Short thesis for the degree of doctor of philosophy (PhD)

Distribution of extracellular matrix molecules in the precerebellar nuclei and olfactory bulb

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The Examination will be held on 26 January, 2021 at 11:00 a.m.

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The Defence will be held on 26 January, 2021 at 1 p.m.

Live online access will be provided. If you wish to join the discussion, please send an e-mail to hunyadi.a2@gmail.com not later than 12 p.m. on the day before the discussion (25 January, 2021). After the deadline, for technical reasons, it is no longer possible to join in to the defence.

1. Introduction

1.1. Extracellular matrix

Macromolecules of the extracellular matrix (ECM) are present in the extracellular space of the central nervous system providing a microenvironment for the physiological operation of neurons and glial cells. The major components of the ECM are hyaluronan (HA), chondroitin sulfate proteoglycans (CSPGs) including aggrecan, brevican, neurocan, versican, and glycoproteins e.g., tenascin-R (TN-R) and link proteins (HAPLN1). ECM accumulates around different neuronal compartments of the central nervous system or appears in diffuse reticular form throughout the neuropil. In the adult central nervous system the perineuronal net (PNN) surrounds the perikarya and dendrites of various neuron types, whereas the axonal coats (ACs) are aggregations of ECM around the individual synapses, and the nodal ECM is localized at the nodes of Ranvier. According to recent observation, the molecular and structural heterogeneity of the ECM in various regions of the central nervous system is correlated with the morphological, neurochemical and functional properties of neurons.

1.2. Precerebellar nuclei

In our previous study we have mapped the distribution, organization and molecular composition of ECM in the vestibular nuclei of brainstem, and observed a number of differences between the individual nuclei associated with their different functions. The vestibular nuclei receive labyrinthine, visual, and proprioceptive inputs and after complex information processing, the output is transmitted directly or indirectly to the eye moving and spinal motoneurons to maintain the posture and ocular stability during the head and body displacement also.

Information are transmitted indirectly to the cerebellum by precerebellar nuclei of the brainstem through the mossy and climbing fiber system. Mossy fiber-

generating neurons are found in several nuclei in the brainstem. The prepositus hypoglossi nucleus (PHN) is one of the source of mossy fibers, while the climbing fibers originate exclusively from the inferior olive (IO). Mossy fiber neurons extend their glutamatergic projection to granule cells conveying peripheral and cortical information to the cerebellum. The IO receives several inputs from the cerebral cortex, the red nucleus, spinal cord and other brain stem nuclei, and extend their glutamatergic projection to Purkinje cells.

1.2.1. Inferior olive

The inferior olive (IO) is a climbing fiber-generating precerebellar nucleus, which is functionally related to the vestibular nuclei. The mammalian IO is composed of the principal olive (PO), dorsal (DAO) and medial accessory olive (MAO) subnuclei. In addition, minor subgroups of neurons such as the dorsal cap (DC), nucleus β, ventrolateral outgrowth (VLO), and dorsomedial cell column (DMCC) are also distinguished. Functionally, the olivary nuclei are divided into somatosensory, visuomotor-vestibular and integrative groups and project to the cerebellum in topographically organized pattern. The olivary neurons have a peculiar structure, the cell bodies emit 2–7 dendrites which usually begin to divide at a 10–20-μm distance from the perikaryon, whereas the synaptic clusters or glomeruli are established on the distal dendrites. The morphologically and functionally uniform population of olivary neurons and the characteristic glomeruli throughout the entire neuropil would suggest a homogeneous ECM pattern. However, based on the heterogeneous function of olivary subnuclei, we suppose that it is reflected in different organization and composition of ECM.

1.2.2. Prepositus hypoglossi nucleus

The prepositus hypoglossi nucleus (PHN) is a mossy fiber-generating precerebellar nucleus of the brainstem, regarded as one of the neural integrators of the vestibulo-ocular reflex. PHN extends from the rostral pole of the hypoglossal nucleus till the genu of facial nerve. Based on diameter, dendritic arbor and location of soma, PHN is segregated into magnocellular and parvocellular subnuclei. The magnocellular subnucleus of PHN is located ventromedially and contains mostly large perikarya, while the dorsolateral parvocellular subnucleus of PHN has small sized neurons. The PHN neurons are either excitatory or inhibitory in function. The PHN is the common integrator for the horizontal eye movements including the gaze stabilizing vestibulo-ocular reflex as well as it influences the activity of spinal motoneurons.

1.3. Olfactory bulb

The olfactory bulb (OB) has a laminar structure, it is divided into the following layers: the glomerular layer (GL), the external plexiform layer (EPL), the mitral cell layer (MCL), the internal plexiform layer (IPL) and the granular layer (GCL). The distinct layers have different neuron types categorized conventionally on the basis of localization of cell bodies. The first set of neurons are located in the GL, referred to as juxtaglomerular cells. The juxtaglomerular cells cells are further categorized into periglomerular, superficial short axon cells and external tufted neurons. The EPL and MCL contain mostly the somata of various tufted and mitral cells, which are the major projection neurons of the OB. The IPL contains the axons of mitral and tufted cells with some of the dendrites of the granule cells. The GCL is mostly populated by morphologically heterogeneous interneurons, the granule cells. Several other neurons of the OB have not been classified into these categories. The neuronal network of the OB is continuously reorganized throughout life. The olfactory sensory neurons have a continuous turnover and their ingrowing axons integrate into the existing neuronal assembly of the glomerulus. On the other hand, the structure

of the olfactory neuronal network is also modified by the incorporation of newborn neurons. These processes result in a high degree of neural plasticity.

2. Aims

In previous study we observed that the lesion of vestibular receptors and subsequent compensation of postural and ocular deficits were accompanied by modification of ECM expression pattern in the vestibular nuclei. Since the vestibular nuclei have extended connections with the precerebellar nuclei of the brainstem, therefore a vestibular lesion-induced alteration of ECM composition is also expected in such remote areas. Knowledge on the organization and distribution of the ECM molecules in the intact precerebellar nuclei is required to follow the possible changes in the ECM expression to understand the role of ECM in the background of vestibular compensation. One of the goal of present study is to describe the unknown molecular composition and distribution pattern of ECM in two precerebellar nuclei, the PHN and the IO.

Several lines of evidence suggested that the ECM is modified in activity-dependent manner and in turn, it modulates the activities of pre- and postsynaptic receptors, therefore, it is involved in various forms of neural plasticity. Therefore, our study was extended to the area of OB, its synaptic network undergoes a lifelong reorganization. In the OB only the role of TN-R was examined. To understand the possible contribution of the other components of the ECM in the plasticity of the olfactory system detailed knowledge on their distribution is required. Therefore, the aim of this study is to describe the molecular composition and organization of these ECM molecules in various layers of the OB in the rat.

3. Materials and methods

3.1. Animals and tissue processing

The study protocol was reviewed and approved by the Animal Care Committee of the University of Debrecen, Debrecen, Hungary according to national laws and European Union regulations [European Communities Council Directive of 24 November 1986 (86/609/EEC)], and was properly conducted under the control of the University's Guidelines for Animal Experimentation (IO, PHN license number: 11/2011/DEMAB; OB license number: 6/2017/DEMAB). The experiments were performed on adult female (12–14 week-old) Wistar rats from Charles River Laboratory (Strain Crl: WI), weighing from 250 to 300 g each. The animals were terminally anesthetized with an intraperitoneal injection of 10% urethane and immediately perfused transcardially with physiological saline. The brainstems were removed and immersed into Sainte-Marie's fixative (99% absolute ethanol and 1% glacial acetic acid). The specimens were embedded in paraffin and transverse sections were made with a microtome at a thickness of 8 μm.

Prior to histochemical and immunhistochemical reactions, in the case of IO and PHN, specimens were blocked in the following reagents: 1% bovine serum albumin (BSA) (HA; *Wisteria floribunda* agglutinin, WFA), 1% BSA + 10% normal goat serum (NGS) (aggrecan), 2% BSA (versican; Hyaluronan and Proteoglycan Link Protein 1, HAPLN1), 3% normal horse serum (NHS) (neurocan), 1% BSA + 10% NHS (brevican), and 1% BSA + 10% normal rabbit serum (NRS) (TN-R).

Before the histochemical and immunhistochemical reactions, in the case of OB specimens were blocked in 3% BSA (HA; WFA; versican), 3% BSA + 10% NGS (aggrecan), 3% BSA + 10% NRS (mouse monoclonal anti-chondroitin sulfate proteoglycan, Cat-301; neurocan), 3% BSA + 10% normal donkey serum (NDS) (brevican; TN-R; HAPLN1).**3.2. Histochemical reactions**

3.2.1. Inferior olive, prepositus hypoglossi nucleus

Distribution of HA was detected by applying biotinylated Hyaluronan Binding Protein. WFA histochemistry was performed using biotinylated *Wisteria floribunda* agglutinin, a lectin binding to *N*-acetylgalactosamine residues of CSPG-glycosaminoglycan chains and glycoproteins, and therefore is regarded as a marker of PNNs. Visualization of labeling was accomplished by incubating the samples with ExtrAvidin Peroxidase complex diluted in PBS followed by 3,3′-diaminobenzidine-tetrahydrochloride with H_2O_2.

3.2.2. Olfactory bulb

Detection of HA distribution and WFA histochemistry were performed according to 3.2.1. chapter. Reactions were visualized by incubating the samples with Streptavidin AlexaFluor 555.

3.3. Immunohistochemical reactions

3.3.1. Inferior olive, prepositus hypoglossi nucleus

After blocking the samples the following primary antibodies were used: rabbit polyclonal anti-aggrecan, mouse monoclonal anti-versican, mouse monoclonal anti-neurocan, mouse monoclonal anti-brevican, goat polyclonal anti-TN-R and goat polyclonal anti-HAPLN1. For better antigen exposure of aggrecan, versican, and brevican molecules, sections were pre-incubated with chondroitinase ABC.

The following secondary antibodies were used: biotinylated goat-anti-rabbit IgG (aggrecan), biotinylated horse-anti-mouse IgG (versican, neurocan, brevican) or biotinylated rabbit-anti-goat IgG (TN-R, HAPLN1). Reactions were visualized as described for HA and WFA-staining.

3.3.2. Olfactory bulb

After blocking slides were incubated in the following primary antibodies: rabbit polyclonal anti-aggrecan, Cat-301, mouse monoclonal anti-versican, mouse monoclonal anti-neurocan, sheep polyclonal anti-brevican, goat polyclonal anti-TN-R, goat polyclonal anti-HAPLN1. For better antigen exposure of aggrecan, Cat-301, versican and brevican molecules, sections were digested with chondroitinase ABC.

The following secondary antibodies were used: goat-anti-rabbit IgG AlexaFluor 555 (aggrecan), rabbit-anti-mouse IgG AlexaFluor 555 (Cat-301, versican, neurocan), donkey-anti-sheep IgG AlexaFluor 555 (brevican), and donkey-anti-goat IgG Alexa Fluor 555 (TN-R, HAPLN1).

3.3.2.1. Double fluorescent labeling

Double fluorescent labeling was made using neurofilament or microtubule-associated protein 2 (MAP2) antibodies in combination with specific ECM markers. The following fluorescent labelings were combined: mouse monoclonal anti-neurofilament + WFA, mouse monoclonal anti-neurofilament + aggrecan, rabbit polyclonal anti-MAP2 + Cat-301, rabbit polyclonal anti-neurofilament + versican, mouse monoclonal anti-neurofilament + brevican, rabbit polyclonal anti-MAP2 + brevican, mouse monoclonal anti-neurofilament + HAPLN1, rabbit polyclonal anti-MAP2 + HAPLN1.

Prior to the incubation with neurofilament or MAP2 primary antibodies, specimens were blocked in 3% BSA + 10% NGS (mouse anti-neurofilament), 3% BSA + 10% NDS (rabbit anti-neurofilament, rabbit anti-MAP2). Primary antibodies were diluted in PBS with 1% BSA + 3% NGS (mouse anti-neurofilament) and 1% BSA + 3% NDS (rabbit anti-neurofilament, rabbit anti-MAP2).

Visualization of reactions was by goat anti-mouse IgG AlexaFluor 488 (mouse anti-neurofilament) or donkey anti-rabbit IgG AlexaFluor 488 (rabbit anti-neurofilament, rabbit anti-MAP2).

3.4. Documentation

3.4.1. Inferior olive, prepositus hypoglossi nucleus

Images were recorded using a Nikon Eclipse E800 conventional light microscope and processed by Photoshop CS4 v11.0 with minimal adjustments of contrast and background.

3.4.2. Olfactory bulb

Images were recorded using Olympus CX31 epifluorescent light microscope with DP27 digital camera and processed by Photoshop CS4 v11.0, with minimal adjustments of contrast and background. Higher magnification images were taken with confocal microscope (Olympus, FV-3000).

3.4.3. Semiquantitative assessment of histochemical and immunohistochemical reactions

After performing all ECM reactions, slides containing the identical cross sectional levels of braintsem and sections of OB were selected from three animals. Staining intensity of ECM reactions was evaluated by using five-grade scaling (–: no staining, +: weak staining, ++: moderate staining, +++: strong staining, ++++: very strong staining). The condensed forms of ECM were indicated by layers in the case of OB.

4. Results

4.1. Inferior olive

Using histochemical and immunohistochemical methods, we described for the first time the distribution and molecular organization of the ECM in the IO of the rat. Reactions revealed that the PNNs were not present in the IO but the neuropil showed positive ECM reactions throughout the nucleus. The most conspicuous feature of the ECM organization was that the stained neuropil surrounded very faint or almost unstained holes up to 20 μm. The areas of the ECM negative holes might correspond to the perikarya and the round-shaped or ovoid territory of the proximal dendrites. This unique organization pattern might be associated with the peculiar neuronal morphology and synaptic architecture of the IO. The synapses are rarely present on the cell bodies and proximal dendrites, therefore it is tempting to assume that the lack of PNNs is due to the absence of synapses on these parts of olivary neurons.

The majority of synapses are located on the distal dendrites of olivary neurons, where synaptic clusters or glomeruli are established. In the stained neuropil, darkly stained ring-like structures of 2–4 μm in diameter were observed with all ECM reactions studied and may indicate the accumulation of the ECM around the glomeruli. These unique form of condensed ECM were described as ACs in perisynaptic position around the preterminals and boutons of the excitatory or inhibitory synapses. We suppose that the ring-like structures presented here indicate the ACs. The other characteristic feature of the condensed ECM was observed by using versican antibody as darkly stained dots which may represent the accumulation of versican around the nodes of Ranvier.

The uniform morphology of the olivary neurons including the characteristic glomerular synaptic organization would suggest a uniform ECM pattern in each olivary subnucleus. Indeed, the organization of ECM was the same throughout the IO, but the molecular composition and staining intensity of ECM reactions was quite heterogeneous.

The olivary nuclei are functionally divided into visuomotor-vestibular, somatosensory and integrative parts on the basis of their afferent connections. The visuomotor-vestibular nuclei, i.e., the DMCC, the DC, the nucleus β showed the most variable ECM expression pattern. The most striking result was the absence of aggrecan reaction in the DC, whereas the DMCC and nucleus β showed intense aggrecan staining. Since aggrecan is an essential component of the ACs in various parts of the central nervous system and it inhibits the structural and dynamic plasticity of central nervous system by the stabilization of synapses, aggrecan-free neuropil in the DC may allow rapid modulation of synaptic events during the oculomotor activity. Another noticeable finding of ECM expression pattern was the strong neurocan staining in the nucleus β versus a very weak or negative staining in DC and DMCC. The full length neurocan, as part of juvenile type ECM, is highly expressed prenatally and in the early postnatal period. Its level is decreased from the second postnatal week, by cleavage of the core protein, and up-regulated again as a consequence of central nervous system injury. There are increasing numbers of data that the transition of juvenile matrix to the mature form restricts the plasticity of adult central nervous system. In the somatosensory nuclei, the DAO and horizontal lamella of MAO, only minor differences were observed in the intensity of ECM reactions. Similarly, the ECM expression pattern was identical in the integrative parts of IO even though their inputs are significantly different from each other.

4.2. Prepositus hypoglossi nucleus

Our results revealed different expression patterns of the ECM molecules in the parvocellular and magnocellular subdivisions of PHN. The most characteristic finding was the presence of PNNs with conspicuous differences between the two subdivisions. In the magnocellular subdivisions of PHN we have detected CSPGs, HA, TN-R, and HAPLN1 link protein in the PNN. The PNNs were not detected with the neurocan, and brevican staining. Using anti-versican antibody, we also observed

heavily stained dots in the pericellular area. The PNNs were rarely observed in the parvocellular subdivision of PHN, only the aggrecan and TN-R antibodies showed very weak staining around the perikarya.

The CSPGs are considered as essential components of the PNNs in the central nervous system of mammalian species however, the PHN neurons express only aggrecan. We hypothesize that our result may be explained by the pivotal integratory function of PHN in the horizontal component of the vestibulo-ocular reflex when the continuous adjustment of gaze fixation needs a high degree of plasticity in the underlying neuronal network. Due to the lack of brevican, neurocan, and versican the less condensed ECM network in the pericellular area might be in favor of ECM remodeling which is an important element of synaptic plasticity. It is well established that CSPGs have an inhibitory effect on neural plasticity and regeneration, thus the lower amount of inhibitory molecules may contribute to higher synaptic plasticity.

In the neuropil, all ECM reactions were positive in the entire PHN showing more intense staining in the magnocellular division of PHN in most cases. The staining intensity of neuropil revealed regional differences within the subnuclei, mostly in the magnocellular subdivision. Within the stained neuropil, mostly in the magnocellular subdivision of PHN, ovoid or round-shaped darker structures of 2–4 μm in diameter were observed with all ECM reactions representing ACs. The versican reaction revealed a large number of heavily stained small dots within a less intense neuropil, indicating the appearance of nodal ECM.

The regional differences in the staining intensity of PNNs and neuropil might reflect the uneven distribution of functionally different neurons in the PHN.

4.3. Olfactory bulb

In the present study, we have described the localization and organization of major ECM molecules, the HA, the lecticans, TN-R and HAPLN1 link protein in the OB

of the rat. Using histochemical and immunohistochemical methods, we detected all of these molecules in the OB showing differences in the molecular composition, staining intensity and organization of ECM between the layers and in some cases within a single layer. The most typical form was a diffuse staining in the neuropil which, depending on the type of reaction contained small, ring-like structures representing the ACs and darkly stained dots corresponding to the nodal ECM. The PNN was rarely present in the OB and appeared as the thin form.

In the **glomerular layer**, each ECM reaction, except the versican, was positive, however staining intensities varied in case of different reactions. Since the versican and TN-R are essential components of the nodal ECM the absence of versican and the very weak TN-R reaction may be related to the absence of Ranvier nodes in the glomeruli due to the presence of nonmyelinated olfactory nerve axons. A characteristic feature of the layer was the mixture of strongly and weakly stained glomeruli shown with WFA, aggrecan and HAPLN1 reactions. The other characteristics of the ECM reactions was an inhomogeneous staining, irregular islands of darker, lighter or even unstained patches were observed within the glomeruli. Since this staining pattern resembles to the two compartments of the olfactory glomeruli this unequal distribution of ECM staining may suggest that a given ECM molecule contributes differently to the synaptic plasticity in the two compartments of the glomeruli. The variable expression pattern of ECM molecules in the periglomerular areas supposed to be related to the morphological, functional and neurochemical characteristics of the periglomerular cells.

To reveal whether the aggrecan, brevican and HAPLN1 staining is associated with neuronal elements, we combined these molecules with MAP2 or neurofilament antibodies to detect the dendrites or axons, respectively. The MAP2 reaction revealed a large number of dendrites, whereas the neurofilament staining labeled only a few axons in the glomeruli. The condensed ECM was represented with the ACs shown by aggrecan, brevican and HAPLN1 reactions in the glomeruli.

All the ECM reactions studied, except versican, were positive in the **external plexiform layer**. The overall staining intensity was strongest with the HA, aggrecan, neurocan and HAPLN1, followed by moderate WFA and brevican reactions and weak TN-R immunoreactivity. The EPL is conventionally divided into an outer/superficial and inner/deep parts, on the basis of differences in the position of mitral- and tufted cell somata and territories of their secondary dendrites. Given the experimental data that the secondary dendrites of mitral and tufted cells establish different synaptic contacts in the different sublayers it is tempting to assume that the unequal distribution of ECM molecules provides a special microenvironment for the local synaptic circuits. We found that the HA, aggrecan, brevican and neurocan reactions were more intense in the outer part of the external plexiform layer. At this point we have comment the role of HA and brevican on the maturation and involvement of parvalbumin positive cells in neural plasticity. Although the parvalbumin positive neurons are found throughout the olfactory bulb, the highest expression was shown in the outer part of the external plexiform layer.

Interestingly, the localization of WFA reaction was inverse to those of the aggrecan staining. Thus, the aggrecan staining was strong in the outer, whereas the WFA reaction was intense in the deep part of the external plexiform layer. This result is in contrast to the previous finding that the WFA staining is mostly dependent on the presence of aggrecan in mammals and most likely specific for its N-acetylgalactosamin carbohydrate epitopes. At present, we cannot give explanation for this contradiction.

The ECM reactions did not reveal an intermediate sublayer in the external plexiform layer, however the very strong brevican positive zone underneath the glomeruli, where the cell bodies of the secondary dendrite-bearing external tufted cells are located, may suggest an ECM-based sublayer in the external granular layer. Among the lecticans, the brevican antibody gave a characteristic beaded appearance.

Its colocalization with the neurofilament reaction may indicate that the large amount of brevican molecules is located at the nodes of Ranvier.

In the external plexiform layer the neurocan staining was strong. This molecule is an essential component of juvenile ECM and its level is dramatically decreases during the early postnatal life in the brain extract. The persistent expression of neurocan in the olfactory bulb is consistent with the high degree of plasticity in the olfactory system.

The **internal plexiform layer** showed positivity with each ECM reaction studied. The layer is populated mostly by axons, the few cell bodies represent one of the subtypes of deep short-axon cells (dSA), the GL-dSA.

The WFA reaction was strong in the IPL, where heavily stained bands, with lighter intervals, were shown running perpendicular to the surface of the OB. Anti-neurofilament labeling revealed immunoreactivity within these bands which may represent the axons of mitral cells, tufted cells, bulbar interneurons or can belong to axons from centrifugal fibers. The WFA staining is continued to the MCL and forms PNN around the somata of a mitral cell. Similarly to the WFA staining, in the IPL, aggrecan positive darker bands appeared and some of them extended into the MCL to form thin PNNs.

The TN-R expression was the highest in the IPL. Similar distribution was described in adult mouse and important role of TN-R was established in OB neurogenesis. These observations showed that the TN-R induces the radial migration of newly formed neuroblasts into the OB and increases the spine density on the dendrites of newborn neurons. The stronger expression of TN-R in the deeper parts of the OB correlate with the direction of radial migratory pathway and the finding that the majority of newborn neurons differentiate into granular and periglomerular cells.

The **granule cell layer** showed the most intense immunostaining with brevican reaction. However, the aggrecan and HAPLN1 antibodies did not label the layer. The

strong brevican reaction and the negative aggrecan staining indicate that the main ECM component of the perisynaptic ECM is the brevican. In the IPL and GCL characteristic dot-like appearance of the versican reaction was detected. They were present in higher number in the outer part of the GCL where alternating darker and lighter columns were shown due to the higher or lower number of versican positive dots, respectively. Neurofilament co-labeling revealed a similar columnar organization; the versican positivity showed a partial overlap with the neurofilament staining in the GCL. In the inner part of the GCL, versican positive dots were present in lower density and here the neurofilament staining was very weak.

5. Summary

In the present study we have described the localization and organization of major ECM molecules, the HA, the lecticans, TN-R and HAPLN1 link protein in the IO, PHN and OB of the rat. By using histochemical and immunohistochemical methods, we detected all of these molecules showing differences in the molecular composition, staining intensity, and organization of ECM between the layers or subnuclei and in some cases within a single layer or subnucleus of the examined structures.

Our results showed that the organization of ECM molecules is conspicuously different in the two precerebellar nuclei, PHN and IO. In case of the IO, the most characteristic finding was the lack of PNNs, presumably due to the absence of synapses on the perikarya and proximal dendrites of IO neurons. We detected markable differences between the magno- and parvocellular divisions of the PHN. PNNs were well developed in the magnocellular subdivision of PHN, whereas the pericellular positivity was almost absent in the parvocellular subdivision of PHN, here a diffuse ECM was observed. We suppose that the difference in the ECM pattern of the two precerebellar nuclei and between the two subnuclei of the PHN could be associated with their different afferent and efferent connections, cytoarchitecture, physiological properties and with their different functions in gaze and posture control. The knowledge on the distribution of various ECM molecules in the intact IO and PHN may help to follow the yet unknown changes of the ECM expression in the neuronal network of vestibular system during the vestibular compensation.

In the OB, one of the striking features of ECM staining pattern was that the reactions were shown dominantly in the neuropil. PNNs were present only in the MCL with the WFA and aggrecan staining and they exhibited only the thin appearance, whereas the other forms of condensed ECM were not recognizable. These results are in agreement with the life-long plasticity of the olfactory system which includes formation and elimination of synaptic contacts and continuous generation and migration of interneurons into the OB. The PNNs limit the plasticity in adulthood by altering new neuronal contacts, acting as a scaffold for molecules that can inhibit synapse formation, and limiting receptor motility at synapses. As the PNN limits the neural plasticity, its rare appearance may be related to the high degree of plasticity in the OB.

Our results might contribute to understand the recently highlighted importance of ECM in the neural plasticity and regeneration.

List of Publications

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List of publications related to the dissertation

1. **Hunyadi, A.**, Gaál, B. Á., Matesz, K., Mészár, Z. M., Morawski, M., Reimann, K., Lendvai, D., Alpár, A., Wéber, I., Rácz, É.: Distribution and classification of the extracellular matrix in the olfactory bulb.
   Brain Struct. Funct. 225 (1), 321-344, 2019.
   DOI: http://dx.doi.org/10.1007/s00429-019-02010-8
   IF: 3.298
2. Kecskés, S., Gaál, B. Á., Rácz, É., Birinyi, A., **Hunyadi, A.**, Matesz, K.: Extracellular matrix molecules exhibit unique expression pattern in the climbing fiber-generating precerebellar nucleus, the inferior olive.
   Neuroscience. 284, 412-421, 2015.
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3. Gaál, B. Á., Kecskés, S., Matesz, K., Birinyi, A., **Hunyadi, A.**, Rácz, É.: Molecular composition and expression pattern of the extracellular matrix in a mossy fiber-generating precerebellar nucleus of rat, the prepositus hypoglossi.
   Neurosci. Lett. 594, 122-126, 2015.
   DOI: http://dx.doi.org/10.1016/j.neulet.2015.03.056
   IF: 2.107

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Total IF of journals (all publications): 8,636
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