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ROLE OF TENASCIN-R AND BREVICAN IN COMPENSATIONS FOLLOWING VESTIBULAR LESION

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23rd of January, 2025; 13:00

INTRODUCTION

Numerous physiological and pathophysiological factors contribute to the reorganization of CNS neuron networks. This intricate process involves a series of interdependent intra- and extracellular events, in which the extracellular matrix (ECM), as observed by our lab and other researchers, plays a crucial role and fundamentally contributes to the establishment of a new equilibrium within the affected neuron network. Additionally, the past decade has witnessed the recognition that ECM remodeling is not uniform across the morphologically and functionally diverse regions of the CNS, and the triggering cause also plays a significant role. The extent and potential for recovery of impaired function following direct CNS injury depends on various factors. It is well established that embryonic neural tissue possesses remarkable plasticity, but this ability gradually diminishes after birth (Carulli et al., 2006; Galtrey et al., 2008). Despite the growing body of experimental data, the molecular mechanisms underlying the presumed morphological and functional changes, cell surface receptor expression, and altered neurotransmitter production associated with CNS injury-induced regenerative and plastic processes remain only partially understood (Oohashi et al., 2015)

Unilateral vestibular lesions can be induced by ablation of the inner ear, which houses the receptors of the vestibular sensory system. Vestibular deafferentation manifests in dynamic and static symptoms: it causes postural and eye movement disorders, is accompanied by spontaneous nystagmus and asymmetric muscle tone, and involves abnormal functioning of the autonomic nervous system (nausea, dizziness). This occurs despite the fact that the vestibular system in mammals does not regenerate after lesion, and the former synaptic activity of the primary afferent vestibular fibers is likely lost to the neuron network. However, in contrast to the silenced primary afferents, the function of somatosensory, visual, and cerebellar afferents terminating in the vestibular nuclei, as well as vestibular commissural nerve fibers, is preserved. Experimental data demonstrate that the normalization of symptoms after vestibular lesion begins before the onset of axonal sprouting, suggesting that a synaptic

reorganization occurs at the level of neuron networks (Deák et al., 2012; Gaál et al., 2015; Faralli et al., 2016).

Vestibular Apparatus in the Brainstem

The sensory organs of the vestibular system are housed in the labyrinth of the inner ear within the petrosal bone. The membranous labyrinth runs within the bony labyrinth, following its shape. The linear and angular acceleration sensors, known as hair cells, are located in the wall of the membranous labyrinth. The *crista ampullaris* of the *ampullae* of the three semicircular canals detect angular acceleration, while the *maculae* of the *utriculus* and *sacculus* detect linear acceleration (Paxinos and Watson 1998). The peripheral processes of the bipolar neurons of the vestibular ganglion (Scarpa) synapse with the hair cells, and their central axons terminate in the vestibular nuclear complex of the brainstem. The vestibular nerve leaves the Scarpa ganglion as a single bundle and runs into the brainstem, on the ventral surface of the pontomedullary angle, just below the inferior cerebellar peduncle.

The vestibular nuclear complex (VNC) of the rat consists of four nuclei located in the dorsolateral part of the open portion of the *medulla oblongata*. In processing our findings, we used the morphometric description published by Suarez et al., 1993, highlighting the following baseline values.

1. Superior Vestibular Nucleus (SVN) (Bechterew)

Average rostrocaudal length of the nucleus is 0.72 ± 0.16 mm. Cell distribution displays 25% small cells (<20 μm diameter); 64% medium-sized cells (20-35 μm); 6% large neurons (>35 μm). Similar neuron diameter distribution was seen in the rostral and caudal parts of the nucleus.

Perineuronal nets surrounding medium- and large-sized neurons of the NVS exhibited the most intense ECM accumulation based on semiquantitative and optical density measurements performed in our lab (Rácz et al., 2014)

2. Medial Vestibular Nucleus (MVN) (Schwalbe)

Largest rostrocaudal extent of the nucleus is 1.58 ± 0.21 mm. Its cell distribution displays 53.5% small cells (<20 μm diameter); 45.5% medium-sized cells (20-35

μm diameter); and 2% large neurons (>35 μm). Distinct functional roles of medium-sized neurons located in the magnocellular area centrally and rostrally and small cells of the parvocellular region located primarily peripherally (Johnston et al., 1993; Saito et al., 2008; Takazawa et al., 2004)

Multiple descriptions and our own lab's findings (Rácz et al., 2014) also indicate that the NVM's large neurons are covered by a perineuronal net, as well as certain parvocellular populations.

3. Lateral Vestibular Nucleus (LVN) (Deiters)

The largest neuron group forming the NVL has a length of 0.85 ± 0.16 mm (Suárez et al. 1993). Its neuron distribution displays 9% small cells (<20 μm diameter); 45.5% medium-sized cells (20-35 μm); 41% large (35-50 μm) and giant neurons (>50 μm). Unlike in the MVN, neurons of different sizes are not segregated within the LVN. Staining differences are also observed for perineuronal nets (PNNs), with well-stained PNNs only observed around magnocellular neurons (Rácz et al., 2014). The PNN of the LVN is particularly dense around magnocellular neurons, suggesting that these neurons may be particularly susceptible to changes in synaptic plasticity.

4. Descending Vestibular Nucleus (DVN) (Roller)

The length of the DVN is shorter than that of the MVN, averaging 1.18 ± 0.2 mm, but it is the most caudal of the four vestibular nuclei. Neuron distribution displays 26% small cells (<20 μm diameter); 62% medium-sized cells (20-35 μm); 12% large (35-50 μm) and giant neurons (>50 μm). Large and giant cells are typically detected in the rostral region, medium-sized cells in similar proportions, and small cells mainly in the caudal region (Suárez et al., 1993).

The staining intensity of perineuronal nets (PNNs) is stronger in the rostral region than in the caudal region, which is likely related to the higher proportion of large cells in the rostral region and the different efferent connections (Rácz et al., 2014).

Extracellular Matrix in the Central Nervous System

The extracellular matrix (ECM) is a network of macromolecules that fills the spaces between cells in the central nervous system (CNS). It accounts for approximately 20%

of the CNS volume (Nicholson and Syková, 1998). The ECM stabilizes the position of cells, optimizes the transport of cytokines, neurohormones, and growth factors, and maintains the ionic balance of the extracellular space.

The neural tissue matrix is composed of the following macromolecules:

1. **Hyaluronic acid (HA)**: A large, negatively charged polysaccharide that provides structural support and hydration.
2. **Chondroitin sulfate proteoglycans (CSPGs)**: A group of proteoglycans that play a role in cell adhesion, migration, and signaling.
3. **Glycoproteins**: A diverse group of proteins that are involved in many different functions, including cell adhesion, signaling, and enzyme activity.
4. **Link proteins**: A group of proteins that connect different ECM components together, particularly stabilize the HA-to-proteoglycan bonds.

Collagen type I is a major component of the ECM in most other tissues, but it is not found in the CNS. Synthesis of HA, link proteins, and aggrecan are synthesized exclusively by neurons. Oligodendroglia and astrocytes also play a role in the production of other ECM components.

Hyaluronic Acid in the Extracellular Matrix

Hyaluronic acid (HA) is a macromolecular polymer composed of D-glucuronic acid and N-acetyl-D-glucosamine dimers linked by alternating β-(1→4) and β-(1→3) glycosidic bonds. The number of disaccharide monomers ranges from 2000 to 25,000, and the total length of the chain can be 2–25μm (Necas et al., 2008). In addition to its high molecular weight, further structural features of hyaluronic acid include its non-sulfated but carboxylated (COO^-) double helical conformation, which is non-branched (Toole, 2004).

Tissue levels of hyaluronic acid in vestibular lesions were not investigated in this study.

Proteoglycans in the Extracellular Matrix

Proteoglycans are large macromolecules with a long core protein axis to which glycosaminoglycan (GAG) side chains of varying length and number are attached. GAG

chains are linear polymers of repeating disaccharides, each disaccharide monomer consisting of a uronic acid and an N-acetyl glucosamine or N-acetyl galactosamine.

The presence and function of proteoglycans in the CNS are complex and play a key role in numerous normal and pathological processes. This makes them potential targets for research in neurological diseases (Köwitsch et al.,2018).

In addition to its role in vestibular compensation, brevican is being investigated in other human-related contexts. **(I)** Role in glioma where its expression is increased, and may play a role in tumorigenesis (Jaworski et al., 1996). **(II)** Role in Alzheimer's disease where its levels are decreased, suggesting that it may play a role in the development of the disease (Morawski et al., 2011). **(III)** Role in regeneration after CNS injury where it is involved in the regeneration process after direct CNS injury (Jones et al., 2003).

Chondroitin Sulfate Proteoglycans (CSPGs)

CSPGs are a diverse group of proteoglycans that play important roles in many biological processes, including cell adhesion, migration, signaling, and inflammation. They are found in a variety of tissues, including the nervous system.

The hyaluronan-binding CSPGs, also known as lectins, are a subset of CSPGs that are characterized by their ability to bind to the polysaccharide hyaluronan. There are four giant molecules in this group: *aggrecan*, *versican*, *neurocan*, and *brevican*.

This thesis focuses on **brevican**, thus the detailed description focuses on this molecule.

Aggrecan In conjunction with HA, aggrecan forms a massive spatial network that exceeds 200 MDa. Aggrecan is detectable in both the perineuronal network (PNN) and the neuropil. According to previous observations in our laboratory, aggrecan is the most abundant CSPG in the PNNs of vestibular neurons in rats (Racz et al., 2014).

Versican Based on the localization of GAG side chains and the length of the core protein, as well as considering the developmental stage, four isoforms are distinguished: V0, V1, V2, and V3 (Zimmermann and Ruoslahti, 1989). In the mature CNS, the V2 isoform is expressed by oligodendrocytes. Its presence in

immunohistochemical labeling is punctate, which can be recognized in the PNN area as well as in the Ranvier constrictions.

Neurocan The core protein of neurocan has seven sites for sulfated GAG binding (Iozzo and Schaefer, 2015). Its molecular weight is 245kDa. It is only present in the CNS, where it inhibits the *in vitro* growth of neurites in adulthood.

Brevican Along with aggrecan, brevican has dominant presence in the neuropil, PNNs, and perisynaptic spaces (Bruckner et al., 2008; Frischknecht and Seidenbecher, 2012). According to the description of our laboratory (Racz et al., 2014), its high expression characterizes the perineuronal networks of the three nuclei of the vestibular nuclear complex in rats (SVN, MVN, LVN), predominantly present in the PNNs of medium and large neurons.

In newborn rats, brevican expression gradually increases and reaches a plateau in adults (Seidenbecher et al., 1998). Brevican is produced by both glial cells and neurons (Seidenbecher et al., 1998, Yamada et al., 1994, John et al., 2006).

Brevican has a molecular weight of 140 kDa. Its globular N-terminal domain (G1) can bind to HA, and it also contains an immunoglobulin-like loop and two link protein segments. The middle section of the core protein is heterogeneous and carries only 1-5 chondroitin sulfate side chains. The C-terminal domain, located far from HA, contains an EGF (epidermal growth factor) module, a lectin-like module, and a complement regulatory protein, which has TN-R binding affinity (Celio, 1998;).

Brevican is the most abundant PNN component after aggrecan, and it is present on the direct surface of neurons. Its perisynaptic presence is known, and the scientific literature agrees that it has a non-permissive effect on axon regrowth in the mature CNS (Frischknecht and Seidenbecher, 2012; Celio, 1998).

Glycoproteins in the Extracellular Matrix

In the complex lattice-like structure of the nervous ECM, HA-CSPG aggregates are cross-linked by tenascin-R glycoproteins. The HA-CSPG connection is further stabilized by so-called binding proteins or link proteins, which belong also to the glycoprotein family.

Tenascins

Based on their localization, tenascins can be divided into four subtypes in mammals: Tenascin-R (TN-R), Tenascin-C, Tenascin-X, and Tenascin-W (Chiquet-Ehrismann and Tucker, 2011).

This thesis focuses on TN-R, so the detailed description focuses on this molecule.

Tenascin-R has a complex structure with 4.5 EGF repeats and 9 fibronectin III repeats. It exists as a homotrimer in the ECM. Tenascin-R binds to a variety of ECM molecules and cell surface ligands, including integrins, heparan sulfate proteoglycans, cell adhesion molecules from the immunoglobulin superfamily (contactin, axonin TAG-1, neurofascin), annexin II, and receptor tyrosine phosphatase. It also interacts with other ECM partners through the fibrinogen-like domain located at its C-terminus, such as aggrecan, brevican, and neurocan via the G3 domain of the aforementioned lectins (Jones and Jones, 2000; Jang et al., 2020; Zimmermann and Dours-Zimmermann, 2008).

Tenascin-R and tenascin-C molecules play a significant role in the final arrangement of the perineuronal network (PNN). This is evidenced by studies on tenascin-R knockout mice, which observed abnormal aggregation of CSPGs and associated migration defects (Carulli et al., 2006; Galtrey and Fawcett, 2007). In human oncology research, tenascin-C expression in CNS tumors has been associated with poor prognosis due to increased metastasis in the stroma of certain tumors (Chiquet-Ehrismann and Tucker, 2011).

Organizational Forms of the ECM in the Central Nervous System

The extracellular matrix is a complex and dynamic network of macromolecules.

1. **Basal Lamina** The basal lamina is a specialized ECM layer that surrounds blood vessels and capillaries in the CNS. It forms a barrier between the CNS tissues and the cerebrovascular system, regulating the passage of molecules and cells. The basal lamina is composed of a variety of proteins, including laminins, collagen IV, and proteoglycans.

2. **Perineuronal Net (PNN)** The PNN is a dense and highly organized ECM structure that typically surrounds the cell body (perikaryon) of neurons. It also extends to enwrap dendrites and axon terminals, as described in several brainstem regions by our laboratory (Racz et al., 2014, Ritok et al., 2022). The composition and function of the PNN have been extensively studied in mammals, amphibians, and birds by numerous independent research groups (Celio et al., 1998; Matesz et al., 2005; Szigeti et al., 2006; Meszar et al., 2008; Balmer et al., 2009; Morawski et al., 2012; Racz et al., 2014; Gaal et al., 2014). The formation of the PNN is strongly linked to the size and function of neurons, which are closely related to the synaptic traffic of the given neuron. Immunohistochemical labeling clearly highlights the soma and its processes, and it can be sharply distinguished from the surrounding less dense neuropil matrix environment. In terms of ECM composition, hyaluronic acid, several CSPGs (aggrecan, brevican, neurocan, versican), tenascin-R, and several link proteins can be detected (Celio et al., 1998; Kwok et al., 2011; Racz et al., 2014; Ritok et al., 2022).
3. **Interstitial Matrix** The interstitial matrix is a loosely organized ECM found in the spaces of the neuropil. It is continuous with the basal lamina or PNN, though it can be easily distinguished from these matrices morphologically and during microscopic analysis.
4. **Axonal Coat (AC)** It is a very fine matrix sheath that covers axons and their preterminal segments. It has only been accepted as an ECM component in the past decade.
5. **Nodal ECM Accumulations** Periodically appearing nodules, which are called nodal ECM accumulations, can be well observed in the white matter tracts of the CNS by immunohistochemical labeling.

This experimental work describes and quantifies the presence of brevican, and tenascin-R, a glycoprotein, in the above-described compartments during functional compensation following labyrinthine lesions.

Synaptic Plasticity

Synaptic plasticity refers to the modification of the strength and efficiency of signal transmission in chemical synapses. In a physiological stimulus environment, it can adapt the function of neural networks within a very wide range. This mechanism is the neurobiological basis of learning and forgetting, experience, but it also appears in pathological processes, neurological and psychiatric disorders, and after strokes, traumatic brain injuries, and sensory deafferentation (Michaluk and Kaczmarek, 2007). It has been demonstrated that a common phenomenon in these brain events with diverse etiologies is a temporary or permanent modification of the PNN composition.

Over the past two decades or so, the importance of extracellular proteases in synaptic plastic adaptations, including vestibular compensation, has been established.

Vestibular Compensation

The symptoms following unilateral inner ear damage have been known for nearly two centuries (Flourens 1842; Goltz, 1870; Ewald, 1982). These observations contributed to the combined cellular and network-level understanding of the phenomenon of 'vestibular compensatio' (Magnus, 1924). Furthermore, it was first recognized that the vestibular organs in mammals do not regenerate, but rather that posture, skeletal muscle function, eye movements, food intake, etc. were restored as a result of extensive adaptation in the central vestibular apparatus. Since then, labyrinthine lesions have been an accepted model of functional changes in synapses in neuroscience.

OBJECTIVES

Building upon the previous findings of our laboratory, we aimed to conduct a morphological analysis of the extracellular presence of Tenascin-R and Brevican molecules in the perineuronal networks (PNNs) surrounding the brainstem neurons of the vestibular apparatus, following surgical ablation of the inner ear sensory organs. Similar to our first study, we set the observation period at 14 days post-surgery.

I. Surgical Precision

Optimizing the surgical precision of unilateral inner ear ablation was necessary to reduce the number of experimental animals used. Additionally, this study involved adapting and videotaping symptomatic criteria.

II. Tenascin-R Expression in PNNs

The Tenascin-R molecule is responsible for crosslinking the ECM network. Therefore, we hypothesized that TN-R expression would be reduced in the PNNs of vestibular neurons during postoperative compensation. Our observations attempted to answer the following questions:

- Does the extracellular presence of TN-R indeed decrease within 14 days post-surgery?
- In which nuclei of the brainstem vestibular nuclear complex is this change observed?
- Is the decrease in TN-R expression quantifiable using semi-quantitative methods? Is there a significant difference in the decrease between different survival stages, reflecting the compensation process?
- Over what time frame can the change in TN-R be tracked, considering the limitations of the chosen methods?
- Within how many days does vestibular function compensate? To what extent does function return?
- Does the decrease in TN-R expression correlate with the size or function of vestibular neurons?

III. **Brevican**, along with aggrecan and hyaluronic acid, is one of the most common structural molecules of PNNs. We investigated its expression in the PNNs of neurons in the superior vestibular nucleus (SVN) during the 14-day period following unilateral labyrinthine lesion. Based on our current understanding of brevican, it is involved in fast saltatory impulse conduction, which is particularly important in the eye movement pathways originating from the SVN. Using fluorescent immunohistochemistry, morphometric, and statistical analyses, we sought to answer the following questions:

- Does the extracellular presence of brevican indeed decrease within 14 days post-surgery?
- Is there a difference in brevican expression between the lesioned (ipsilateral) and intact (contralateral) SVN?
- To what extent does brevican expression decrease in PNNs with strong, moderate, and weak immunoreactivity in the intact state? Over what time frame do these changes exist?
- Can our histological observations be correlated with the recovery of lesion symptoms?

MATERIALS AND METHODS

Ethical Approval

Our animal experiments were conducted in accordance with procedures reviewed and approved by the Debrecen University Workplace Animal Welfare Committee under the following permit numbers: 6/2017/DEMAB; 11/2011/DEMAB. The surgical interventions were approved by the Hajdú-Bihar County Government Office, Food Chain Safety and Land Office under permit number HB/06/ÉLB/2270-10/2017.

Experimental Animals

For the study of brevican expression, n=15 adult, for tenascin-R (n=12) and as control group n=3 female Wistar rats (Charles River Laboratory; Strain Crl: WI) were included in the experiment. All animals taken from 12-14 weeks old age groups.

Unilateral Labyrinthine Lesion and Histological Processing

Unilateral labyrinthine lesion (UL) was performed under general anesthesia. The procedure involved removing part of the inner ear (vestibular labyrinth) on one side of the rat's head. This lesion is known to disrupt vestibular function, leading to symptoms such as dizziness, imbalance, and nystagmus (eye movements).

Animal Survival and Tissue Preparation

Rats were allowed to survive for 1, 3, 7, and 14 days following the UL procedure. At each survival time point, the rats were euthanized and their brainstems were removed. The brainstems were then sectioned into 8µm thick frontal slices. These slices were used for immunohistochemical staining to visualize the expression of Tenascin-R and Brevican.

Immunohistochemistry for Tenascin-R

To investigate the localization of **Tenascin-R** expression, we performed immunohistochemistry using a goat-derived polyclonal anti-Tenascin-R IgG antibody (1:300; R&D Systems, Minneapolis, MN, USA). The antibody binding was visualized using diaminobenzidine-tetrahydrochloride (DAB; Sigma-Aldrich).

Before immunohistochemical labeling of **Brevican**, enzymatic digestion was demanded using chondroitinase-ABC (1:100; 0.02 U/ml; Sigma-Aldrich).

For the first primary antibody in the double immunofluorescence labeling, we used rabbit-derived polyclonal anti-NeuN (1:1000; Merck Millipore, Temecula, CA, USA) antibody to label neuronal nuclei. This antibody was visualized using donkey anti-rabbit IgG AlexaFluor 488 (1:1000; Life Technologies, Eugene, Oregon, USA) fluorescent secondary antibody.

For the second primary antibody, we used mouse-derived monoclonal anti-brevican (1:200; BD Biosciences, San Jose, CA, USA) antibody specifically targeting brevican. The visualization of the reaction was performed by secondary and tertiary incubation, using biotinylated horse anti-mouse IgG secondary antibody (1:1000; Vector Laboratories, Burlingame, CA, USA), followed by Streptavidin AlexaFluor 555 (1:1000; Life Technologies) conjugated tertiary reagent.

Image Acquisition and Analysis

Fluorescent images were captured using an Olympus CX31 epifluorescence microscope equipped with an Olympus DP74 camera (Olympus Ltd., Tokyo, Japan). For quantitative analysis of PNNs, ImageJ v1.46 (National Institute of Health, Bethesda, MA, USA) software was used.

Semi-quantitative Assessment and Statistical Analysis of Tenascin-R Expression Changes

Light microscopy images acquired with identical settings (brightness, contrast, magnification) were then quantified based on the independent judgment of two researchers, and their evaluation was verified by a third person. The intensity of the reaction was determined on a four-point scale in the perineuronal networks: (-) no

staining; (+) weak staining; (++) moderate staining; (+++) strong staining. The entire vestibular nuclear complex of 3-4 rats was quantified in each survival group, separately for the PNNs of the ipsilateral and contralateral sides.

Quantitative Assessment and Statistical Evaluation of Brevican Expression Changes

Optical densitometry was used to quantify the intensity of the brevican reaction in the PNNs of neurons in the NVS (Faralli et al., 2016). The NVS of 3-4 rats in each survival group was quantified, separately for the PNNs of the ipsilateral and contralateral sides.

Quantitative Determination of Perineuronal Networks

The quantitative data of the morphological findings were subjected to statistical analysis according to the aspects described in the 'Results' section.

RESULTS

Considering the different localizations of ECM, this scientific observation described matrix changes in four extracellular compartments within approximately two weeks post-surgery following UL. **Perineuronal Networks (PNNs):** The ECM accumulates heavily around certain perikaryons, forming a structure identified as the perineuronal network. **Neuropil Matrix:** In neuronal processes and glia-filled spaces, the neuropil, a diffuse network-like matrix occupies the majority of the compartments. **Axonal Coat:** Within the neuropil, we also observed the so-called 'axonal coat' form, which consists of 2-4μm diameter, ring-shaped axon sheaths. **Nodal ECM:** The fourth form of ECM accumulation is the intense punctate immunohistochemical labeling of nodal ECM, which is present both perisomatically and in the neuropil. This last form became visible only with the immunoreaction against brevican.

The description of our findings is presented separately for the two studied ECM components (tenascin-R and brevican).

Tenascin-R

Following unilateral labyrinthine lesion (UL), Tenascin-R expression transiently decreases in the perineuronal networks and neuropil of the vestibular nuclear complex.

Statistical Analysis of Tenascin-R Semiquantitative Intensity Values

Superior Vestibular Nucleus: Statistical comparison revealed a significant difference in semiquantitative intensity values between the 3rd and 7th survival days ($P < 0.001$). No statistical significance was found when comparing values between the 1st and 3rd days, or between the 7th and 14th days.

Medial Vestibular Nucleus – Magnocellular part: Statistical analysis showed no significant changes in intensity values among the survival stages within the examined postoperative period.

Lateral Vestibular Nucleus and Descending Vestibular Nucleus – Rostral part: Intensity change was only significant between the 1st and 3rd days ($P < 0.001$). No

significant difference was found in intensity values among the remaining survival days.

Brevican

Following labyrinthine lesion, the perineuronal networks of the NVS exhibited transient morphological changes.

Statistical Analysis of Brevican Optical Densitometry Intensity Values

After UL, the proportion of brevican-positive perineuronal networks among the strong-moderate-weak categories transiently changed.

Operated Side (Ipsi-lateral): On the 1st post-op day, the proportion of PNNs with strong immunostaining decreased dramatically to only 12.43%, 23.07% on the 3rd day, and 6.23% on the 7th day. The proportion of PNNs showing moderate staining intensity increased significantly by about 50% compared to control values; their proportion was 63.8% on the 1st post-op day, 71.15% on the 3rd day, and 46.29% on the 7th day. The proportion of PNNs with weak staining also increased significantly, with proportions of 23.07% on the 1st day, 5.77% on the 3rd day, and 47.47% on the 7th day. Significance on the 1st, 3rd, and 7th post-operative days ($\chi 2$ test, $\chi 2$ (2, n=156 - 337) = 21.05 - 157.36, $P < 0.001$) ipsilaterally.

Intact Side (Contra-lateral): Similar to the PNNs on the operated side, there was a radical shift in the expression pattern. Strong staining was observed in 6.99% of PNNs on the 1st day, 17.39% on the 3rd day, and 5.45% on the 7th day, representing a very strong decrease compared to control animals. PNNs with moderate staining intensity were present in 76.2% on the 1st day, 72.1% on the 3rd day, and 49.4% on the 7th day. The proportions of weakly stained PNNs also showed a very strong increase, representing 16.8% on the 1st day, 10.4% on the 3rd day, and 45.15% on the 7th day among neurons covered with PNNs. Significance on the 1st, 3rd, and 7th post-operative days ($\chi 2$(2, n= 143 - 330) = 41.99 - 157.87, $P < 0.001$) contralaterally.

Effect of Labyrinthine Lesion on Average Optical Density Values of Brevican-Positive Perineuronal Networks in the Nucleus Vestibularis Superior

On the 1st day after UL, a radical decrease in average densitometric values was observed on both sides, with a value of 70% on the operated side and 70.3% on the intact side. The immunoreactivity of PNNs increased bilaterally by the 3rd day of survival, reaching 84% on the operated side and 76.78% on the intact side. Average values measured on the 7th day decreased to half of the values of the control groups (56.3% on the non-operated side and 56.38% in the intact nucleus). On the 14th day, the average values on the operated side increased to the level of the control value, 100.7%, and 89.44% on the contralateral side.

According to the statistical analysis, there was a significant change in optical density in the NVS of both the operated and intact sides, presumably as a consequence of the lesion (Kruskal-Wallis one-way ANOVA and Ranks test, $H(4)=329.20$, $P < 0.001$ (ipsilateral) and $H(4)=317.27$, $P < 0.001$ (contralateral) (number of elements n=118-337/survival day/side)). Optical density values showed a decrease compared to control values on the 1st, 3rd, and 7th postoperative days (Dunn's post hoc test $P < 0.001$). On the 14th day, average optical density values were again measured that were the same as the control values (Dunn's post hoc test, $P > 0.999$ (ipsilateral) and $P = 0.024$ (contralateral)).

CONCLUSIONS

Possible Role of Tenascin-R in Vestibular Compensation

1. Tenascin-R is known to activate microglia cells, which in response secrete cytokines and growth factors, including BDGF (brain-derived neurotrophic factor) and NGF (neurotrophic growth factor) (Liao et al., 2005).
2. Under physiological conditions, the extracellular matrix inhibits the formation of a significant amount of new synaptic connections by stabilizing synapses.

   The decreasing staining intensity, or decreasing presence of non-permissive tenascin-R, is thought to stimulate synaptic plasticity, or synaptogenesis according to some assumptions, which cannot be proven in this experimental approach.
3. The possible role of tenascin-R in vestibular compensation is the possible inhibition of commissural pathways between the bilateral vestibular nuclei (Holstein et al., 1999; Bergquist et al., 2008; Malinvaud et al., 2010).
4. Tenascin-R accumulation has been recognized by several groups in the Ranvier nodes of large myelinated axons (Apostolova et al., 2006; Bekku et al., 2009). Tenascin-R is a modulator of the beta subunit of voltage-dependent Na^+ channels and desensitizes them in the axon membrane (Srinivasan et al., 1998; Xiao et al., 1999).

Possible Role of Brevican in Vestibular Compensation

1. It is likely that the reduced brevican expression accelerates the adaptation of synapses between non-vestibular afferents and NVS neurons (Gacek et al., 1988; Dieringer, 1995; de Waele et al., 2000).
2. On the 14th postoperative day, the optical density measured in the operated animals returned to control values on both the ipsi- and contralateral sides. This suggests that brevican is involved in stabilizing the rearranged synaptic state (Frischknecht et al., 2009; Blosa et al., 2013; Favuzzi et al., 2017).
3. Brevican also regulates the plastic adaptation of synapses by anchoring potassium channels and AMPA receptors in the hippocampus (Favuzzi et al., 2017). The role

of brevican in fast synaptic transmission has also been established in the auditory system (Blosa et al., 2015; Sonntag et al., 2018). Based on the common embryonic origin of the auditory and vestibular systems, as well as their similar morphological, physiological, and neurochemical properties, we hypothesize that the increased brevican expression in the NVS on the 14th postoperative day is also related to the restoration of fast synaptic transmission.

LIST OF PUBLICATIONS

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/126/2024.PL
Subject: PhD Publication List

Candidate: Ágnes Magyar
Doctoral School: Doctoral School of Dental Sciences

List of publications related to the dissertation

1. **Magyar, Á.**, Rácz, É., Matesz, K., Wolf, E., Kiss, P., Gaál, B. Á.: Lesion-induced changes of brevican expression in the perineuronal net of the superior vestibular nucleus.
   Neural Regen. Res. 17 (3), 649-654, 2022.
   DOI: http://dx.doi.org/10.4103/1673-5374.320988
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2. Gaál, B. Á., Jóhannesson, E. Ö., Dattani, A., **Magyar, Á.**, Wéber, I., Matesz, K.: Modification of tenascin-R expression following unilateral labyrinthectomy in rats indicates its possible role in neural plasticity of the vestibular neural circuit.
   Neural Regen Res. 10 (9), 1463-1470, 2015.
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List of other publications

3. **Magyar, Á.**: Ajak-Szájpadhasadékkal született gyermekek kezelése, rehabilitációja.
   In: A gyermek-rehabilitáció sajátosságai /Szerk. Vekerdy-Nagy Zsuzsanna, Medicina Könyvkiadó Zrt., Budapest, 145-150, 2019.

4. Buglyó, G., **Magyar, Á.**, Biró, S., Csízy, I., Beyer, D., Molnár, K., Oláh, É.: Nucleotide Transition 390C-T in the Wilms' Tumor 1 Gene: a Risk Factor of Hypospadias?
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5. Szakszon, K., Szegedi, I., **Magyar, Á.**, Oláh, É., Andrejkovics, M., Balla, P., Lengyel, A., Berényi, E., Balogh, I.: Complete recovery from psychosis upon miglustat treatment in a juvenile Niemann-Pick C patient.
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6. **Magyar, Á.**, Csízy, I.: Csecsemő-, gyermek- és serdülőkori ovárium ciszták sebészi kezelése. *Gyermekgyógyászat. 62* (3), 148-152, 2011.

7. Józsa, T., **Magyar, Á.**, Cserni, T., Szentmiklósi, J. A., Erdélyi, K., Kincses, Z., Rákóczy, G., Balla, G., Rőszer, T.: Short-term adaptation of rat intestine to ileostomy: implication for pediatric practice.
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8. Cserni, T., **Magyar, Á.**, Németh, T., Paran, S., Csízy, I., Józsa, T.: Atresia of the ileocecal junction with agenesis of the ileocecal valve and vermiform appendix: report of a case.
Surg. Today. 36 (12), 1126-1128, 2006.
DOI: http://dx.doi.org/10.1007/s00595-006-3302-x
IF: 0.698

Total IF of journals (all publications): 11,102
Total IF of journals (publications related to the dissertation): 7,068

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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