IBRO Reports 4 (2018) 22–37

Contents lists available at ScienceDirect

IBRO Reports

journal homepage: http://www.journals.elsevier.com/ibro-reports

Research Paper

Expression of aggrecan components in perineuronal nets in the mouse cerebral cortex

Hiroshi Ueno[a,b,c,*], Kazuki Fujii[c,d], Shunsuke Suemitsu[e], Shinji Murakami[e], Naoya Kitamura[e], Kenta Wani[e], Shozo Aoki[e], Motoi Okamoto[b], Takeshi Ishihara[e], Keizo Takao[c,d]

[a] Department of Medical Technology, Kawasaki University of Medical Welfare, Okayama, 701-0193, Japan
[b] Department of Medical Technology, Graduate School of Health Sciences, Okayama University, Okayama, 700-8558, Japan
[c] Life Science Research Center, University of Toyama, Toyama, 930-0194, Japan
[d] Department of Behavioral Physiology, Graduate School of Innovative Life Science, University of Toyama, Toyama, 930-0194, Japan
[e] Department of Psychiatry, Kawasaki Medical School, Okayama, 701-0192, Japan

ARTICLE INFO

Article history:
Received 2 September 2017
Accepted 27 January 2018

Keywords:
Aggrecan
Brain region-specific
Chondroitin sulfate proteoglycan
Extracellular matrix
Perineuronal nets
Plasticity

ABSTRACT

Specific regions of the cerebral cortex are highly plastic in an organism's lifetime. It is thought that perineuronal nets (PNNs) regulate plasticity, but labeling for Wisteria floribunda agglutinin (WFA), which is widely used to detect PNNs, is observed throughout the cortex. The aggrecan molecule—a PNN component—may regulate plasticity, and may also be involved in determining region-specific vulnerability to stress. To clarify cortical region-specific plasticity and vulnerability, we qualitatively analyzed aggrecan-positive and glycosylated aggrecan-positive PNNs in the mature mouse cerebral cortex. Our findings revealed the selective expression of both aggrecan-positive and glycosylated aggrecan-positive PNNs in the cortex. WFA-positive PNNs expressed aggrecan in a region-specific manner in the cortex. Furthermore, we observed variable distributions of PNNs containing WFA- and aggrecan-positive molecules. Together, our findings suggest that PNN components and their function differ depending on the cortical region, and that aggrecan molecules may be involved in determining region-specific plasticity and vulnerability in the cortex.

1. Introduction

To preserve region-specific functions, certain areas of the cortex are associated with high and low plasticity over the course of development (Craig and Commins, 2006; Kolb, 2009). One molecule that is crucial in maintaining cortical plasticity is the perineuronal net (PNN), a highly condensed extracellular matrix (ECM) molecule in the central nervous system (Sorg et al., 2016). The PNN is a mesh-like structure that surrounds the cell body, proximal dendrites, and axonal initial segment of specific neurons. Approximately 15% of neurons in the mature brain are surrounded by PNNs (Guimarães et al., 1990; McRae et al., 2007), most of which are parvalbumin (PV)-positive GABAergic interneurons, and a small portion of which are pyramidal cells (Härtig et al., 1994; Wegner et al., 2003).

Maturation of GABAergic circuitry in the sensory cortex implies the onset of a critical period that is associated with cortical plasticity (Hensch and Fagiolini, 2005; Maffei and Turrigiano, 2008). The formation of PNNs around PV-positive interneurons in the sensory cortex indicates the end of this so-called critical period (Pizzorusso et al., 2002; McRae et al., 2007). In the visual cortex of

Abbreviations: FrA, frontal association cortex; DLO, dorsolateral orbital cortex; LO, lateral orbital cortex; VO, ventral orbital cortex; Cg, cingulate cortex; PL, prelimbic cortex; IL, infralimbic cortex; DP, dorsal peduncular cortex; M1, primary motor cortex; M2, secondary motor cortex; MPtA, medial parietal association cortex; LPtA, lateral parietal association cortex; S1Tr, primary somatosensory cortex–trunk region; S1BF, primary somatosensory cortex–barrel field; S2, secondary somatosensory cortex; V2MM, secondary visual cortex–mediomedial area; V2ML, secondary visual cortex mediolateral area; V1M, primary visual cortex monocular area; V1B, primary visual cortex binocular area; V2L, secondary visual cortex lateral area; Au1, primary auditory cortex; AuD, secondary auditory cortex dorsal area; AuV, secondary auditory cortex ventral area; TeA, temporal association cortex; Ect, ectorhinal cortex; PRh, perirhinal cortex; DIEnt, dorsintermed entorhinal cortex; DLEnt, dorsolateral entorhinal cortex; RSD, retrosplenial dysgranular cortex; RSGc, retrosplenial granular cortex c region; RSGb, retrosplenial granular cortex b region; RSGa, retrosplenial granular cortex a region.

* Corresponding author at: Department of Medical Technology, Kawasaki University of Medical Welfare, 288, Matsushima, Kurashiki, Okayama, 701-0193, Japan.
E-mail addresses: dhe422007@s.okayama-u.ac.jp (H. Ueno), kfujii@cts.u-toyama.ac.jp (K. Fujii), ssue@med.kawasaki-m.ac.jp (S. Suemitsu), muraka@med.kawasaki-m.ac.jp (S. Murakami), n-kitamura@med.kawasaki-m.ac.jp (N. Kitamura), kenta99101@yahoo.co.jp (K. Wani), shoaoki@med.kawasaki-m.ac.jp (S. Aoki), mokamoto@md.okayama-u.ac.jp (M. Okamoto), t-ishihara@med.kawasaki-m.ac.jp (T. Ishihara), takao@cts.u-toyama.ac.jp (K. Takao).

https://doi.org/10.1016/j.ibror.2018.01.002

the mature brain, it has been shown that treatment of PNNs with the enzyme, chondroitinase ABC, can restore plasticity (Pizzorusso et al., 2002). Similar mechanisms have been described for sensory input-dependent plasticity in other brain regions (Balmer et al., 2009; Gogolla et al., 2009).

Although the detailed function of PNNs is not clear, it is thought that their main roles involve neural plasticity, synaptic stability, and neuroprotective function (Sorg et al., 2016). The main constituents of PNNs include hyaluronic acid, tenascin-R, and the lectican family of chondroitin sulfate proteoglycans (CSPGs) (i.e., aggrecan, versican, brevican, and neurocan) (Bandtlow and Zimmermann, 2000; Yamaguchi, 2000). The plant-derived lectin, *Wisteria floribunda agglutinin* (WFA), has been widely used to detect PNNs through binding of *N*-acetylgalactosamine (Brückner et al., 1993; Schweizer et al., 1993; Seeger et al., 1994; Giamanco et al., 2010). Another method used to detect PNNs is through antibodies against aggrecan, the main PNN component (Matthews et al., 2002). These antibodies include AB1031 and Cat-315, with the former recognizing the central protein domain of the chondroitin sulfate glycosaminoglycan binding region of aggrecan (Giamanco et al., 2010; Lendvai et al., 2013), and the latter recognizing the HNK-1 carbohydrate epitope of aggrecan (Matthews et al., 2002; Dino et al., 2006; McRae et al., 2007).

While there is general agreement that PNNs regulate plasticity, WFA-labeled PNNs have been found throughout the cortex of the mature mouse (Brückner et al., 2000; Horii-Hayashi et al., 2015). Moreover, WFA-positive PNN labeling does not appear to vary over development (Horii-Hayashi et al., 2015). Considering that the plasticity of specific brain regions is highly variable over the span of an organism's lifetime, it is unlikely that WFA-positive PNNs control plasticity. Some studies have suggested the possibility that aggrecan molecules regulate plasticity as, during postnatal development, aggrecan expression is delayed when sensory input is deprived (McRae et al., 2007; Ye and Miao, 2013; Ueno et al., 2017b). Furthermore, when mice are housed in enriched environments after experimental cerebral ischemia, Cat-315-positive PNNs decrease (Madinier et al., 2014). In fact, it has been suggested that aggrecan molecules are not ubiquitously expressed throughout the cortex (Morawski et al., 2012a, 2012b; Ueno et al., 2017a). However, a quantitative analysis of aggrecan-positive PNNs in the cortex has not been conducted.

Along with their possible role in developmental plasticity, it has been suggested that aggrecan molecules are necessary for mediating the neuroprotective function of PNNs (Suttkus et al., 2014). It is well-established that certain brain regions are more susceptible to damage in neuropsychiatric disorders and neurodegenerative diseases. Interestingly, postmortem studies of patients with schizophrenia and autism show selective PNN abnormalities in the prefrontal and entorhinal cortices (Pantazopoulos et al., 2010; Mauney et al., 2013; Berretta et al., 2015). One theory explaining the cause of neuropsychiatric disorders is oxidative stress, which aggrecan-positive PNNs show resistance to (Gawryluk et al., 2011; Cabungcal et al., 2013; Suttkus et al., 2014). Indeed, in Alzheimer's disease, aggrecan-expressing PNNs are less susceptible to tau protein-induced damage (Morawski et al., 2010). It is therefore possible that aggrecan molecules are involved in neurological disorders, which target specific brain regions.

In this study, we focused on the quantitative measurement of aggrecan-positive PNNs and glycosylated aggrecan-positive PNNs in the mature mouse cortex. We examined the region-specific presence of aggrecan using three antibodies (i.e., AB1031, Cat-315, and Cat-316) that recognize different components of the aggrecan molecule (McRae et al., 2007, 2010; Foster et al., 2014; Madinier et al., 2014; Suttkus et al., 2014; Carstens et al., 2016; Morikawa et al., 2017). Note that Cat-316 recognizes the *o*-linked chondroitin sulfate epitope of aggrecan (Lander et al., 1997; Matthews et al., 2002). We believe that our findings will contribute to clarifying the state of region-selective vulnerability and plasticity in the cortex of individuals with neuropsychiatric disorders.

2. Materials and methods

2.1. Animals

Five adult male mice (C57BL/6J) were used for these experiments. Animals were purchased from Charles River Laboratories (Kanagawa, Japan), and housed in cages (3–5 animals per cage) with food and water available *ad libitum* under a 12 h light/dark cycle at 23–26 °C. All efforts were made to minimize the number of animals used and their suffering. All experimental protocols were performed in accordance with the U.S. National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised in 1996), and were approved by the Committee for Animal Experiments at Kawasaki Medical School Advanced Research Center and the Institutional Animal Care and Use Committee of University of Toyama.

2.2. Tissue preparation

For tissue preparation, animals were deeply anesthetized with a lethal dose of sodium pentobarbital (120 mg/kg, i.p.), and transcardially perfused, first with ice-cold phosphate buffered saline (PBS) for 2 min and then with 4% paraformaldehyde in PBS (pH 7.4) for 10 min (10 ml/min). Brains were dissected and postfixed overnight with 4% paraformaldehyde in PBS at 4 °C, and cryoprotected by immersion in 15% sucrose for 12 h followed by 30% sucrose for 20 h at 4 °C. Brains were frozen in O.C.T. Compound (Tissue-Tek; Sakuma Finetek, Tokyo, Japan) using dry ice-cold normal hexane, and serial coronal sections of 40-μm thickness were prepared using a cryostat (CM3050S; Leica Wetzlar, Germany) at −20 °C. Sections were collected in ice-cold PBS containing 0.05% sodium azide.

2.3. Immunohistochemistry

Cryostat sections were treated with 0.1% Triton X-100 in PBS at 20 °C for 15 min. After three washes in PBS, sections were incubated with 10% normal goat serum (ImmunoBioScience Corp, WA, USA) in PBS at room temperature for 1 h, washed three time in PBS, and incubated overnight at 4 °C in PBS containing biotinylated WFA (B-1355, Vector Laboratories, Funakoshi Co., Tokyo, Japan; 1:200) and primary antibodies (described below). After washing in PBS, sections were incubated with corresponding secondary antibodies (indicated below) and streptavidin-conjugated Texas Red (SA-5006, Vector Laboratories) at room temperature for 2 h. Labeled sections were rinsed again with PBS and mounted on glass slides with Vectashield medium (H-1400, Vector Laboratories). Prepared slides were either immediately imaged or stored at 4 °C.

2.4. Antibodies

The following primary antibodies were used for staining: rabbit anti-aggrecan (AB1031, Millipore, Tokyo, Japan; 1:200), mouse anti-aggrecan (Cat-315; MAB1581, Millipore, 1:1000), or mouse anti-aggrecan (Cat-316; MAB1582, Millipore, 1:10 000). The following secondary antibodies were used for visualization: Alexa Fluor 488-conjugated goat anti-rabbit IgG (ab150077, Abcam; Cambride, MA; 1:1000) or FITC-conjugated anti-mouse IgM (sc-2082, Santa Cruz, Texas, USA, 1:1000).

2.5. Microscopy imaging

For the quantification of WFA-, aggrecan-, Cat-315-, and Cat-316-positive PNNs, sections were stained as described previously