Hearing Research 265 (2010) 15–24

Contents lists available at ScienceDirect

Hearing Research

journal homepage: www.elsevier.com/locate/heares

Research paper

Distribution of perineuronal nets in the human superior olivary complex

Elise Schmidt, Thomas P. Wolski Jr., Randy J. Kulesza Jr. *

Auditory Research Center, Lake Erie College of Osteopathic Medicine, Erie, PA 16509, USA

ARTICLE INFO

Article history:
Received 22 July 2009
Received in revised form 17 March 2010
Accepted 17 March 2010
Available online 20 March 2010

ABSTRACT

Perineuronal nets (PNNs) are specialized assemblies of chondroitin sulfate proteoglycans (CSPGs) in the central nervous system that form a lattice-like covering over the cell body, primary dendrites and initial axon segment of select neuronal populations. PNNs appear to play significant roles in development of the central nervous system, neuronal protection, synaptic plasticity and local ion homeostasis. In seven human brainstems (average age = 81 years), we have utilized *Wisteria floribunda* (WFA) histochemistry and immunocytochemistry for CSPG to map the distribution of PNNs within the nuclei of the human superior olivary complex (SOC). Within the SOC, the majority of net-bearing neurons are situated in the most medially situated nuclei, especially the superior paraolivary nucleus and medial nucleus of the trapezoid body. Net-bearing neurons are consistently found in the ventral nucleus of the trapezoid body and posterior periolivary nucleus, but to a lesser extent in the lateral nucleus of the trapezoid body. Finally, perineuronal nets are typically absent from the lateral and medial superior olives.

1. Introduction

Perineuronal nets (PNNs) are composed from the lectican family of CSPGs and constitute a specialized condensation of the extracellular matrix (ECM) in the central nervous system (Härtig et al., 1995; Wintergerst et al., 1996; Yamaguchi, 2000; Viapiano and Matthews, 2006; Pantazopoulos et al., 2008). PNNs exist as an extracellular reticulation, forming a glove-like covering of the soma, dendrites and initial axon segment of select neuronal populations, leaving gaps for synaptic contacts (Hockfield and McKay, 1983; Celio et al., 1998; Yamamoto et al., 1988; Atoji et al., 1989; Brückner et al., 1993; Wagoner and Kulesza, 2009). PNNs appear to function in a multitude of physiological processes (Celio et al., 1998; Morris and Henderson, 2000; Dityatev and Schachner, 2003). During the embryonic period, lecticans function in cell adhesion and direct migration of neurons and their neurites (reviewed by Yamaguchi, 2000). In the post-embryonic period, the formation of PNNs coincides with the development of synaptic maturity and the close of the critical period (Guimarães et al., 1990; Friauf, 2000; Pizzorusso et al., 2002; Galtrey and Fawcett, 2007; Rauch, 2004; McRae et al., 2007). In the mature brain, PNNs function to promote synaptic stability and prevent plasticity; injury or digestion of PNNs has been shown to reactivate plasticity (Sur et al., 1988; Hockfield et al., 1990; Hockfield, 1993; Pizzorusso et al., 2002; Dityatev and Schachner, 2003; Galtrey and Fawcett, 2007; Kwok et al., 2008). PNNs also function in ion homeostasis, where the well-hydrated ECM provides a local anionic environment and can modulate voltage-sensitive ion channels (Brückner et al., 1993; Snow et al., 1994; Härtig et al., 1999, 2001). Further, PNNs appear to reduce oxidative stress and protect certain neuronal populations against neurodegenerative disease (Reinert et al., 2003; Fiedler et al., 2007; Miyata et al., 2007). Recently, it has been shown that environmental enrichment during the early post-natal period expedites the development of PNN in contextually dependant regions of the brain (Simonetti et al., 2009). PNNs can be positively identified via numerous histo- and immunocytochemical techniques, but are only associated with specific neuronal populations and comparative studies provide evidence indicating species-specific distributions of PNNs (Nakagawa et al., 1986; Brückner et al., 1993; Wintergerst et al., 1996; Atoji et al., 1997; Celio et al., 1998; Morris and Henderson, 2000; Härtig et al., 2001; Cant and Benson, 2006; Deepa et al., 2006; Hilbig et al., 2007; Pantazopoulos et al., 2008; Wagoner and Kulesza, 2009; Morawski et al., 2009).

The superior olivary complex (SOC) is a conglomeration of nuclei within the lower brainstem tegmentum that functions in localization of sound sources, encoding temporal features of sound and

Abbreviations: CN, cochlear nucleus; CSPG, chondroitin sulfate proteoglycan; Ctt, central tegmental tract; ECM, extracellular matrix; FN, facial nucleus; L, lateral; LNTB, lateral nucleus of the trapezoid body; LSO, lateral superior olive; M, medial; Ml, medial lemniscus; MNTB, medial nucleus of the trapezoid body; MSO, medial superior olive; P, posterior; PNN, perineuronal net; PPO, posterior periolivary nucleus; SOC, superior olivary complex; SPON, superior paraolivary nucleus; tz, trapezoid body; VCN, ventral cochlear nucleus; VNTB, ventral nucleus of the trapezoid body; WFA, *Wisteria floribunda* agglutinin

* Correspondence to: Randy J. Kulesza, Auditory Research Center, Lake Erie College of Osteopathic Medicine, 1858 West Grandview Boulevard, Erie, PA 16509, USA. Tel.: +1 814 866 8423; fax: +1 814 866 8411.
E-mail address: rkulesza@lecom.edu (R.J. Kulesza).

0378-5955/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.heares.2010.03.077

descending modulation of the cochlear nucleus and cochlea (see reviews by Heffner and Masterton, 1990; Spangler and Warr, 1991; Schwartz, 1992; Thompson and Schofield, 2000; Oliver, 2000). Recently, we have described the organization of the human SOC and provide quantitative evidence that this brainstem center is, in terms of neuronal architecture, similar to what has been described for laboratory animals (e.g. gerbil, cat, rhesus; see Fig. 1; Heffner and Masterton, 1990; Helfert et al., 1991; Schwartz, 1992; Thompson and Schofield, 2000; Oliver, 2000; Hilbig et al., 2007; Kulesza, 2007, 2008). Despite these similarities, many questions still remain regarding function, connectivity and neurochemical profile of neurons in the human SOC.

PNNs have been associated with specific neuronal subtypes in the auditory brainstem nuclei of rat, gerbil, hedgehog, dog, rhesus and human (rat – Friauf, 2000; Härtig et al., 2001; gerbil – Lurie et al., 1997; Cant and Benson, 2006; hedgehog – Morawski et al., 2009; dog – Atoji and Suzuki, 1992; Atoji et al., 1990, 1995, 1997; rhesus – Hilbig et al., 2007; human – Wagoner and Kulesza, 2009). Furthermore, we recently reported that PNNs are restricted to select populations of neurons within the human ventral cochlear nucleus (VCN). Thus, our objective here is to map the location of PNNs within the human SOC and to correlate these results with our previous findings of PNN in the cochlear nucleus.

2. Materials and methods

2.1. Fixation

This report is based on the study of seven human brainstems (six female and one male) from individuals ranging in age from 72 to 94 years of age. Brainstems were obtained from donated cadavers and our IRB approved all procedures. Cadavers were perfused through the right common carotid artery with an embalming solution (King Chemical Inc., St. Louis, MO) and the brain was dissected from the skull. Brainstems were trimmed, bisected and immersed in a buffered solution of 4% paraformaldehyde at 4 °C for at least 1 week. Brainstems were only included in our investigation when: (1) the cause of death was not neurological or metastatic cancer, (2) brains showed no gross signs of degenerative disease, (3) embalming occurred within 24 h of death and (4) there was no evidence of brainstem pathology, trauma or vascular compromise.

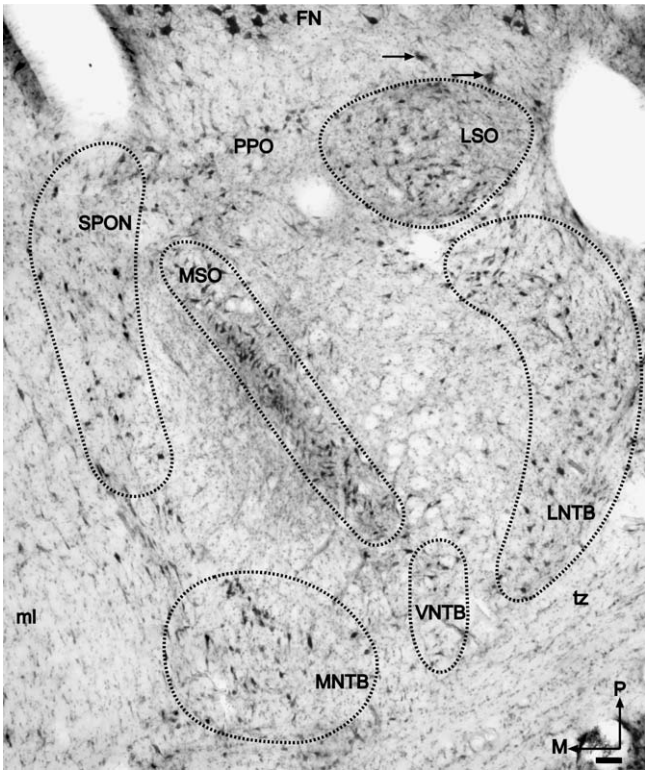

Fig. 1. Organization of the human SOC. This figure shows a Giemsa-stained section through the middle-third of the human SOC; the main SOC nuclei are indicated by dashed lines (as per Kulesza 2007, 2008). There are a number of neuronal profiles situated outside the main nuclear boundaries, especially between the SPON and MNTB. The arrows posterior to the LSO indicate peri-LSO neurons. The scale bar equals 100 μm; magnification = 72×.

2.2. Identification of perineuronal nets

Brainstem blocks, extending from the vestibulocochlear nerve root to the trigeminal nerve root, were cryoprotected in a 30% sucrose solution until saturated (>7 days at 4°C), sectioned on a freezing microtome at a thickness of 40 μm and collected in 0.1 M sodium phosphate buffer. For each brain, an alternating series of sections were stained for Nissl substance with Giemsa for reference (as per our previous reports; Kulesza, 2007, 2008). PNNs were identified by specific binding of biotinylated *Wisteria floribunda* agglutinin (WFA) and an antibody directed against chondroitin sulfate proteoglycans (CSPGs) as described below (WFA – Vector Laboratories; Pantazopoulos et al., 2008; CSPG – anti-CSPG, MAB1581, Millipore). All of the following steps were done at room temperature with agitation. Free-floating tissue sections were rinsed in 0.1 M phosphate buffer, endogenous peroxidase activity was quenched in a solution of 3% hydrogen peroxide, tissue was permeablized in a solution of 5% triton and non-specific binding was blocked with a 1-h incubation in 1% normal donkey serum (NDS). For WFA, sections were incubated for 15–20 h, free-floating, in a solution containing between 5 and 20 μg/ml biotinylated WFA. For CSPG immunocytochemistry, free-floating sections were incubated overnight in a 1:2000 dilution of anti-CSPG, 1% NDS. Sections were washed in phosphate buffer and then incubated for 60 min in a 1:200 solution of biotinylated goat anti-mouse (Millipore). Both WFA and CSPG sections were then rinsed in phosphate buffer and incubated for at least 1 h in ABC solution (Vector Laboratories, Elite Kit). Following this incubation, sections were rinsed in phosphate buffer, then 0.05 M Tris and the chromogenic reaction was developed by incubation in 0.05% diaminobenzidine, 0.01% hydrogen peroxide with heavy metal intensification (Adams, 1981). Tissue sections processed without WFA or anti-CSPG failed to reveal any labeling. Sections were mounted onto glass slides from gelatin alcohol, dried, dehydrated, cleared and coverslipped. Alternating series of sections processed for PNNs were counterstained for Nissl substance with Giemsa or neutral red.

2.3. Morphological analysis

Tissue sections were examined with an Olympus BX45 microscope and photographed with an Olympus DP12 digital camera. Some of the figures included in this report are montages digitally assembled in Photoshop 7.0 (Adobe Systems, Inc.). All figures were subjected to manipulations of contrast and brightness in Photoshop 7.0 and figure labeling was accomplished in Freehand MX 11. For morphometric analyses, neurons were systematically sampled throughout the rostro-caudal extent of each nucleus. Cell bodies (including nuclei) were traced at a magnification of 500× while focusing (to most accurately determine the cell body contour) with the aid of a camera lucida attachment (Olympus). Grayscale tracings were analyzed using ImageJ software (calibrated to a standard scale bar; ImageJ is available at http://rsb.info.nih.gov/ij/). An index of circularity was calculated for each contour using the following equation: