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Research Report

Expression of chondroitin sulfate proteoglycans in barrel field of mouse and rat somatosensory cortex

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ABSTRACT

Chondroitin sulfate proteoglycans (CSPGs) consist of chondroitin sulfate (CS) glycosaminoglycans (GAGs) and core protein and regulate the migration, axonal outgrowth, and synaptogenesis in mammalian brains. In the present study, we investigated the localization of CSPGs, the effects of sensory deprivation on the density of perineuronal nets (PNNs), and the effects of chondroitinase ABC (Chase) on the formation of barrel structures in the posterior medial barrel subfield (PMBSF). In developing mouse and rat brains, the immunoreactivity of chondroitin-6-sulfate containing proteoglycan (CS-6-PG), phosphacan, and neurocan was stronger at barrel septa as compared with barrel hollows and surrounding cortex, while the labeling of *Wisteria floribunda* agglutinin (WFA) was observed at barrel hollows. In adult brains, CS-6-PG-immunoreactive and WFA-labeled PNNs were observed mainly at barrel hollows of mouse, but they were seen chiefly at barrel septa of rats. Sensory deprivation of facial vibrissae reduced the number of WFA-labeled PNNs at barrel hollows but not at barrel septa. Intracerebral injection of Chase did not affect the formation of barrel structures in the PMBSF. These data indicate species-dependent heterogeneity of CSPG expression and activity-dependent formation of PNNs in the PMBSF, but CS GAGs have no crucial function in constructing the barrel structures during early postnatal development.

1. Introduction

The rodent somatosensory system is an excellent model to study neuronal pattern formation and neuronal plasticity (Fox, 1992; Frostig, 2006; for review, see Peterson, 2007). In the primary somatosensory cortex, periphery-related patterns of sensory receptors are recapitulated in layer IV as an array of multineuronal structures called “barrels” (Woolsey and Van der Loos, 1970). In barrels, neurons are arranged in cylindrical or oval-shaped rings that are separated from

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Abbreviations: Chase, chondroitinase ABC; ConA, concanavalin A; CO, cytochrome oxidase; CS, chondroitin sulfate; CSPGs, chondroitin sulfate proteoglycans; CS-6-PG, chondroitin-6-sulfate containing proteoglycan; ECM, extracellular matrix molecules; GAG, glycosaminoglycan; GFAP, glial fibrillar acidic protein; NeuN, neuron-specific nuclear antigen; PMBSF, posterior medial barrel subfield; PN, postnatal day; PNNs, perineuronal nets; PNA, peanut agglutinin; VVA, *Vicia villosa* agglutinin; WFA, *Wisteria floribunda* agglutinin; WGA, wheat germ agglutinin

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neighbors by hypocellular "septa". Those cell-dense walls surround cell-sparse "hollows" containing thalamocortical afferent terminals (Woolsey and Van der Loos, 1970). The removal of sensory information by whisker follicle cauterization or denervation induces a disorganization of structural barrel pattern. These barrel structures are consolidated during the first few days (critical period) through somatosensory inputs from whiskers, so that the removal of sensory information has little effect upon the organization of barrel structures over postnatal day 5 (PN5) (Van der Loos and Woolsey, 1973; Woolsey and Wann, 1976; Wong-Riley and Welt, 1980; Schlaggar et al., 1993). The organization of barrel structures is primarily determined by a genetic program such as FGF-8 as well as the general patterning of the neocortex (Fukuichi-Shimogori and Grove, 2001). Moreover, refinement of barrel structures is likely to be guided by activity-dependent mechanisms, since barrel structures are less clearly defined or absent in mice with genetic knockout of several genes such as NMDA receptor (Iwasato et al., 2000), phospholipase C β1 (Hannan et al., 2001), adenyl cyclase 1 (Welker et al., 1996), and monoamine oxidase A (Cases et al., 1996).

The extracellular matrix (ECM) molecules are involved in cell–substrate interactions during morphogenesis of the nervous system in developing brains and plastic modifications of neuronal connectivity in adult brains. Chondroitin sulfate proteoglycans (CSPGs) have been recognized as important constituents of the brain's ECM and are considered to participate in neural network formation (for reviews, see Bovolenta and Fernaud-Espinosa, 2000; Galtrey and Fawcett, 2007). CSPGs consist of a core protein and sulfated glycosaminoglycans (GAGs) (Bandtlow and Zimmermann, 2000; Yamaguchi, 2000) and are generally known to inhibit neurite outgrowth (Oohira et al., 1991; Katoh-Senba et al., 1995). Moreover, recent studies have shown that CSPGs are responsible for structural plasticity in adult brains such as functional recovery after spinal cord injury (Bradbury et al., 2002), axonal sprouting of cerebellar Purkinje neurons (Corvett and Rossi, 2005), and reactivation of ocular dominance plasticity in the visual cortex (Pizzorusso et al., 2002, 2006).

Perineuronal nets (PNNs) are a specialized form of ECM that appears at the end of the critical period (Celio et al., 1998). PNNs have been visualized using a variety of methods including the labeling of lectins such as *Wisteria floribunda* agglutinin (WFA) and *Vicia villosa* agglutinin (VVA) that have high affinities to *N*-acetylgalactosamine (Härtig et al., 1992; Brückner et al., 1993), the binding of hyaluronic acid binding protein to hyaluronic acid (Brückner et al., 1998, 2000), and immunological staining for CSPGs (Brückner et al., 1998, 2000). PNNs are frequently seen around parvalbumin-immunoreactive GABAergic interneurons in the cerebral cortex (Brückner et al., 1994; Brückner and Grosche, 2001). During postnatal development, PNNs are first detected at the onset of the period during which the pattern of neuronal activity determines the mature synaptic circuitry and neuronal phenotype in the visual cortex (Köppe et al., 1997; Brückner et al., 2000). It is also shown that dark-rearing from birth prolongs the duration of the critical period and attenuates the expression of several CSPGs (Lander et al., 1997; Pizzorusso et al., 2002). PNNs are well preserved and maintained for 3–10 weeks in slice culture, indicating that the differentiation of PNNs is part of the developmental program maintained in brain tissue *in vitro* (Brückner and Grosche, 2001). It is also shown that the formation of PNNs in brain slice culture is promoted via calcium signaling (Brückner and Grosche, 2001). More recently, we have demonstrated that neurons themselves are able to construct a PNNs structure in dissociated cortical culture without glial cells (Miyata et al., 2005, 2007), and later this was confirmed in hippocampal neuron culture (John et al., 2006).

Various ECM molecules are shown to be expressed specifically in the somatosensory barrel cortex. Lectin histochemistry has revealed that lectin binding is absent from barrel hollows in the early postnatal period using peanut agglutinin (PNA), concanavalin A (ConA), and wheat germ agglutinin (WGA) (Cooper and Steindler, 1986; Steindler et al., 1995). It has been demonstrated that CSPGs such as neurocan and DSD-1-PG are also absent from barrel hollows in the early developing brains (Watanabe et al., 1995; Steindler et al., 1995). Tenascin-C, which is able to interact with CSPGs, is shown to be expressed predominantly at barrel septa, but tenascin-C-deficient mice show normal distribution of DSD-1-PG immunoreactivity and PNA labeling (Steindler et al., 1995; Mitrovic et al., 1996; Cybulska-Klosowicz et al., 2004). A recent study has shown that trimming of facial vibrissae attenuates the expression of Cat-315-immunoreactive aggrecan without changing the number of WFA-labeled PNNs (McRae et al., 2007). Moreover, the deprivation of sensory information from facial vibrissae decreases the number of VVA-labeled PNNs in the PMBSF (Bahia et al., 2008).

At present, however, the functional significance of CSPGs in pattern formation of barrel structures is not completely understood. In the present study, therefore, we examined the spatiotemporal expression of CS GAGs and major CSPGs such as phosphacan and neurocan in the PMBSF of the somatosensory cortex using rats and mice to show the heterogeneity of CSPG expression. Furthermore, we investigated the effects of sensory deprivation of facial vibrissae on

Fig. 1 – Tangential sections showing the histochemical staining of CO and red-fluorescence Nissl and the immunohistochemical staining of NeuN and GFAP in the PMBSF of the somatosenosry cortex using mice and rats. (A–D) In PN10 and adult mice, barrel structures were clearly observed by CO and Nissl stainings and NeuN immunostaining at low magnification view and each barrel hollow was seen clearly by double labeling of Nissl staining and NeuN immunostaining at high magnification view. (E–E″) In rats, barrel structures were clearly observed by CO staining, but each barrel hollow was not clearly seen by NeuN immunostaining and Nissl staining. (F–K′) The immunohistochemistry revealed that aggregates (arrowheads) of GFAP-positive astrocytes distributed in the PMBSF of mice and rats in spite of uniform distribution of them at surrounding cortex (nonbarrel cortex). Scale bars = A and C (500 μm), B, D, and E–K (100 μm).