lesion of vibrissal follicles on a distribution of dendrites in the barrel field. In this experimental model the normal columnar cortical arrangement is disturbed and therefore one might expect an effect on the pattern of the layer V pyramidal cells dendrites.

Four male Swiss-Albino mice were operated on the day of birth (P0). Under a local anaesthesia (1 mg of 2% xylocain) vibrissal follicles of all but the C rows were cut out unilaterally (on one side of the snout) (Sjucińska and Kossut 1994) thus leaving the other whisker pad intact. Adult mice of at least 4 weeks of age were perfused with 0.1M phosphate buffered saline followed by 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4. Brains were removed and postfixed at room temperature for a few days and sectioned tangentially to the barrel field on a vibratome onto 50 μm thick slices. Four sections through layer IV of the barrel cortex were obtained on average from a single hemisphere and submitted to immunohistochemistry using a monoclonal mouse anti-MAP-2 antibody (clone HM-2, Sigma ImmunoChemicals) at a dilution of 1:1500, according to the protocol reported by White and Peters (1993), slightly modified (24 h incubation with the primary antibody). Nissl staining was carried out on some sections to visualize the barrel field cytoarchitecture and confirm the effect of lesion.

The slides were examined under the light microscope 20 x objective magnification and analysed using a computer image analysing system MCID-M4 (Imaging Research Inc., Canada). Two sections (containing an entire well visible barrel C1) from each brain were chosen: one from the hemisphere corresponding to a lesioned side and another one from the contralateral, control hemisphere. Barrel C1 was analysed and a comparison was made between an experimental and a control side. The size of a control and an experimental barrel C1 was compared by measuring their area. The area measured

included barrel hollows and walls. The packing density of MAP-2 immunopositive profiles was determined by counting their number per unit area. Each measurement was repeated three times and the mean value was considered to be each individual measurement. In order to increase chances that the counts include only clusters of layer V pyramidal cells apical dendrites the profiles of a size over 10.0 μm^2 were taken into account.

A comparison of a Nissl stained sections from a control (Fig. 1A) and an experimental (Fig. 1B) hemisphere revealed altered cytoarchitecture of a barrel field corresponding to a lesioned side of the snout as compared to the control barrel field. The mean size of a control barrel C1 was 0.088±0.009 mm^2 whereas the mean size of an experimental barrel C1 reached 0.243±0.011 mm^2. MAP-2 immunostaining showed a barrel-like pattern (Fig. 1C). Examination of an individual barrel revealed a considerably lower packing density of immunoreactive profiles in a barrel hollow (1764±242/mm^2) than in walls (2852±302/mm^2). A size of individual clusters varied both within a barrel, between barrels and between animals without any clear tendency. In an experimental hemisphere (corresponding to the lesioned side) MAP-2 immunostaining reflected changed morphology of an enlarged row C (Fig. 1D). A decrease in the packing density of immunopositive profiles was observed in a barrel C1 hollow as well as in walls. The values dropped on average by

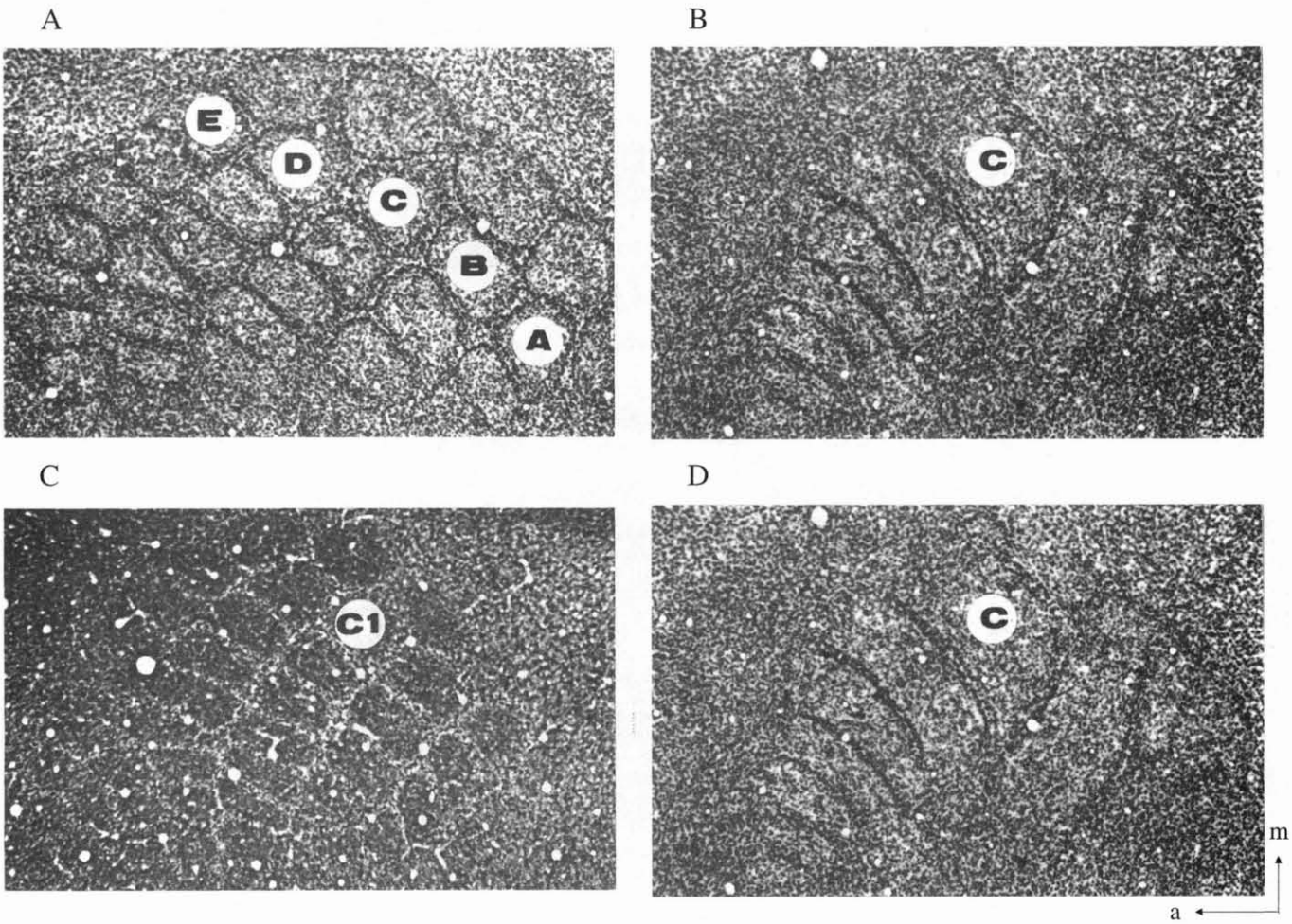

Fig. 1. The effect of a neonatal unilateral partial vibrissectomy of all but the C rows on the cortical representation of whiskers in 4 weeks old mice in sections cut tangentially to the barrel field. Nissl stained sections: (A) control hemisphere, (B) experimental hemisphere. MAP-2 immunostained sections: (C) control hemisphere, (D) experimental hemisphere. A-E indicate barrel rows; a, anterior; m, medial. Bar 100 μm.

29% ($P<0.01$, one-tailed t -test) in a barrel hollow and by 39.5% ($P<0.02$, one-tailed t-test) in barrel walls. The decrease in the packing density was most likely a result of the area enlargement. An increase in the absolute number of clusters was much smaller than the increase in the area occupied by barrels. It should be noted that the result concerned only the profiles of a size over 10.0 μm^2.

There are three main sources of dendrites present in barrels: smooth and spiny stellate cells which form barrels and send dendrites preferentially oriented into the barrel hollow, V and VI layers pyramidal cells sending upward their apical dendrites and II/III layers pyramidal cells which extend their basal dendrites down to layer IV (Keller 1995). The apical dendrites originating from layer V have been

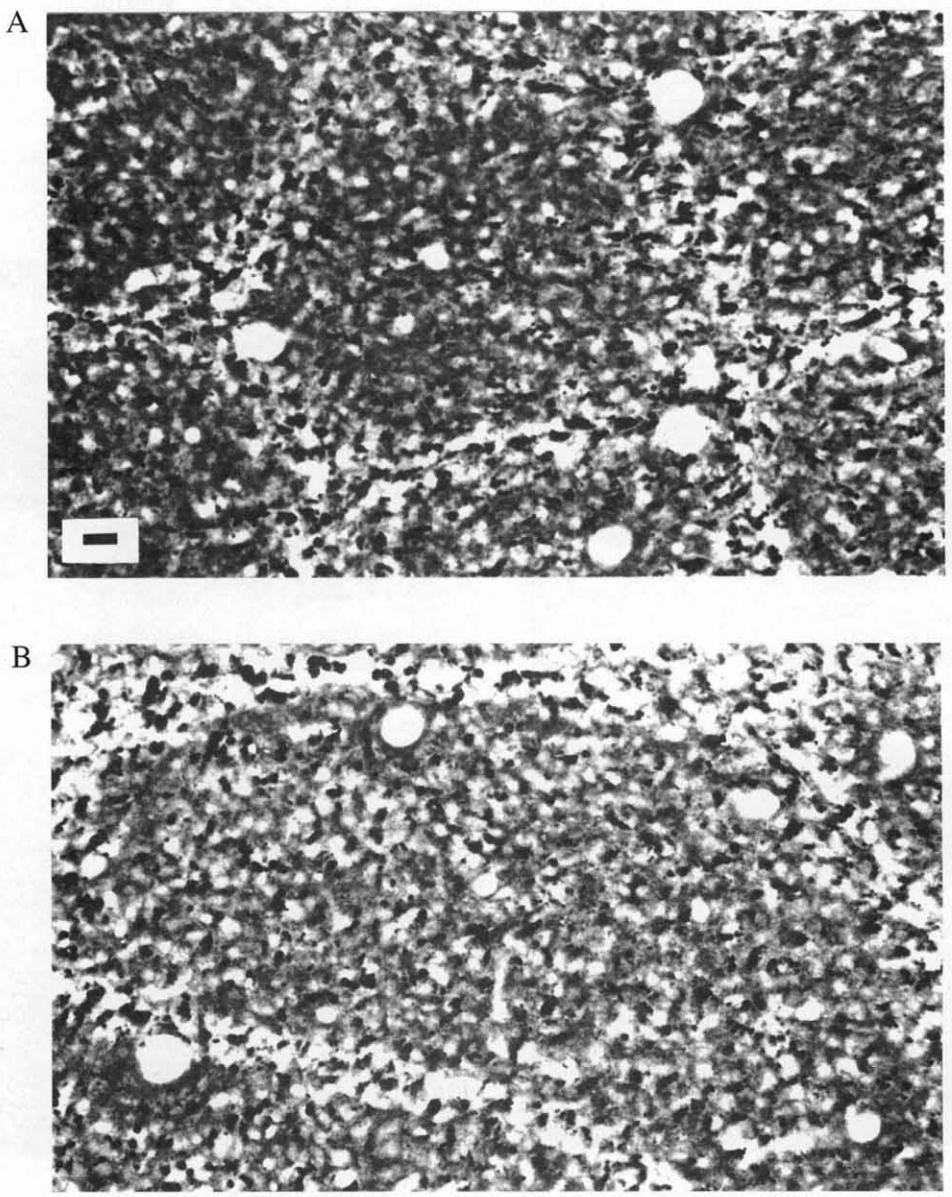

Fig. 2. High power view of dendritic clusters pattern revealed by anti-MAP-2 immunostaining. A, control barrel C1 from a control hemisphere; B, enlarged barrel C1 from an experimental hemisphere. Bar 10 μm.

shown to form clusters ascending into layer II/III where they are joined by apical dendrites of local pyramidal cells (White and Peters 1993).

We considered the positively anti-MAP-2 stained profiles to be dendritic clusters or single dendrites (Fig. 2). A common feature of the dendritic clusters was their higher packing density in a barrel wall than in hollow which was consistent with the previous studies (White and Peters 1993). We also assumed that large dendritic clusters (area over 10.0 μm^2) originate from layer V pyramidal cells according to the description by White and Peters (1993) of the dendritic patterns in cortical modules across the depth of the somatosensory cortex in mice. The presented results suggest a decrease in a packing density of apical dendrites clusters presumably arising from layer V pyramidal cells in an enlarged barrel C1. In the analysed barrels, along with the counted clusters, smaller immunopositive profiles occurred which were not taken into account. These profiles could represent fine terminals of small clusters or single dendrites arising from layer VI pyramidal cells.

A barrel size is directly related to the number of myelinated axons innervating the corresponding vibrissae (Welker and Van der Loos 1986) which in turn determine the amount of terminal afferents along the sensory path including thalamocortical afferents entering barrels (Jacquin et al. 1993). Thalamocortical afferents occupy preferentially barrel hollows (Keller et al. 1985, Bernardo and Woolsey 1987) and they display a barrel-like pattern before the cytoarchitecture is established (Schlaggar and O'Leary 1994). Moreover thalamocortical afferents retain a temporal capacity to reorganize within barrels (Andres and Van der Loos 1985).

Our results show a decrease in the packing density of apical dendrites clusters in an enlarged barrel as compared to the control barrel. It is conceivable that the dendritic clusters appear to be more diffuse in an experimental barrel than in a control barrel due to an increase in the number of thalamocortical afferents entering the enlarged barrel and filling in the space between clusters.

Our preliminary estimation suggests a dendritic reorganization in the mouse barrel cortex after a peripheral selective unilateral lesion. It is too soon, however, to draw conclusions on a specific character of the change and further investigation is required including studies on dendritic clusters course across the depth of barrel cortex after a neonatal lesion.

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