Brain Extracellular Matrix and the Connection with Neuropsychiatric Disorders

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SVEUČILIŠTE U RIJECI
ODJEL ZA BIOTEHNOLOGIJU
Preddiplomski sveučilišni studij
„Biotehnologija i istraživanje lijekova“

Virna Nilović

IZVANSTANIČNI MATRIKS MOZGA I POVEZANOST S NEUROPSIHIJATRIJSKIM POREMEĆAJIMA

Završni rad

Rijeka, 2023.

Mentor: izv.prof.dr.sc Jelena Ban

Bachelor’s thesis was defended on July 18th, 2023.

In front of the Committee:

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This thesis has 40 pages, 14 figures, 4 tables and 38 citations.

SUMMARY

The extracellular matrix (ECM) of the brain is a complex meshwork of molecules that surrounds and supports neuronal and non-neuronal brain cells. The major components of the brain ECM include glycosaminoglycans (GAGs), proteoglycans (PGs), hyaluronan and matrix-degrading enzymes. The most important components are chondroitin sulfate PGs lecticans which form aggregates with other matrix components and contribute to the formation of perineuronal nets (PNNs) that are unique to the central nervous system. PNNs are matrix molecule assemblies surrounding neuronal somata, dendrites and axon initial segments in a lattice-like appearance. PNNs play a pivotal role in maintaining the integrity of the matrix and preserving existing synapses which is crucial for long-term memory consolidation. ECM is part of the "tetrapartite" synapse which also involves pre-synaptic and post-synaptic neurons, as well as astrocytes, and it is the key to maintaining physiological microenvironment at the synaptic cleft and propagation of nerve impulses. The brain ECM plays a central role in various physiological and pathological processes, such as cell proliferation, cell migration, apoptosis, synaptic plasticity, myelination, neuroinflammation, and neurodegeneration. Defects in ECM components or their dysregulation have been implicated in various neurodegenerative and neuropsychiatric disorders, such as Alzheimer's disease, Parkinson's disease, and schizophrenia. Research on the brain ECM is a rapidly evolving area that holds great promise for the development of novel therapeutic strategies for the disorders of the central nervous system.

KEY WORDS: *extracellular matrix, central nervous system, perineuronal nets, lecticans, "tetrapartite synapse", neuropsychiatric disorders*

SAŽETAK

Izvanstanični matriks (ECM) mozga složena je mreža molekula koje okružuju i podupiru neuralne i ne-neuralne moždane stanice. Glavne komponente matriksa mozga uključuju glikozaminoglikane, proteoglikane, hijaluronan i enzime koji razgrađuju matriks. Najvažnije komponente su hondroitin-sulfat proteoglikani zajedničkog imena lektikani koji se akumuliraju s ostalim komponentama te pridonose stvaranju perineuronskih mreža (PNNs) koje su jedinstvene za središnji živčani sustav. PNNs su agregati molekula matriksa koji okružuju tijela neurona, dendrite i inicijacijske segmente aksona. Ove mreže igraju glavnu ulogu u održavanju integriteta matriksa i očuvanju postojećih sinapsi što je važno za učvršćivanje dugoročnog pamćenja. ECM je dio "tetrapartitne" sinapse koja osim molekula matriksa uključuje presinaptičke, postsinaptičke neurone i astrocite te je ključna za održavanje fiziološkog mikrookruženja u sinaptičkoj pukotini i propagaciju živčanih impulsa. ECM mozga igra središnju ulogu u raznim fiziološkim i patološkim procesima, kao što su proliferacija, migracija, apoptoza stanica mozga, plastičnost sinapsi, neuroinflamacija i neurodegeneracija. Defekti u komponentama matriksa ili njihova disregulacija povezani su s različitim neurodegenerativnim i neuropsihijatrijskim poremećajima, poput Alzheimerove bolesti, Parkinsonove bolesti i shizofrenije. Istraživanje matriksa mozga područje je koje se brzo razvija i ima potencijala za razvoj novih terapijskih strategija za poremećaje središnjeg živčanog sustava.

KLJUČNE RIJEČI: *izvanstanični matriks, središnji živčani sustav, perineuralne mreže, lektikani, "tetrapartitna sinapsa", neuropsihijatrijski poremećaji*

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INTRODUCTION

All cell types synthesize and secrete components which form the extracellular matrix (ECM). ECM is a highly dynamic and complex 3D network composed of multidomain macromolecules that surround, support, and give structure to cells and tissues; it helps cells' adhesion and cell-to-cell communication by signal transduction which in turn influences the development of all multicellular organisms. Cells express specific ECM receptors on their surface that play a crucial role in determining and regulating many cell functions (proliferation, migration, differentiation, morphogenesis, apoptosis) [1, 3, 4]. Each tissue and organ has its distinct ECM that differs in both structure and organization and is prone to adapting to environmental stimuli and as a result, crucial in maintaining homeostasis [12]. ECM also acts as a reservoir of growth factors that aids the formation of 'scar tissue' during damaged tissue repair.

CORE MATRISOME

The multidomain macromolecules that build the ECM vary from tissue to tissue (in both structure and function) and tend to cross-link which gives stability to the matrix as a whole [3]. These variations affect the overall structure and biomechanical properties of the ECM. The “core matrisome” [2] includes approximately 300 proteins among which collagens, elastin, proteoglycans, and glycoproteins are the major components.

Collagens are the most abundant and omnipresent ECM proteins designed to give structure and resiliency to tissues. Collagens are made of three polypeptides called α-chains that form a triple helical structure, and they

are divided into a superfamily of 28 distinct genetic types, which are then categorized according to their structural similarities. Generally, there are six categories: fibril-forming collagens, network-forming collagens, beaded filaments and anchoring fibrils, fibril-associated collagens with interruptions in their triple helices, collagens with a transmembrane domain and multiplexin collagens [3, 5]. Many genetic disorders, including osteogenesis imperfecta, Ehlers–Danlos syndrome, multiple epiphyseal dysplasia, Alport syndrome and Ullrich congenital muscular dystrophy, are the result of mutations in collagen genes [4, 5].

Elastin is encoded by only one gene, as opposed to the genetic diversity of collagen superfamily. It is the major protein component of elastic fiber, which is particularly important in tissues that have to be reversibly expendable and deformable, namely lungs, skin and major vascular vessels [3].

ADHESIVE GLYCOPROTEINS

Fibronectin is an insoluble multimer critical for the attachment and migration of cells, functioning as "biological glue"[1]. Fibronectin is assembled into fibrils, that can bind over 40 distinct growth factors and cytokines, and these fibrils play a key role in composition of a provisional ECM during embryonic development and wound healing. Binding of other ECM proteins, namely collagens, elastins, and proteoglycans, to fibronectin fibrils facilitates the maturation and tissue specificity of the ECM [6].

Vitronectin is found in the biggest concentrations in the areas of tissue injury and necrosis [3]. In the nervous system, it participates in neural differentiation, neurogenesis, regulating axon size as well as supporting and guiding neurite extension. It can also reduce the permeability of the blood-brain barrier by interacting with integrin receptors and consequently plays a cardinal role in brain protection [7].

Laminins constitute a family of 20 different glycoproteins that are assembled into a cross-linked web that affect cell proliferation, migration, and differentiation; they are essential for early embryonic development and organogenesis [8].

MATRICELLULAR PROTEINS

"Matricellular" proteins are a group of extracellular proteins that function by binding to matrix proteins and to cell surface receptors, but do not contribute to the structural integrity of the ECM [3].

Tenascins are a family of five ECM glycoproteins: -C, -R, -X, -W and -N; out of the five, only tenascin-C (TNC) and tenascin-R (TNR) are found in the central nervous system (CNS) [11].

Proteoglycans constitute several genetically unrelated families of multidomain proteins that act as a backbone to which polysaccharide side chains are covalently attached. Aforementioned polysaccharides consist of repeating disaccharide subunits, and they are called glycosaminoglycans (GAGs). Proteoglycans are named after the attached GAG chain - chondroitin sulfate (repeating galactosamine and glucuronic/iduronic acid), heparan sulfate (repeating glucosamine and glucuronic/iduronic acid) or keratan sulfate (repeating glucosamine and galactose disaccharide) [3]. They contain one to three negative charges per disaccharide due to the presence of sulfate and carboxylate groups. Highly negative GAG chains allow proteoglycans to bind water molecules and divalent cations creating a gel-like environment which acts as a mechanical support to the matrix [3].

Another constituent that is also composed of glycosaminoglycans but lacks the protein core is hyaluronan (HA). HA is made of repeating unsulfured N-acetylglucosamine and glucuronic acid subunits and it is present in all tissues and body fluids. HA facilitates cell migration and because of that, it

is found in many developing tissues and in pathological conditions such as high-grade tumors (colon cancer, melanoma) [4].

The proteoglycan family of matrix proteins is a heterogenous group divided into four categories based only on distinguishing characteristics, not structural similarities: large proteoglycans that form aggregates by interacting with hyaluronan – lecticans (versican, aggrecan, neurocan, brevican), basement membrane heparan-sulfate proteoglycans (HSPGs: perlecan, agrin), cell surface HSPGs that are further classified into two major families – syndecan-like integral membrane HSPGs (SLIPSs) and glypican-related integral membrane HSPGs (GRIPs) and small leucine-rich proteoglycans (SLRPs: decorin, biglycan, fibromodulin, lumican and epiphycan) [3, 4].

CENTRAL NERVOUS SYSTEM

In the nervous system, both neurons and glial cells produce and secrete ECM components. ECM of the brain is specialized and distributed non-uniformly throughout it; it is estimated to comprise between 10% and 20% of the total brain volume [12, 13, 20]. The brain matrix has some unique components and functions; it is based on aggregates of HA and chondroitin sulfate proteoglycans connected by glycoproteins, and posttranslational remodeling proteases but has relatively small amounts of fibrillar collagens and fibronectin, apart from the basement membrane, meningeal layers, and blood-brain barrier [4, 14, 29]. The most important proteoglycans present in the CNS are mostly secreted chondroitin sulfate proteoglycans (CSPGs) and membrane-bound heparin sulfate proteoglycans (HSPGs). Proteoglycans secreted by neuronal and non-neuronal cells co-aggregate and form extracellular structures called perineuronal nets (PNNs) around cell somata, axon initial segment and dendrites; they are also the major components of the glial scar tissue after CNS injury. PNNs are important in

maintaining the stability of synapses and they have a somewhat of a dual nature: while preserving the existing synapses, they restrict plasticity and synaptogenesis and by forming glial scars, they limit axonal regeneration [14, 26]. PNNs have fundamental functions in the physiological processes of the brain, such as cognitive learning and long-term memory consolidation [14, 25].

The ECM has multiple roles in the CNS physiology (both in development and adulthood) as well as pathology. Abnormal changes in the composition and structure of the ECM molecules are associated with the development and progression of several pathologic conditions and diseases such as neurodegenerative diseases (NDD) and dementia, epilepsy, and neuropsychiatric diseases such as mood disorders and schizophrenia (SZ) [15, 19, 38].

PURPOSE STATEMENT

The purpose of this thesis is to research and summarize the literature on the differences between systemic ECM and the matrix of the central nervous system with emphasis on the brain. The brain is a complex multifunctional organ that controls almost every process that regulates the human body including breathing, temperature, hunger, and superior cognitive processes such as perception, memory, and emotion and as such, has a distinctive composition of the ECM. The components tend to aggregate and form structures unique to the central nervous system. Both the differences in the composition and the presence of unique structures around brain cells are associated with the occurrence and progression of NDD and neuropsychiatric disorders. This thesis assembles the recent findings on specific components of the brain ECM and their contribution to the occurrence of neuropsychiatric disorders. Similar mechanisms might underlie the pathology of schizophrenia and mood disorders which is explained in the last part of the thesis.

MAIN PART

EXTRACELLULAR MATRIX OF THE BRAIN

The brain is a complex organ that has many distinct regions in which the cells are diversely organized due to the different ECM components and the expression of the neuronal and non-neuronal cells' receptors for these components. ECM components are anchored to cell membranes through a variety of cell surface molecules, such as the cell adhesion molecule (CAM) superfamily - integrins, membrane bound proteoglycans (mostly HSPGs) and hyaluronan synthase [29].

The ECM of the brain executes many functions crucial in maintaining homeostasis. It has an essential architectural role in regulating biomechanical properties and creating a framework for the CNS structure. It creates a permissive microenvironment that regulates cell migration, differentiation, i.e. neurogenesis and gliogenesis, axonal outgrowth, guidance and myelination, and synaptogenesis during the nervous system formation (Table 2) [16]. ECM is distributed unevenly throughout the brain and consequently each brain region expresses different ECM proteins and has a unique microenvironment [12,18]. It acts as a physical barrier and controls diffusion rates of membrane-associated receptors, neurotransmitters, and ions, and it also controls the production and distribution of growth and differentiation factors which is essential for cell survival. The ECM of the adult brain provides an inhibitory microenvironment that affects cell survival, plasticity, damage response and regeneration by preventing abnormal remodeling of the neuronal circuits [12, 13, 15].

JUVENILE AND ADULT ECM

The brain possesses a remarkable ability to continuously reshape itself throughout the entire lifespan in a process called plasticity [32]. ECM composition, quantity and structure undergo dynamic remodeling during CNS development. The highest quantity of ECM relative to the cell mass is during the early prenatal development phase, and gradually decreases towards the late prenatal phase. Astrocytes are the predominant source of ECM molecules (Figure 14) which serve both structural and signaling roles in the juvenile form of ECM. The juvenile form of ECM is present in late embryonic phase and early postnatal life and supports neural patterning which involves several stages of neurodevelopment [29, 38]. The first stage encompasses neurogenesis and proliferation which is followed by cell migration. While the other processes of neuronal development continue into postnatal life, migration is finished by the time of birth. After arriving at their final destination, neurons form dendrites and axons that distinguish them from the other cells, and they differentiate into subsets. Glial cells insulate axons with a layer of myelin which is important for the physiological functioning of the CNS. The formation of neurites enables the creation of connections between neurons in a process called synaptogenesis which is crucial for the propagation of action potentials that are accelerated by myelinated axons.

Almost all cell types in the developing CNS secrete CSPGs that provide critical cues for these processes. CSPGs modulate chemical properties required for traction or repulsion of migrating cells and growing axons, and dictate with which growth factors, chemokines, axon guidance molecules and other ECM factors can cells interact (Figure 1). They progressively accumulate around cell body, axon initiation segment, dendrites, and synapses of certain types of neurons and along with other components of the ECM, contribute to the formation of specialized PNNs whose structure varies across different brain regions [14, 29].

The PNNs are established during critical period at relatively late stages of postnatal development; the critical period is a time of activity-dependent stabilization of synaptic connections and neural circuitry [19]. Another process that begins during postnatal development is called synapse pruning, during which excessive synaptic formation is followed by elimination of less active synapses [32]. Together with synapse pruning, PNNs regulate synaptic connectivity and plasticity and the final changes in the composition of the ECM correlate with the closure of the critical period and PNNs maturation in an activity-dependent manner [16].

Maturation of the PNNs induces a shift from juvenile forms of plasticity to more restricted mature forms of the adult brain, in which the ECM provides a non-permissive environment for axonal outgrowth and regeneration [19, 29]. The adult CNS still retains the ability to promote functional plasticity and ECM molecules are the key regulators of the delicate balance between structural remodeling and stabilization of neuronal circuits (Figure 1) [33]. Structural remodeling of the extracellular milieu is performed by matrix-degrading enzymes which can digest ECM components or generate proteolytic fragments that then act as signaling molecules [33]. CSPGs have the main role in stabilization of the neuronal circuits as part of the PNNs or by forming clusters with glial cells [32]. These molecules are important for adult neurogenesis and differentiation [37].

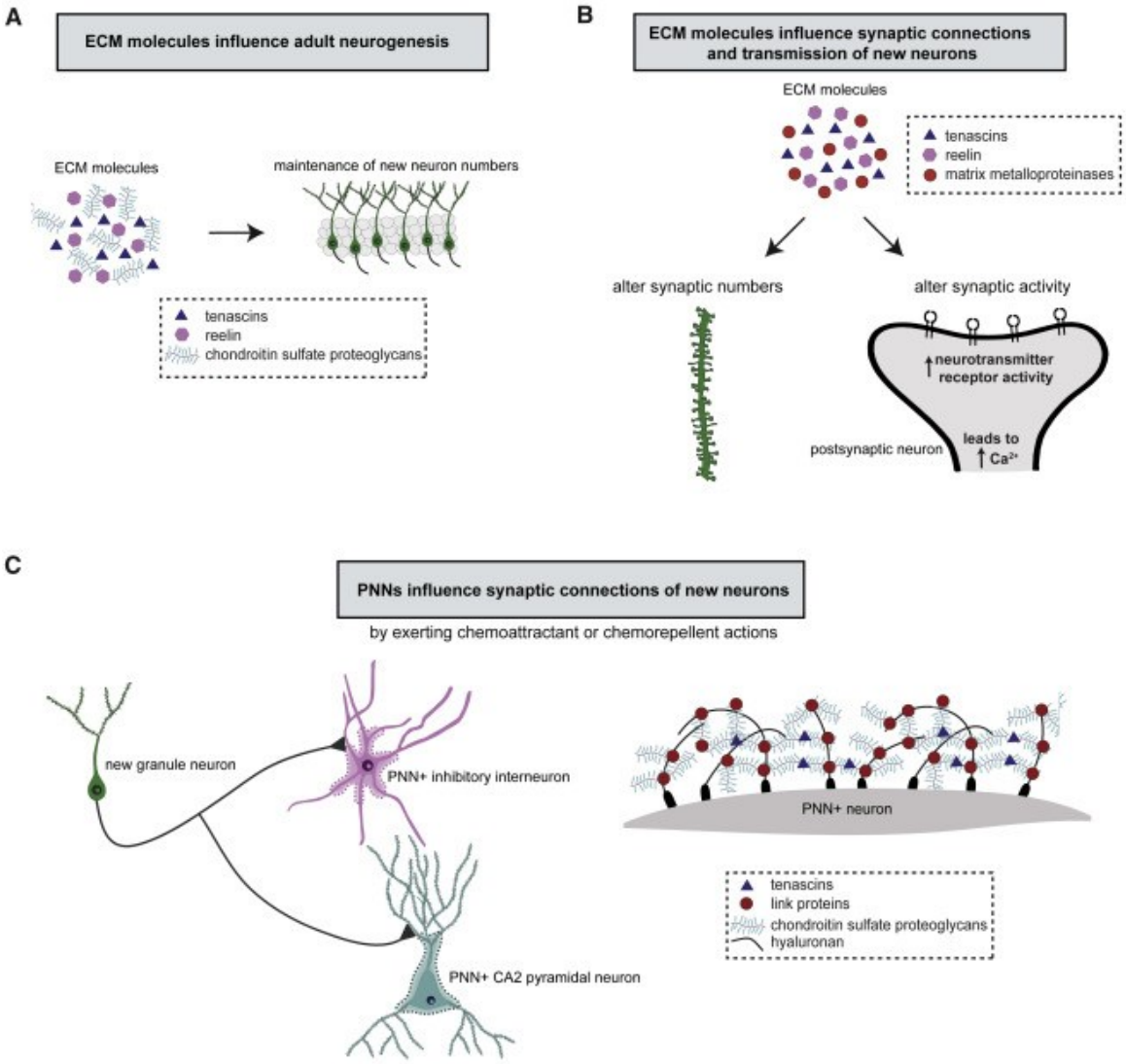


Figure 1 *ECM molecules as the key factors in neural patterning and adult neurogenesis - connections between different neuronal subtypes are shown [37]*

COMPARTMENTS OF THE BRAIN ECM

The brain ECM can be divided into three areas that vary in composition: loosely organized basement membrane around the cerebral vessels, dense perineuronal nets (PNNs) and amply distributed interstitial matrix situated between CNS parenchyma (Figure 2) [12].

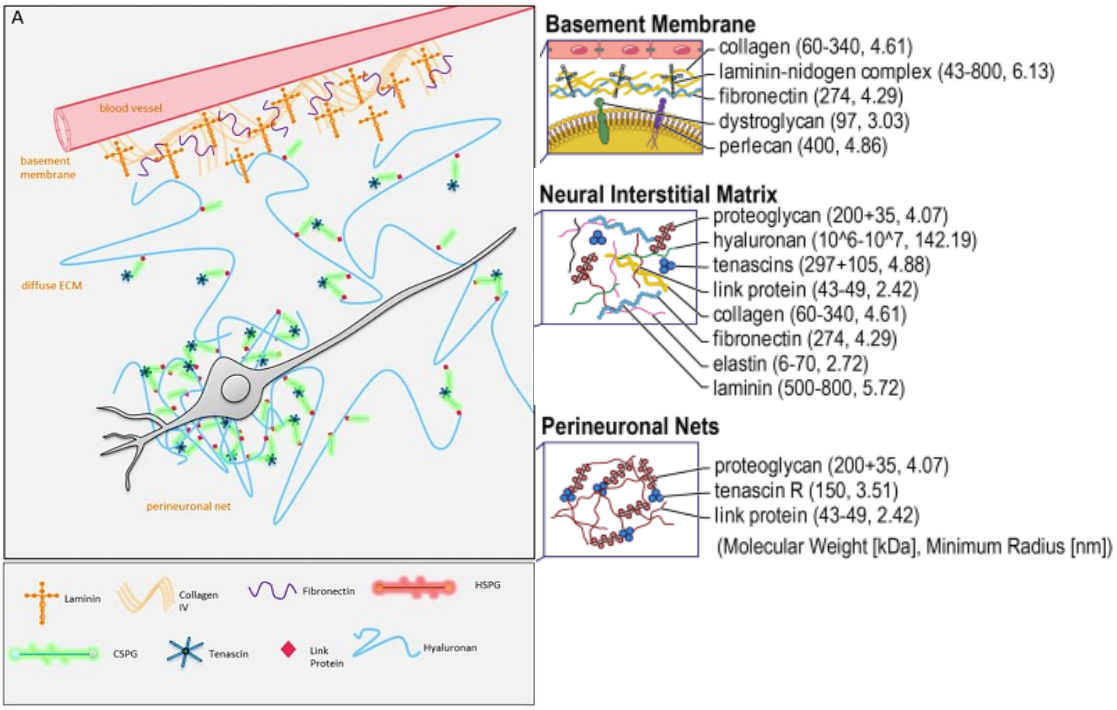


Figure 2 *Three compartments of the brain and the difference in composition [13, 21]*

The ECM of the nervous system is different than that of the systemic ECM because it is dominated by GAGs, especially HA, while ECM of other tissues consists mainly of collagens [13]. Many components of the ECM can be found in other tissues such as collagens or laminins (Figure 3), but some of the proteoglycans are only expressed in the nervous tissue. PNNs as a third compartment of the ECM are unique and can only be found in the adult brain ECM.

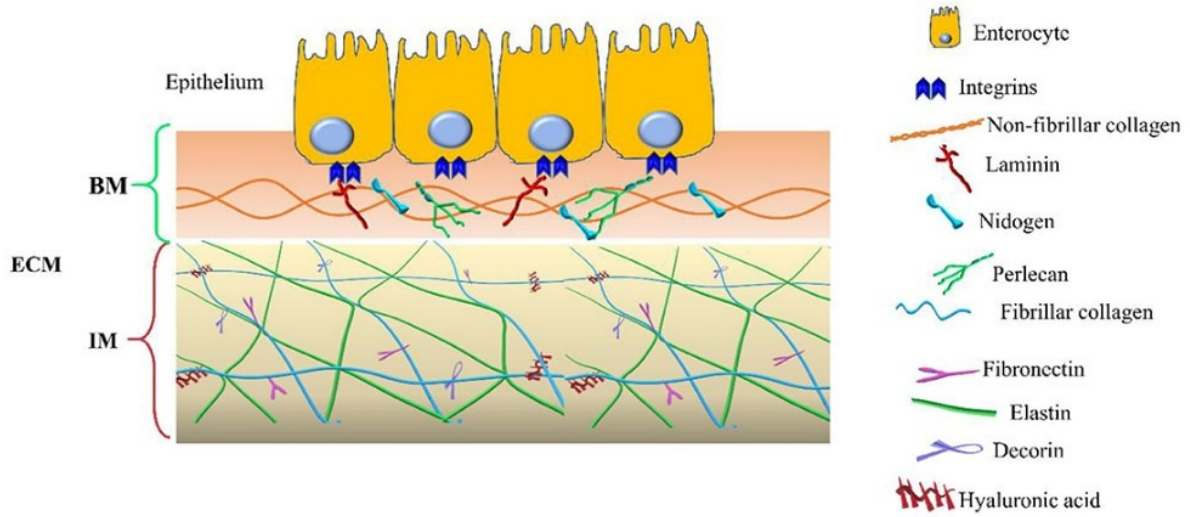


Figure 3 *Two compartments of the intestinal ECM and the abundance of collagens [17]*

The basement membrane is mainly composed of type IV collagen, fibronectin and HSPGs (mainly perlecan and agrin) and preserves the blood-brain barrier (Table 1); it plays a key role in establishing and maintaining cell polarity and differentiation [12,18].

Accumulation of CSPGs along with HA, TNR and link proteins contributes to the formation of PNNs (Table 1) which have a central role in maintaining the integrity of the ECM and neuronal circuitry; at the same time, PNNs inhibit axonal regeneration and restrict structural synapse plasticity; they are numerous within the hippocampus, auditory, visual, and somatosensory cortices [12, 13, 19]. PNNs are highly negatively charged due to the sulfated residues of GAG chains in CSPGs which facilitate interactions with ions and signaling molecules, including growth factors, and provide neuroprotection against oxidative stress [31].

The interstitial matrix is composed of proteoglycans, HA, tenascins, link protein, fibrous proteins (collagens and elastin) and glycoproteins (fibronectin and laminin) and its role is to provide tissue hydration and facilitate crosslinking the matrix components to enhance its integrity and stability [18].

COMPONENTS OF THE BRAIN ECM

Component	Interstitial matrix, PNNs, axonal and synaptic compartments	Basement membrane
Basic scaffold	HA, lectican type CSPGs, tenascin R, link proteins	Type IV collagen, laminin, fibronectin
Associated membrane-bound and secreted PGs	Cell surface CSPGs (RPTP-β*), phosphacan, membrane-bound HSPGs (syndecan and glypican)	Agrin, perlecan

**Receptor-type protein-tyrosine phosphatase*

Table 1 *Basic components of the brain ECM – adapted from [19]*

BASIC SCAFFOLD – PNNs, INTERSTITIAL MATRIX

GLYCOSAMINOGLYCANS

HA is the backbone of the ECM synthetized mostly by astrocytes and it is widely distributed in both diffuse interstitial matrix and in PNNs (Figure 7). Astrocytes also express HA-degrading enzyme hyaluronidase and other matrix-degrading enzymes and play a central part in both the synthesis and catabolism of HA [38]. Its concentration in the brain is abundant at all times but it is even higher in pathological conditions, such as high-grade gliomas [4, 13]. Many of its functions are due to its hydrophilic nature and therefore space-filling and hydrating properties; these properties secure the organization of the brain compartments and maintenance of the ionic gradients necessary for optimal cell function. HA facilitates cell migration and wound repair and maintains the integrity and stability of the ECM by forming clusters (aggregates) with different matrix proteins that lead to the formation of dense protective PNNs [4, 12, 25].

HYALURONAN-BINDING PROTEOGLYCANS – LECTICANS

CSPGs are distributed throughout the CNS and their functions vary as a result of interactions with different core proteins, glycation and the sulfation of GAGs. Lecticans (hyalectans) are the most abundant secreted CSPGs present in interstitial matrix responsible for the formation of the PNNs; they consist of N-terminal domain that binds HA and C-terminal domain that binds TNR. There are four types present in the brain: aggrecan, versican, neurocan and brevican [4]. Lecticans are the organizers of the brain ECM and, as the main component of the PNNs which inhibit axonal regeneration, the key regulators of neural plasticity [28, 30].

Aggrecan is a very large proteoglycan expressed by astrocytes and neurons and found almost exclusively in PNNs. It is heterogeneously glycosylated and different glycoforms are expressed across various regions which suggests that differential glycosylation potentially modulates its functions, which are not yet completely understood, but research points to a few possible roles. Aggrecan potentially secures high-rate synaptic transmission and mechanically stabilizes synapses which leads to adult ECM being the key in providing a non-favorable milieu for plasticity; it could also be responsible for the neuroprotective role of the ECM and reduction of oxidative stress [35].

Versican occurs in four isoforms present in almost all tissues. Isoforms V0 and V1 are expressed during prenatal phase of brain development and V2 is expressed from the neonatal period to adulthood by oligodendrocytes and their precursors. It is present in dense complexes with other ECM molecules around the nodes of Ranvier, which are the gaps in myelin sheath that encloses axons, and it is commonly involved in neuronal adhesion and axonal growth, but its specific role is not completely clear yet [11, 12].

Brevican and neurocan are found only in the nervous tissue [12]. Brevican is released by both neuronal and non-neuronal cells and its concentration

is at its highest during gliogenesis in the development phase of the brain, following brain injury or in primary brain tumors; it potentially modulates axonal outgrowth and mediates plasticity and synaptic transmission by regulating the localization of potassium channels [14].

Neurocan is expressed by neurons, and it is highly synthetized during late embryogenesis and its expression declines promptly in the postnatal phase. It contributes to the development of mature PNNs by modulating transcriptional and translational processes of various ECM molecules that contribute to the formation of PNNs, such as brevican and aggrecan [36].

ADDITIONAL GLYCOPROTEINS

HAPLN

Link proteins are small glycoproteins that stabilize the interaction between HA and lecticans by binding to both independently (Figure 4) [4]. Link proteins belong to the same *Hapln* gene family which includes four members, among which, three are expressed primarily in the brain (Hapln1/Crtl1, Hapln2/Bral1 and Hapln4/Bral2). Hapln1/Crtl1 and Hapln4/Bral2 are found in PNNs, while Hapln2/Bral1 is present around the nodes of Ranvier [34].

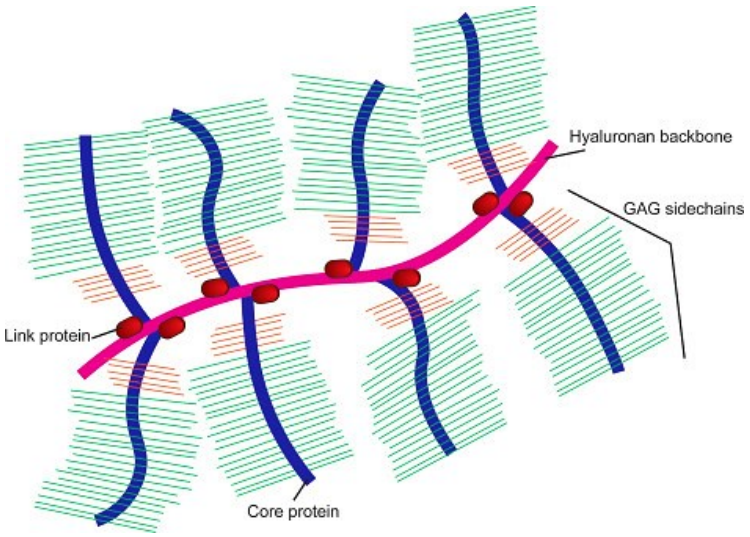


Figure 4 *Lectican-hyaluronan cluster [20]*

TENASCIN-R

TNR is only synthesized in the CNS by subsets of interneurons and oligodendrocytes during myelination [20, 22]. It affects neurite outgrowth and synaptic transmission and contributes to the formation of quaternary complexes with HA, lecticans and link proteins which are the key to the assembly of the PNNs [30, 36]. Lectican properties such as size and glycosylation and their interactions with the ligands of the quaternary complexes affect compactness and therefore the functions of the PNNs (Figure 7) [34]. The matrix becomes less stiff if these interactions are altered or if the amount of HA increases which provides a favorable microenvironment for the plasticity of the brain (Figure 5) [30, 36].

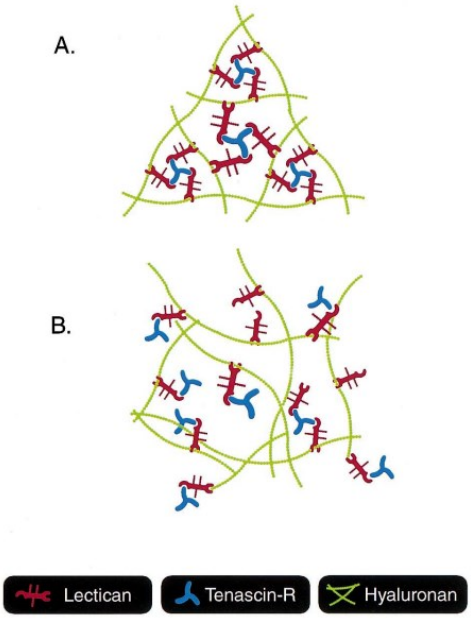
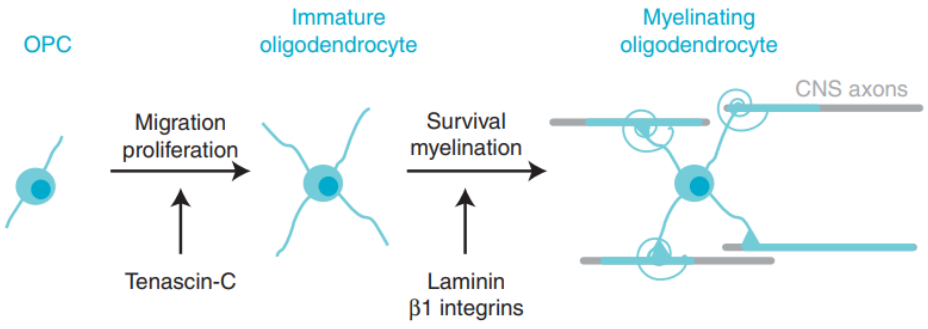


Figure 5 *Quaternary complexes form PNNs (link proteins not shown) [30]*

TENASCIN-C

TNC is expressed both in systemic ECM and in the ECM of CNS, in neural and non-neural cells during development of the CNS and tissue repair [13]. It forms aggregates with HA, aggrecan and link protein Hapln1 in PNNs [11, 36]. TNC has a key role in the first stage of generating mature oligodendrocytes that are responsible for myelination of axons in the CNS (Figure 6) [22].



**OPC = oligodendrocyte precursor*

Figure 6 *Tenascin-C and myelination in the CNS [22]*

BASIC SCAFFOLD - BASEMENT MEMBRANE

Collagens are not abundant in the ECM of the CNS, but type IV collagen is present in the basement membrane surrounding cerebral vessels (Table 1). Type IV collagens form a more flexible triple helix which acts as an insoluble scaffolding and integrates laminins and fibronectin [4, 13].

Laminins are not abundant in the brain, except in the basement membrane and interstitial matrix where they help maintain the integrity and regulation of the blood-brain barrier [9]; laminins are critical for layer formation in the developing neocortex and play roles in differentiation, cell migration and the survival and maturation of myelinating glia during development [4, 19, 22].

Fibronectin is produced by astrocytes during the development of the CNS and participates in the mechanisms of migration, axon elongation and guidance, but its role in the adult brain is still unknown, as it is not highly expressed [19]; it has been observed in different brain tumors, such as glioblastomas, and it is upregulated in case of injury [4].

NON-HYALURONAN BINDING PROTEOGLYCANS

MEMBRANE-BOUND AND SECRETED PROTEOGLYCANS - PNNs AND INTERSTITIAL MATRIX

Receptor-type protein-tyrosine phosphatase (RPTP) family of enzymes encompasses at least eight sub-families of which, a variant called RPTP-β is exclusively synthetized in the nervous system. RPTP-β is involved in oligodendrocyte survival and in memory formation in the hippocampus [14]. Phosphacan, a product of alternative splicing of RPTP-β, is a secreted CSPGs synthesized by astrocytes. It is mainly expressed in the brain and

strongly inhibits neurite outgrowth; it is a high affinity ligand for TNC and TNR and it can stimulate proliferation of the cells [4, 12].

Membrane-bound HSPGs include syndecan and glypican. Syndecan acts as a receptor for growth factors, and it is involved in growth factors signaling that controls neural migration. Glypican is important during neuronal development because it binds several critical proteins, such as growth factors and axon guidance molecules (Figure 7) [19].

MEMBRANE-BOUND AND SECRETED PROTEOGLYCANS – BASEMENT MEMBRANE

Agrin is a HSPGs synthesized by motor neurons and expressed in high concentrations in the developing brain, and it is believed to promote formation and specialization of synapses and differentiation of presynaptic motor neurons. It can bind to tenascins, laminins, and thrombospondin [4, 12, 22]. Agrin and perlecan are crucial in the organization of the basement membrane and in signaling pathways [19].

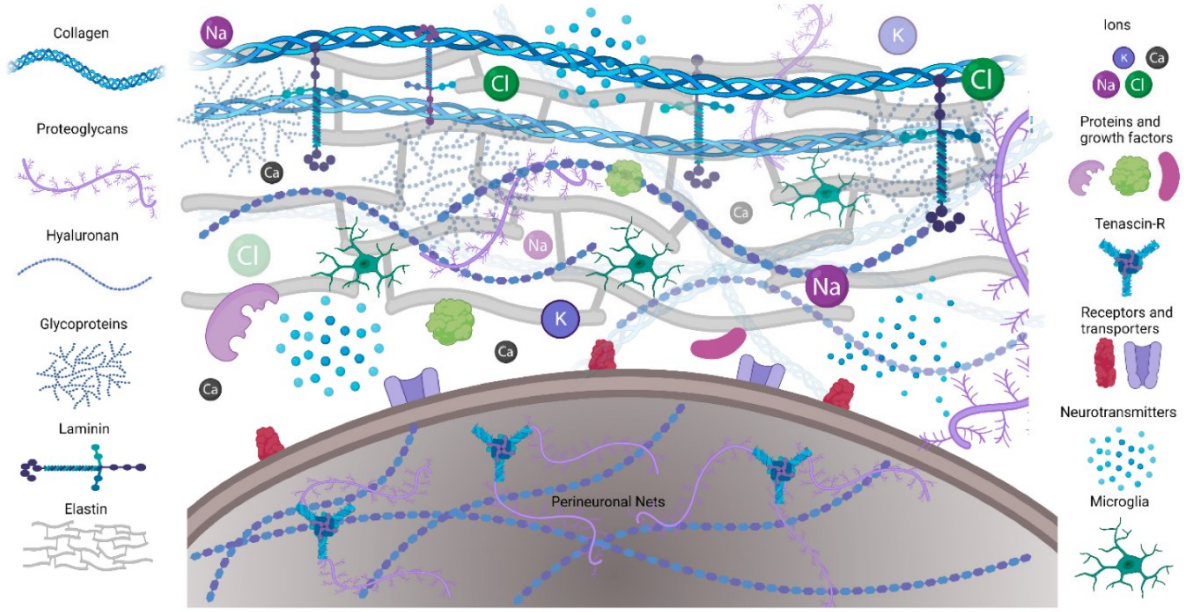


Figure 7 *Composition of the basic scaffold and PNNs in the ECM of the CNS [31]*

ECM AROUND NEURONS

PERINODAL ECM

The nodes of Ranvier enable spreading of action potentials because they are rich in voltage-gated sodium channels. These gaps expose axons to the ECM molecules which stabilize the nodes and regulate communication between neurons and adjacent astrocytes (Figure 8). The ECM is more complex around larger diameter axons and includes lecticans, phosphacan, HA, TNR and link proteins. Versican or neurocan and brevican co-localize and the complex between them and HA and TNR is stabilized by a brain-derived link protein called Bral1 (Hapln2) [14, 23]. A simpler matrix surrounds the nodes of smaller axons, and it is comprised of only versican, HA and Bral1 (Figure 9). Bral1 is vital for neuronal conductivity, and it has been identified as a contributor to the pathology of several neurological disorders such as Parkinson's disease and SZ (Figure 10) [23]. Brevican deficiency causes a reorganization of the nodal matrix which suggests that it conditions the axon diameter-dependent specialization of the hyaluronan-lectican clusters [29].

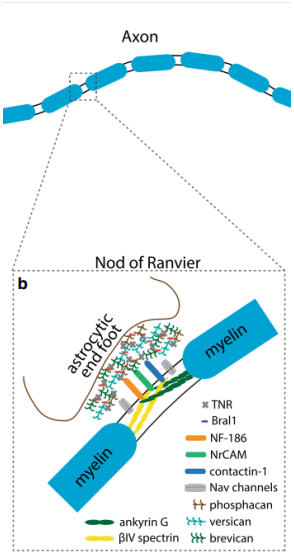


Figure 8 *ECM stabilizes and regulates neuron-glia communication [14]*

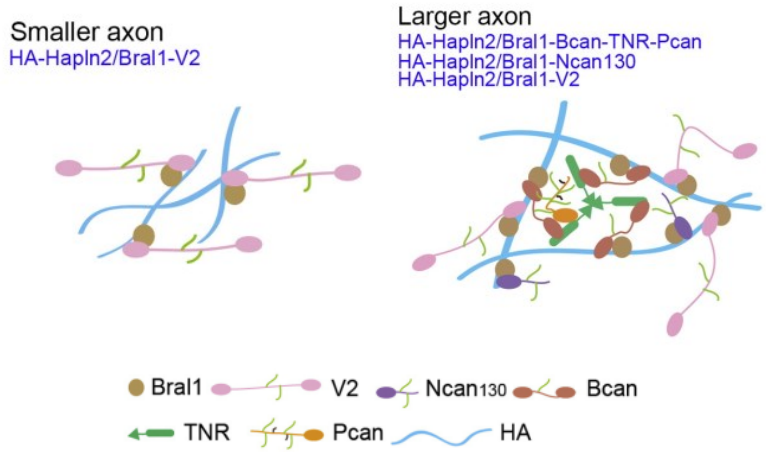


Figure 9 Axon diameter-dependent composition of the nodal ECM [35]

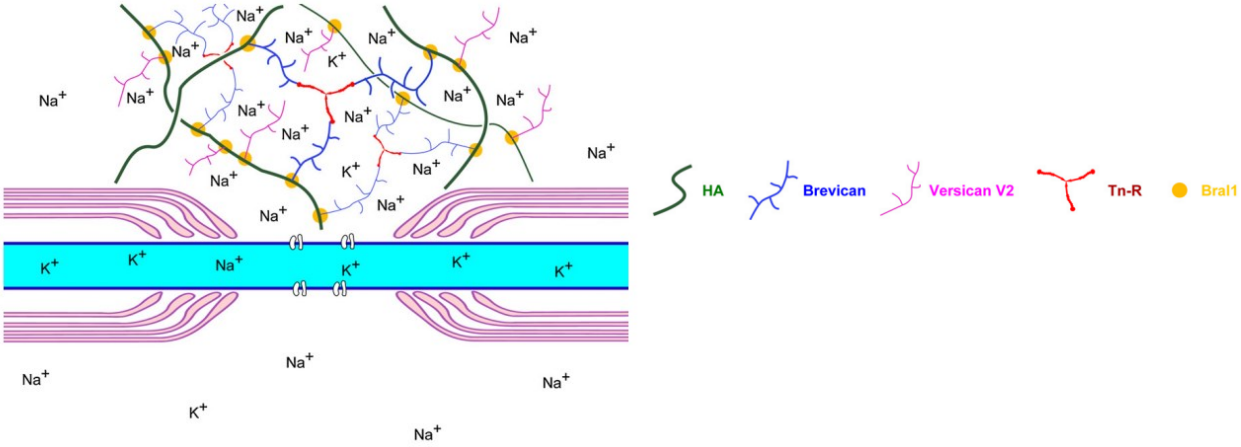


Figure 10 *Decreased concentration of positive ions in the absence of a Bral1-associated ECM cluster [24]*

TETRAPARTITE SYNAPSE

Synaptic regulation is an important ECM function. Several ECM molecules, including reelin, CSPGs, TSPs, laminins and tenascins, together with the main group of ECM receptors, integrins and neural CAMs (N-CAMs), cooperate to regulate synaptogenesis, synaptic stabilization and maintenance, plasticity, and homeostasis [29]. Those interactions between ECM, glial and neuronal elements are fundamental for synaptic functions, and it has been suggested that ECM molecules participate in the formation of "tetrapartite" synapse (Figure 11), as opposed to "tripartite" (presynaptic and postsynaptic elements and astrocytes) which omits the contribution of ECM [20, 29].

Neuroglycan C (NGC) is a transmembrane proteoglycan expressed only in the CNS where it is involved in synaptogenesis and neurite outgrowth [14].

Thrombospondins (TSPs) are a family of multifunctional calcium-binding glycoproteins that interact with neuronal receptors and bind components of the ECM. They are secreted by astrocytes and mainly expressed during the early postnatal period when synapses form between neurons; TSP1 and TSP2 are expressed in immature astrocytes, and they can induce

synaptogenesis both *in vitro* and *in vivo*. TSP4 is involved in local signaling in the nervous system and contributes to spinal sensitization and neuropathic pain [14,18].

Reelin is a secreted signaling glycoprotein that is largely expressed in the brain. During development, reelin is synthetized by Cajal-Retzius cells and is critical because it acts as the main regulator of corticogenesis and migration of cells. Cajal-Retzius cells almost completely disappear when the migration of cortical neurons is completed, and GABAergic interneurons start expressing reelin. In the adult brain, it modulates synaptic signaling pathways and regulates synaptic plasticity and neurite outgrowth; it seems to be essential for superior cognitive functions including learning and memory consolidation [14, 26, 29].

Growing evidence points to synaptic pathology across several brain disorders, including SZ, mood disorders, autism spectrum disorder and Alzheimer's disease; distinction between synapses in different brain regions and mechanisms underlying synaptic pathology of a spectrum of brain disorders may explain the varied pathophysiological manifestations of the diseases [32].

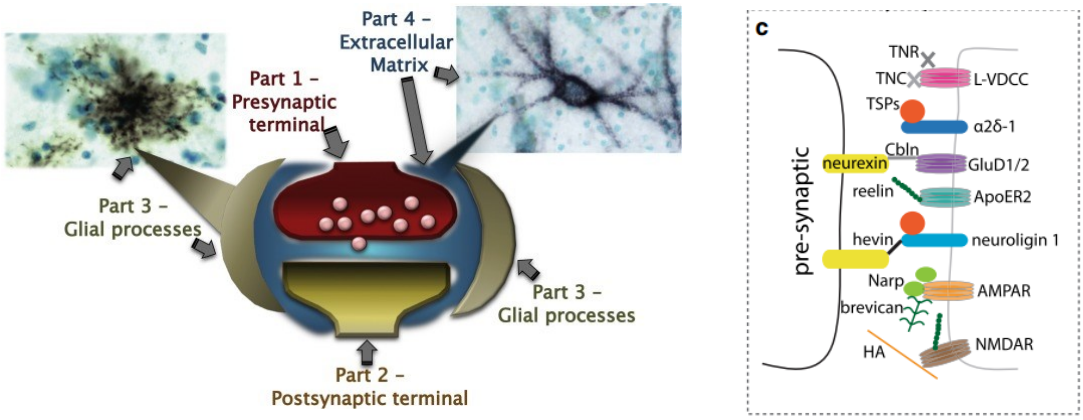


Figure 11 *"Tetrapartite synapse" and its components [14, 32]*

COMPONENTS OF THE ECM AND THEIR FUNCTIONS

ECM functions	ECM components
Neuronal migration	Laminin Proteoglycans Tenascin Thrombospondin Reelin
Axon outgrowth and myelination	Fibronectin Vitronectin Laminin Collagen Proteoglycans Tenascin Thrombospondin
ECM functions	**ECM components**
Dendritic spine formation and synapse control	Laminin Fibronectin Tenascin Proteoglycans (Agrin) Thrombospondin Reelin Lectins MMPs
Neuromuscular junction development and synaptogenesis	Proteoglycans—Agrin and chondroitin sulfate proteoglycans (CSPGs) Laminin Collagen IV Reelin Thrombospondin

Table 2 *Components of the ECM of the brain and CNS classified according to the function [18]*

ECM REMODELING

The composition and characteristics of the ECM are constantly changing; the components of the ECM are deposited, degraded, or modified under physiological settings and aging, and under pathological conditions. Matrix-degrading enzymes and their inhibitors (TIMPs - tissue inhibitors of MMPs) are present at an equilibrium concentration to maintain homeostasis. The concentration and activation of two major metalloprotease families must be regulated and tightly coordinated [18].

MATRIX METALLOPROTEINASES

Metalloproteinases are zinc-containing endopeptidases, synthetized by neurons, astrocytes (Figure 14), microglia, and endothelial cells [38]. Proteolytic enzymes called matrix metalloproteinases (MMPs) are a family of 24 enzymes divided into aggrecanases, collagenases, gelatinases, stromelysins and membrane-type MMPs (Table 3). MMP substrates are wide-ranging and nonspecific, they can degrade all protein components of the ECM as well as growth factors, cell adhesion molecules and receptors. MMP-3 degrades CSPGs after CNS injury, but at the same time degrades collagens, fibronectin, and laminins. Aggrecan can be degraded by twelve members of this family. Some MMPs restrict the formation of an inhibitory glial scar by degrading proteoglycans; MMP-2 specifically degrades neurocan and versican, while MMP-3 degrades TNC, brevican and phosphacan [13].

Name	Examples	Functions
Aggrecanases	ADAMTS -1, -4, -5,- 9, -15	Cleave and fragment aggrecan core protein
Collagenases	MMP-1, -8, -13	Cleave fibrillar collagen
Gelatinases	MMP-2, -9	Degrade type IV collagen, fibronectin, laminin, aggrecan, elastin
Stromelysin	MMP -3, -10	Digest noncollagenous matrix proteins and degraded collagen
Matrilysin	MMP -7	Degrade aggrecan

Table 3 *Main proteinases and functions – adapted from [18]*

ADAMTS

More specific matrix remodeling enzymes that belong to the same superfamily of metalloproteinases as MMPs are ADAMTS (*a disintegrin and metalloproteinase with thrombospondin motifs*). ADAMTS is a family of 19 secreted enzymes that generally have the same linear structure of prodomain, metalloprotease domain, disintegrin domain, central TSP-1-like domain, cysteine-rich region, and a variable number of TSP repeats at the

C-terminus (Figure 12). The presence of specific structural domains differentiates the members of the family, out of which, some participate in the degradation and subsequential renewal and alterations of proteoglycans of the ECM in the CNS. ADAMTS enzymes have been detected by different techniques in most CNS structures, including the hippocampus, striatum, cortex, temporal lobe, brain stem, and spinal cord; they are synthesized (Figure 14) mostly by astrocytes (specifically after a brain injury), although microglia and neurons also express them [26].

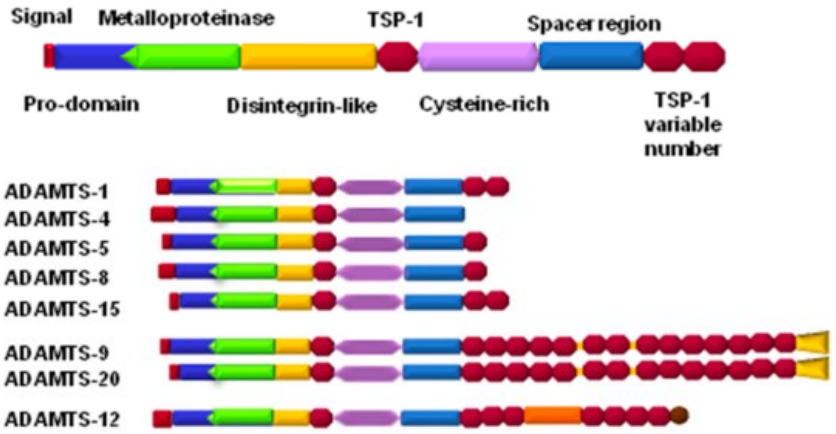


Figure 12 *Schematic representation of ADAMTSs involved in proteolysis of the ECM components of the CNS [26]*

ADAMTS – HYALECTANASES

ADAMTS-1, -4, -5, -8, -9, -15, and -20 are considered to be hyalectanases, enzymes responsible for the degradation of lecticans (Table 4) [26]. Aggrecan can be degraded by several members of the ADAMTS family of proteinases, but ADAMTS-4 is expressed in the highest concentrations of all ADAMTS in the adult brain and along with ADAMTS-5, is the most effective aggrecanase [28]. Versican can be processed by various members of the family, including ADAMTS-1, -4, -5, -9, -15, -20. The cleavage of versican by ADAMTS-1 generates a bioactive fragment called versikine that is involved in apoptosis and the migration of immune cells. Brevican can be degraded by ADAMTS-1, -4 and -5 and the proteolysis generates two

possible fragments that have been connected to the pathological pathways of brevican. The overexpression of enzymes -4 and -5 has been detected in glioma cells, and a connection between increased brevican degradation by enzymes -1 and -4 and the loss of synaptic density has been described. Neurocan is considered to be a substrate for ADAMTS-12 because it has been observed that in the absence of this enzyme, neurocan accumulates in specific regions of ADAMTS-12-deficient mice's brain [26].

ADAMTSs can additionally degrade other proteoglycans such as phosphacan and reelin. ADAMTS-4 processes reelin and blocks its cellular signaling pathways and consequently causes defects in synaptic plasticity. Additionally, ADAMTS-2 and ADAMTS-3 can perform a specific proteolytic cleavage of reelin (*N-t cleavage*) that eliminates its biological activity and leads to pathological conditions [26].

ADAMTS enzymes contribute to the repair of damaged tissue after brain injuries; they degrade proteoglycans which are the main component of the glial scarring, and by degrading glial scars, they stimulate axonal growth and synaptic plasticity after injury. These enzymes have inflammatory and antiangiogenic properties, and because of those, they also lead to the progression of neurodegenerative disorders (Figure 14). The expression of these enzymes is upregulated in response to certain diseases, neuronal disorders, or CNS lesions such as Alzheimer's disease, amyotrophic lateral sclerosis, ischemic stroke, SZ, and spinal cord injury, but their role is complex, and has not yet been completely understood [26, 28].

ADAMTS	Known Substrates	Neuronal Process/Disorder
ADAMTS-1	Versican, brevican	Neuroplasticity; inflammation; Alzheimer's disease
ADAMTS-3	Reelin	Alzheimer's disease; SZ
ADAMTS-4	Versican; aggrecan: reelin; brevican	Neuroplasticity; myelination; inflammation; Alzheimer's disease, SZ
ADAMTS-5	Versican; aggrecan; reelin; brevican	Neuroplasticity; inflammation; Alzheimer's disease
ADAMTS-9	Versican	Inflammation
ADAMTS-12	Neurocan	Inflammation; SZ

Table 4 *ADAMTSs and substrates in the CNS – adapted from [26] - for additional NDD diseases related to ADAMTS, see the original version (ref 26)*

NEUROPSYCHIATRIC DISORDERS

Neuropsychiatric disorders are complex disorders that encompass both neurological and psychiatric conditions and they are characterized by variable symptoms, heterogeneous genetic variations, and widespread changes in anatomical pathology. Most neuropsychiatric diseases, including autism spectrum disorder (ASD), SZ, mood disorders and attention deficit hyperactivity disorder (ADHD), have a complex genetic component, but the underlying biological mechanisms are still mostly unknown [15]. It has been proposed that psychiatric disorders may share the neurobiological factor of impaired neurodevelopmental and adult plasticity processes, including cell migration, synaptogenesis, and synapse pruning. There is also growing evidence that ECM is an important regulator of neuronal adaptation and plasticity. Furthermore, changes in the composition of the ECM and environmentally induced ECM-mediated neural processes may underlie the development and progression of psychiatric disorders [15].

SCHIZOPHRENIA

SZ is a polygenic disorder characterized by positive symptoms such as hallucinations, delusions, hyperactivity, and negative symptoms such as apathy, flat affect, social and cognitive deficits. Onset of these clinical symptoms generally occurs during late adolescence or early adulthood and coincides with the maturation of brain areas responsible for the cognitive functions affected in SZ namely prefrontal cortex and medial temporal lobe. This has led to the hypothesis that impaired maturation of neural circuits due to impaired synaptic pruning and prolonged neuronal plasticity could be a major cause of this disorder [29]. The brain ECM and changes in the expression of its components may play a key role in pathophysiology of SZ and may contribute to different aspects of functional impairment in this disease. Results from human genetic and *post mortem* studies reveal genetic susceptibility for genes encoding several key ECM molecules, such as CSPGs, reelin, integrins, and remodeling enzymes as well as dysregulated expression of these molecules and disruption of PNNs [27].

CONNECTION BETWEEN SCHIZOPHRENIA AND ECM

PNNs AND SZ

Changes in the structure of the synapses and in the expression of presynaptic proteins as well as presence of fewer synaptic vesicles and shrinkage of the axon terminals have been reported in several brain regions. Substantial aberrations affecting CSPGs expression in glial cells and PNNs have been detected in the medial temporal lobe of patients with SZ. Expression of genes encoding for five major CSPGs in the brain (lecticans and phosphacan), has been increased in amygdala and entorhinal cortex [27, 29] that are interconnected regions involved in emotion-related learning and associative sensory information processing, while the density of PNNs has been significantly reduced (Figure 13). In contrast, a healthy

brain contains the highest density of PNNs in these regions. Changes in the CSPGs expression did not depend on altered cell numbers or risk factors (such as age of onset, the duration of illness, substance abuse) which suggests that this is an inherent feature of the disease. Several studies have associated SZ with genes encoding for neurocan and neuroglycan C, which implies that the abnormal CSPGs expression may be due to a genetic predisposition [27].

If CSPG abnormalities and dysregulation are present during development, it may interfere with neuronal migration, axon outgrowth and synaptic maturation within the amygdala and entorhinal cortex of the brain. Disruption of the accumulation of CSPGs and the formation of the PNNs during later stages of development may lead to failure to stabilize neural circuitry, incomplete maturation of neuronal populations, and improper transition to an adult form of plasticity. These defects in the amygdala may affect the ability to attribute appropriate emotional values to sensory inputs and form stable, contextualized memories, while the defects in entorhinal cortex may impair the establishment and strengthening of long-term memory and perception [29]. As a result of these defects, SZ is considered a neurodevelopmental disorder [15].

REELIN AND SZ

Extensive research supports the involvement of reelin in the disorder [29]. Several studies have shown halved levels of reelin mRNA and protein in SZ patients, which points to its potential role in the progression of the disease [15]. Reelin downregulation as a result of aberrant epigenetic mechanisms and genetic abnormalities has been detected in various brain regions (Figure 13), including the hippocampus, prefrontal and temporal cortices, and cerebellum. Findings of overexpression of DNA methyltransferases (enzymes that catalyze methylation of RELN gene promoter region) and of increased expression of methyl donor molecules, such as S-adenosyl

methionine (SAM), indicate causality between pathological epigenetic modifications and the downregulation of reelin [27, 29].

Reelin downregulation is typically coupled with decreased expression of glutamic acid decarboxylase 67 (GAD67). It has been observed that GAD67 promoter regions are also affected by hypermethylation. GAD67 is one of the two enzymes involved in the biosynthesis of GABA, which is a major inhibitory neurotransmitter of the brain. This suggests a strong link between reelin expression, the balance between excitatory and inhibitory neurotransmission and altered neuronal migration [15, 27].

Reelin downregulation has also been detected in bipolar disorder patients during a period of mania with psychosis that led to the hypothesis that the two disorders share a molecular mechanism contributing to psychosis [15].

MATRIX-DEGRADING ENZYMES AND SZ

Several studies have reported that mRNA expression of MMPs and ADAMTSs is altered in SZ; higher concentrations of MMP-9 and its inhibitor have been detected in blood samples from patients with SZ and elevated levels of the enzyme have also been observed in the blood serum of treatment resistant patients [27].

MOOD DISORDERS

Mood disorders are characterized by altered emotional state and they are generally divided into major depressive disorder (MDD) and bipolar disorder and include several related conditions. MDD is clinically characterized by the extended occurrence of a number of symptoms, including sadness, anhedonia, avolition, and cognitive deficiencies, and can be triggered by stress and traumatic emotional experiences. In bipolar disorder, depressive

symptoms alternate with periods of mania, characterized by euphoria and elevated energy, hyperactivity, and sometimes psychosis [15, 27].

The neurobiology behind bipolar disorder partially overlaps with that of MDD and is thought to affect similar neural processes and circuitry, such as synaptic plasticity in the prefrontal cortex and other areas that regulate cognition. Bipolar disorder has been associated with altered morphologies and densities of interneurons in the hippocampus and prefrontal cortex and has been proposed as a progressive neurodevelopmental disorder [15].

CONNECTION BETWEEN MOOD DISORDERS AND ECM

A link between a genetic variation of neurocan and bipolar disorder has been identified and could potentially be the cause for the period of hyperactivity/mania associated with the disease [27].

The role of reelin is more established than that of other ECM components; a reduced number of reelin-expressing cells has been found in brains of bipolar patients compared to healthy controls, while there has been a nonsignificant reduction in patients with MDD without psychosis [15]. Reelin levels have been observed to be decreased in the prefrontal cortex and in the cerebellum of bipolar patients with psychosis (Figure 13), while reelin in MDD patients remained unaffected. Bipolar disorder has been connected to a genetic variant of the reelin gene which is particularly associated with susceptibility in females. As reelin is also strongly associated with schizophrenia, it is possible that reduced reelin expression might underlie psychosis or result from it (Figure 13).

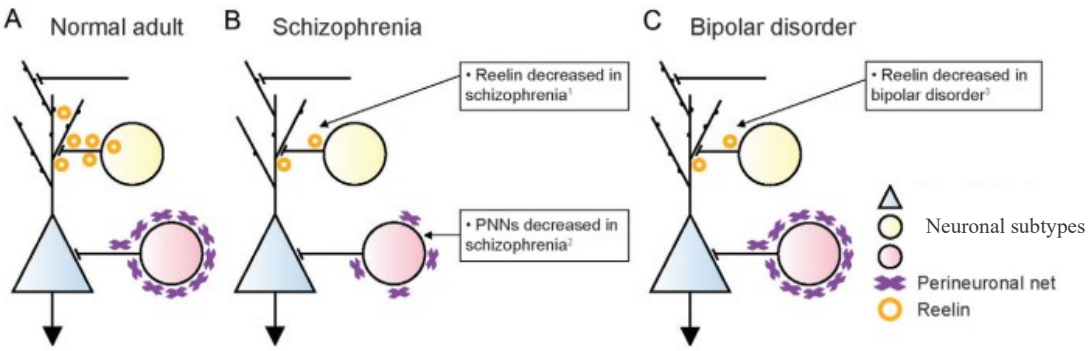
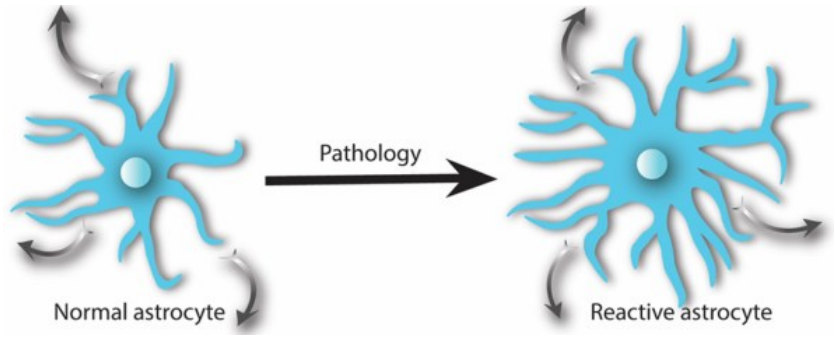


Figure 13 *Decrease in reelin expression in both disorders and PNN reduction in SZ – adapted from [15]*

Evidence for the role of the ECM in MDD is currently lacking. Animal models that examine the stress component of depression showed a strong link between reelin expression and depression in a way that reelin might contribute to resilience to stress. Stress induced with corticosterone, led to a reduction in hippocampal neurogenesis, reduced reelin-expressing cells in the hippocampi and an increase in immobility in behavioral tests; however, wild-type animals under no-stress conditions showed no difference in hippocampal neurogenesis compared to animal models. This revealed that reduced reelin expression does not affect neurogenesis at its own but might contribute to resilience to stress as it has been shown that overexpression of reelin in animals, subjected to chronic corticosterone injection, resulted in increased mobility in behavioral tests. Whether reelin affects depressive-like behavior in humans remains to be further investigated and validated [15].



Formation and Remodeling of ECM and PNNs	**Neurodegeneration**
CSPGs, lecticans, TNR, TNC, MMP-9, -3, -2, ADAMTSs	Aggrecan, neurocan, brevican, phosphacan, syndecans, glypicans, agrin, TNR, TNC, HA MMP-9, -2, ADAMTSs, TIMPs
Formation of interstitial, perinodal and perisynaptic matrix, PNNs; maintenance of ionic homeostasis, ECM remodeling, neuroplasticity	Glial scar, PNN distruption, neuronal and glial dysfunctions, ionic disbalance, inflammation, maladaptive plasticity

Figure 14 *Astrocytes synthetize main ECM components and are disrupted in NDDs - adapted from [38] - for more information on the other roles of astrocytes, see the original version (ref 38)*

CONCLUSION

ECM of the brain is a highly dynamic microenvironment undergoing constant structural modifications during remodeling processes and it plays a critical role in modulating brain development, maintaining physiological conditions and controlling plasticity. The maintenance of the balance between synthesis of the matrix components and proteolytic cleavage by matrix-degrading enzymes is of utmost importance [33]. An increasing number of studies have correlated disruptions in this balance and the changes in matrix composition with the occurrence of various neurodegenerative and neuropsychiatric disorders, highlighting the importance of understanding its role in brain health and diseases. In diseased states, increased proteolytic cleavage of PNNs disrupts their structural integrity and reduces their overall quantity but leads to restoration and an increase in plasticity of the adult CNS which can be important for the potential regeneration after injury. Several studies have also reported that preventing PNN disruption by blocking the activity of the MMPs largely alleviates disease symptoms [38]. Further research is needed to fully understand the mechanisms by which matrix influences the brain thus making ECM molecules a potential target for novel therapeutic treatments.

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EDUCATION

2015 – 2019 secondary education – Prva rijecka hrvatska gimnazija – **language major** **https://prhg.hr/**
October 2019 – NOW **Undergraduate programme of Biotechnology and Drug Research**, Department of Biotechnology, University of Rijeka **https://www.biotech.uniri.hr/en/**

ACTIVITIES

2017 – 2022 Peek&Poke Computer Museum – **volunteering**
summer 2022 – **internship** at a local plant pharmacy and learning the basics of pharmacognosy
2/2023 – **student assistant on the course** "Basics of Molecular Medicine"

SKILLS

• English – fluent (IELTS band 8) • Italian – intermediate • German – basic	• basic use of bioinformatic programs (Avogadro, ChemaxonMarvin, VMD) • excellent use of the MS Office package • excellent use of online communication tools (Skype, Zoom, MS Teams)