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AGGRECAN-BASED EXTRACELLULAR MATRIX IS AN INTEGRAL PART OF THE HUMAN BASAL GANGLIA CIRCUIT

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Abstract—The extracellular matrix is known to be involved in neuronal communication and the regulation of plastic changes, and also considered to protect neurons and synapses against damage. The goal of this study was to investigate how major extracellular matrix components (aggrecan, link protein, hyaluronan) constitute the pathways of the nigral system in the human basal ganglia circuit affected by neurodegeneration in Parkinson's disease. Here we show that aggrecan- and link protein–related components form clear regional distribution patterns, whereas hyaluronan is widely distributed in gray and white matter. Two predominant phenotypes of the aggrecan-based matrix can be discriminated: (1) perineuronal nets (PNs) and (2) axonal coats (ACs) encapsulating preterminal fibers and synaptic boutons. Clearly contoured PNs are associated with GABAergic projection neurons in the external and internal division of the globus pallidus, the lateral and reticular part of the substantia nigra, as well as subpopulations of striatal and thalamic inhibitory interneurons. Dopaminergic nigral neurons are devoid of PNs but are contacted to a different extent by matrix-coated boutons forming subnucleus-specific patterns. A very dense network of ACs is characteristic especially of the posterior lateral cell groups of the compact substantia nigra (nigrosome 1). In the subthalamic nucleus and the lateral thalamic nuclei numerous AC-associated axons were attached to principal neurons devoid of PNs. We conclude from the region-specific patterns that the aggrecan-based extracellular matrix is adapted to the fast processing of sensorimotor activities which are the therapeutic target of surgery and deep brain stimulation in the treatment of advanced stages of Parkinson's disease.

Key words: basal ganglia, perineuronal net, chondroitin sulfate proteoglycan, synapse, Parkinson's disease.

The extracellular matrix is an integral part of the neural tissue. Aggregating proteoglycans, complexed with hyaluronan, tenascins and link proteins form a macromolecular scaffold organized in region and cell type–dependent patterns (Delpech et al., 1989; Brückner et al., 1993; Asher et al., 1995; Yamaguchi, 2000; Viapiano and Matthews, 2006; Carulli et al., 2007). The most conspicuous form of extracellular matrix organization is the perineuronal nets (PNs) associated with many types of neurons in the brain and spinal cord. Different proportions of hyaluronan and aggregating proteoglycans, the predominance of certain glycosylation forms of proteoglycans, as well as the degree of sulfation may influence the physicochemical properties of the extracellular space, e.g. diffusion parameters, ion buffering capacity or binding properties for a variety of regulatory factors (Syková, 2004). It is therefore reasonable to assume that the cellular microenvironment is adjusted to major functional properties of neurons, such as their firing patterns, as well as to the plasticity of synapses and the sprouting capacity of axons retained in the adult state (Dityatev and Schachner, 2003; Rauch, 2004; Viapiano and Matthews, 2006; Galtrey and Fawcett, 2007). Moreover, the extracellular matrix may protect neurons against damage induced by oxidative stress and other environmental factors (Morawski et al., 2004, 2005).

The highly specified extracellular matrix scaffold in the normal brain suggests that a disturbed matrix integrity can severely affect the physiological properties and the plastic capacity of individual neurons and complex neuronal circuits. It has been shown that dramatic changes can be related to the additional expression of matrix components in glial scars in response to acute brain injuries, such as stroke (Hobohm et al., 2005) and spinal cord injury (Silver and Miller, 2004; Galtrey et al., 2007; Massey et al., 2007). In contrast, a long-lasting decomposition of the extracellular matrix scaffold may occur in chronic pathological states such as viral infections (Belichenko et al., 1997, 1999; Medina-Flores et al., 2004; Vidal et al., 2006). An accelerating influence of extracellular matrix components, especially of heparan sulfate proteoglycans, in the Alzheimer's disease amyloidopathy has also been suggested (van Horssen et al., 2003).

Studies on the organization of the extracellular matrix in human brain regions most frequently affected in neurodegenerative diseases have been focused on the cerebral cortex (Belichenko et al., 1997, 1999; Brückner et al., 1999; Baig et al., 2005) and basal forebrain (Adams et al., 2001). In the substantia nigra complex, despite its central role in Parkinson's disease (PD), knowledge is restricted to the detection of hyaluronan (Yasuhara et al., 1994). Our studies in the rat (Hobohm et al., 1998) and the Chilean mouse opossum (Brückner et al., 2006), using lectin staining and chondroitin sulfate proteoglycan (CSPG) antibod-

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Abbreviations: AC, axonal coat; BHABP, biotinylated hyaluronic acid-binding protein; CRTL1, cartilage-related aggrecan-binding link protein; CSPG, chondroitin sulfate proteoglycan; DAB, diaminobenzidine; DAB-Ni, nickel-enhanced diaminobenzidine; GAD, glutamic acid decarboxylase; PBS, phosphate-buffered saline; PD, Parkinson's disease; PN, perineuronal net; TH, tyrosine hydroxylase.

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ies, revealed region-specific patterns of extracellular matrix organization in the substantia nigra complex. Notably, the absence of PNs around dopaminergic neurons in the substantia nigra and the ventral tegmental area appeared to be a phylogenetically conserved principle.

The general goal of the present study was to analyze whether the extracellular matrix contributes to region-specific properties of the basal ganglia circuit. Specifically, we intended to investigate if neurons known to be affected by degeneration in PD, the dopaminergic nigral neurons and cholinergic neurons of the tegmental pedunculopontine nucleus, differ with respect to extracellular matrix organization from neurons that are relatively resistant to degeneration in PD (Hirsch et al., 1987; van Domburg and ten Donkelaar, 1991; Hardman et al., 1996, 1997; Ma et al., 1996; Hirsch, 1999; Pahapill and Lozano, 2000; Jellinger, 2002; Halliday et al., 2005; Wakabayashi et al., 2006; DeLong and Wichmann, 2007). Therefore, we focused our study on major intrinsic components of the basal ganglia circuit, including the compact part of the substantia nigra, the ventral tegmental area, the lateral and basal part of the reticular substantia nigra, the external and internal division of the globus pallidus, the caudate nucleus and putamen, the subthalamic nucleus, as well as the thalamus and the tegmental pedunculopontine nucleus receiving basal ganglia output signals. The data may have possible clinical relevance to the pharmacological treatment of PD. They may also be considered in the discussion of functional benefits induced by invasive therapies, since the globus pallidus, the subthalamic nucleus, the motor thalamus and the pedunculopontine nucleus are the preferred interventional targets in severe cases of PD (Hamani et al., 2006; Moro and Lang, 2006; Temel and Visser-Vandewalle, 2006).

EXPERIMENTAL PROCEDURES

Autopsied cases

Human brains were provided by the brain bank of the Interdisciplinary Centre for Clinical Research at the University of Leipzig, Leipzig, Germany. All procedures of acquisition of the patient's personal data, autopsy and the handling of the autopsy material have been approved by the Ethical Committee of Leipzig University. The brains were obtained from five individuals of both sexes aged 48–88 years and showed no signs of pathological alterations (Table 1). The brains were dissected and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for 3–4 weeks at 4 °C.

Tissue preparation

After fixation, 15 mm-thick slices were prepared in the frontal plane according to the atlas of the human brain (Mai et al., 1997). Tissue blocks containing the regions of interest were cryoprotected in 30% sucrose in 0.1 M phosphate-buffered saline, pH 7.4 (PBS). Series of 30 μm-thick sections were cut on a freezing microtome and collected in PBS containing 0.1% sodium azide.

Identification of anatomical regions and applied nomenclature

Anatomical regions were identified using Nissl-stained sections and the atlas of the human brain (Mai et al., 1997). The nomenclature of brain regions was mainly adopted from brain atlases of human (Mai et al., 1997) and rhesus monkey (Paxinos et al., 2000). The identification of anatomical divisions and cell groups of the substantia nigra is based on studies of Braak and Braak (1986), Fearnley and Lees (1991), van Domburg and ten Donkelaar (1991), McRitchie et al. (1995), and Damier et al. (1999a). We additionally used the data of detailed anatomical descriptions of the basal ganglia complex (Morel et al., 2002; Waldvogel et al., 2004, 2007), the striatum (Waldvogel and Faull, 1993), the thalamus (Morel et al., 1997; Münkle et al., 2000), and the pedunculopontine nucleus (Mesulam et al., 1989).

Cytochemistry

Antigen retrieval. All sections were pre-treated with an initial antigen retrieval step (Evers and Uylings, 1997) by boiling the sections three times for 10 s in a microwave (at 900 W) in 0.1 M citrate buffer, pH 2.5, followed by rinsing with PBS.

Detection of extracellular matrix components. To abolish endogenous peroxidase activity cytochemical procedures except the fluorescence methods were started by the treatment with 1% H_2O_2 in PBS-T (0.05% Tween) for 30 min followed by rinsing with PBS-T. A subsequent blocking step with 2% bovine serum albumin, 0.3% casein and 0.1% gelatin in TBS-T was carried out for 1 h at room temperature to prevent non-specific antibody binding to the tissue.

For demonstration of extracellular matrix components sections were incubated with the following antibodies: rabbit anti-CSPG (Quartett, Berlin, Germany; 1:2000) raised against bovine nasal cartilage (Bertolotto et al., 1991) and introduced as stable marker for PNs in human brain (Bertolotto et al., 1991; Hausen et al., 1996; Brückner et al., 1999; Adams et al., 2001), goat anti-human CRTL1 polyclonal antibody (R&D Systems Inc., Minneapolis, MN, USA; 1:400) raised against recombinant human hyaluronan and proteoglycan link protein 1 (HAPLN1), known to stabilize the connection between proteoglycans (mainly aggrecan) and hyaluronan (Neame and Barry, 1994; Carulli et al., 2007), and monoclonal aggrecan antibody (clone HAG7D4, Acris, Hiddenhausen, Germany; 1:10) raised against human cartilage aggrecan. The presence and distribution of hyaluronan were detected by using biotinylated hyaluronic acid-binding protein (BHABP, Cape Cod Inc., East Falmouth, MA, USA; 1 μg/ml). To test the

Table 1. Profile of the human subjects used in this study

Case	Gender	Age (yr)	Postmortem delay (h)	Cause of death	Neuron no. nigrosome 1
1	M	48	72	Pancreatitis	19,800
2	M	72	72	Lymphoma	30,233
3	F	57	24	Peritonitis	33,350
4	M	88	26	Pancreatitis	21,533
5	M	65	24	Myocardial infarction	23,750