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NG2 cells are uniformly distributed and NG2 is not required for barrel formation in the somatosensory cortex

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ABSTRACT

The somatosensory barrel cortex in the rodent forms during the first postnatal week setting up a periphery related map with each whisker represented as a bundle of thalamocortical axons (TCAs) in layer IV. The centers of each barrel (hollows) contain the densely packed TCAs, while the areas between each barrel (septa) form a boundary between each barrel. NG2 chondroitin sulfate proteoglycan (CSPG) expressing cells (NG2 cells, polydendrocytes) make up a unique population of glial cells that receive synaptic like input and form close contacts with growing axons. In the present study we investigated the developmental distribution of NG2 cells in the barrel cortex to determine if they display preferential septa distribution similar to other extracellular and cell surface CSPGs. Immunohistochemistry for NG2 and platelet-derived growth factor receptor alpha (PDGFRα) in NG2DsRedBAC transgenic mice showed uniform distribution of NG2 cells and processes in barrel hollows and septa at postnatal (P) days 5, 6, 7, 8, 14, and 30. Changes in the barrel pattern formation caused by cauterization of one row of whiskers at P1 resulted in corresponding changes in extracellular and cell surface CSPG distribution at P7 but no detectable changes in NG2 cell bodies and processes. Furthermore, no abnormalities in barrel formation or reorganization were detected in NG2 knockout mice. These observations suggest that NG2 cells are unlikely to play an inhibitory boundary role on TCA growth and that NG2 expression is not necessary for normal barrel formation.

Introduction

The somatosensory barrel cortex in the rodent is organized as a topographic map where axons projecting from the ventral posterior medial nucleus (VPM) of the thalamus form bundles that represent individual mystacial vibrissae (Woolsey and Van der Loos, 1970; Petersen, 2007). These projections set up two functional domains: 1) the barrel hollows that are the bundles of axons within each barrel that preferentially respond to individual whiskers and 2) the barrel septa that are the boundaries between each hollow. This pattern forms during the first postnatal week and can be altered during a developmental critical period, by changes in sensory input by whisker removal before postnatal day 3 (Wong-Riley and Welt, 1980).

The end of the critical period for large-scale structural plasticity in the barrel cortex coincides with the unequal distribution of axon growth inhibitory extracellular matrix (ECM) and cell surface molecules at the septa. ECM molecules such as lectins, tenascin, aggrecan, neurocan and other chondroitin sulfate proteoglycans (CSPGs), which are generally known to be repulsive to growing axons (Snow et al., 1990), show increased expression in the septa during the first postnatal week (Cooper and Steindler, 1986a; Steindler et al., 1989; Bahia et al., 2008; Nakamura et al., 2009), when the thalamocortical axon (TCA) branches elaborate and locate their targets within each barrel (Erzurumlu and Jhaveri, 1990).

Cells that express the NG2 CSPG molecule on their surface (NG2 cells, polydendrocytes) comprise a unique population of glial cells in the central nervous system (CNS) separate from astrocytes, oligodendrocytes, and microglia (Nishiyama et al., 2009). NG2 cells, also known as oligodendrocyte progenitor cells (OPCs) exist widely in both gray and white matter of developing and mature CNS (Dawson et al., 2003). Furthermore, they receive synaptic input from neurons in both gray (Bergles et al., 2000; Jabs et al., 2005; Ge et al., 2006) and white matter (Ziskin et al., 2007; Kukley et al., 2007) into adulthood. These data indicate that axon terminals interact intimately with NG2 cells possibly influencing axon growth.

Chondroitin sulfate molecules are generally known to be repulsive to growing axons, (Snow et al., 1990). The NG2 CSPG has shown inhibitory action on neurite outgrowth (Dou and Levine, 1994) and increased expression after CNS injury (Levine, 1994). Other studies have demonstrated however that NG2 cells, unlike the NG2 molecule, are conducive to and may even provide “guide posts” for growing axons (Yang et al., 2006, Busch et al., 2010). If NG2 cells were

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repulsive to growing axons it could be hypothesized that they would be located at the septa of each barrel during somatosensory cortex development. It would be unlikely that axon growth inhibitory cells would be found in the center of a densely packed bundle of axons.

The objective of this study was to determine whether NG2 cells could be localized to the septa of the barrel cortex when thalamocortical axons are finding their targets. We performed immunohistochemistry for NG2 glial cells on tangential sections through barrel cortex at different developmental stages in normal, NG2 knockout and whisker deprived mice and rats. Interestingly, unlike extracellular and cell surface chondroitin sulfate proteoglycans, we demonstrate that NG2 cells are uniformly distributed in barrel hollows and septa, and deletion of NG2 had no effect on the formation or reorganization of the barrels.

Results

Chondroitin sulfate proteoglycan expression during barrel development

Histochemical reaction for the mitochondrial enzyme cytochrome oxidase (CO) with diaminobenzidine (DAB) was performed on tangential sections through layer IV of the somatosensory barrel cortex in order to reveal the whisker pattern. The barrel pattern was reliably detected in all animals between the ages P5 and P30 and was used on every other section in order to identify sections that exhibited a clear barrel pattern and could be used for immunohistochemistry (Figs. 1A, D, G, and J).

Immunohistochemistry for chondroitin sulfate glycosaminoglycan chains using the CS-56 monoclonal antibody was performed on tangential sections through the somatosensory barrel cortex adjacent to those used for CO detection at postnatal days 5, 6, 7, 8, 14 and 30. As previously reported (Miller et al., 1995, Nakamura et al., 2009), more intense CS-56 immunoreactivity was observed in the barrel septa (Figs. 1B and E) compared to the barrel hollows until P7. CS-56 immunoreactivity in the septa declined thereafter, and by P14 and P30 immunoreactivity in the septa was only marginally higher than that in the hollows (Figs. 1H and K). In the present study, CS-56 immunoreactivity was used to identify barrel septa in the early postnatal somatosensory cortex as described below.

5HTT expression during barrel development

Serotonin has been shown to be important for normal barrel development and both the serotonin receptor $5HT_{1B}$ and serotonin

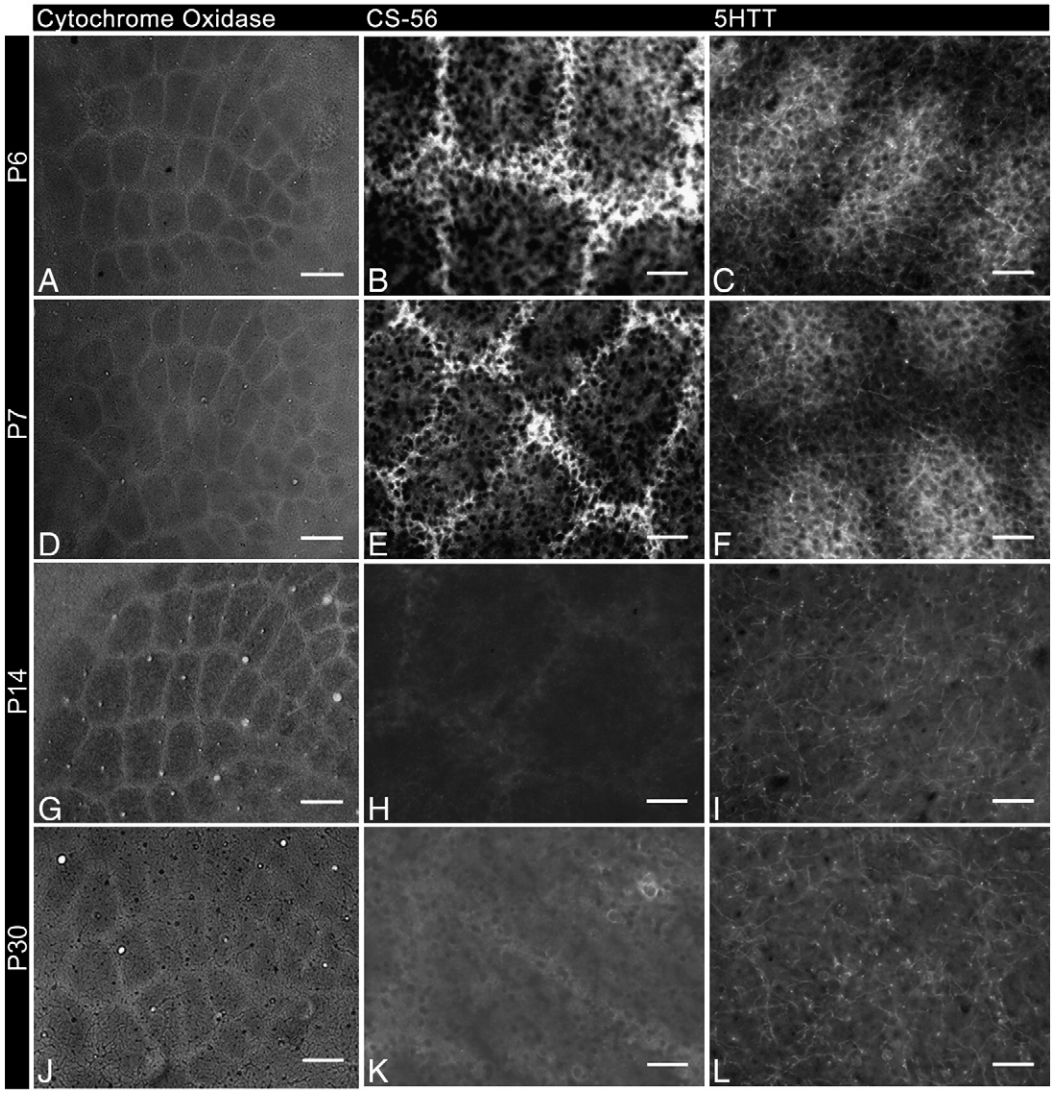

Fig. 1. Changes in expression levels of chondroitin sulfate and serotonin transporter in tangential sections during barrel cortex development from P6 to P30. A, D, G, and J: Cytochrome oxidase histochemistry. B, E, H, and K: Immunolabeled with CS-56 antibody. C, F, I, and L: Immunolabeled with antibody to 5-HTT. Cytochrome oxidase histochemistry reveals the barrel pattern at all ages examined. Adjacent tangential sections stained for chondroitin sulfate proteoglycans (CS-56) and serotonin transporter (5HTT) show transient expression in barrel septa and hollows respectively from P6 to P7. Scale bars 100 μm in A–D and 50 μm E–L.