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The Perineuronal Net Component of the Extracellular Matrix in Plasticity and Epilepsy

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Introduction

Formation, stabilization, and maintenance of synapses require complex interactions with the extracellular environment. Therefore, the molecules that comprise the extracellular space likely play critical roles in these processes. The extracellular space of the central nervous system (CNS) is organized into a loose scaffold termed the extracellular matrix (ECM). This matrix plays a particularly important function during development of the CNS by organizing the space so that other molecules and cells within the space can function optimally (Pearlman and Sheppard, 1996; Wright et al., 2002). The ECM contributes to multiple neuronal functions including proliferation, migration, morphological differentiation, synaptogenesis, synaptic stability, and cell signaling cascades (Dityatev et al., 2003; Sandvig et al., 2004). Neurons extend processes that weave their way through a complex maze to make precise connections on a target cell. These precise connections are formed, pruned and stabilized via unique and precise interactions with the cell surface and surrounding matrix.

The perineuronal net (PN) is a unique ECM structure that is most prominently displayed around GABAergic interneurons, with parvalbumin (PV) expressing cells having the highest level of co-localization (Brückner et al., 1993; Celio and Chiquet-Ehrismann, 1993; Wintergerst et al., 1996; Morris and Henderson, 2000; McRae et al., 2007). During development the PN forms a lattice-like structure around the synapses on the cell body and proximal dendrites of interneurons, and is therefore uniquely positioned to influence synaptic development and stabilization (Hockfield et al., 1990). The appearance of the PN seems to signify the maturation of the CNS concurrent with a decrease in plasticity. Although the exact function of the PN is unknown, it is likely involved in the stabilization of existing synapses, the prevention of new synapses on mature neurons, the linkage of the ECM with the cytoskeleton, and may facilitate neuron-astrocyte interactions (Frischknecht et al., 2009; Kwok et al., 2011). The PN may inhibit growth and synapse formation (Hensch, 2003) and the perisynaptic localization of PNs around interneurons suggests a role for these structures in synaptic stabilization.

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Seizures are the result of an imbalance in excitation and inhibition and faulty GABAergic circuitry may have an essential role in initiating and ultimately maintaining the seizure prone condition of the brain. Temporal lobe epilepsy (TLE) is the most common focal epilepsy and is frequently associated with a history of prolonged seizures in childhood or other neuronal insults. Animal models provide most of our knowledge about the process of epileptogenesis, which results from various brain insults and culminates in recurring spontaneous seizures. As reviewed by Pitkänen and Lukasiuk (2009), there are numerous changes in the brain that occur during the latent period between brain injury and development of spontaneous seizures including neuronal loss, synaptic reorganization, and mossy fiber sprouting, followed by progression to chronic epilepsy.

The mechanisms for synaptic rearrangement and loss of inhibition during the development of epilepsy are still under investigation. Based on the role of the PN in synaptic stability and its location around GABAergic interneurons this structure may contribute to the progression of epilepsy. This review will provide an overview of 1) the ECM of the CNS 2) the specialized PN component of the ECM 3) the importance of activity in establishing the PN 4) the role of the PN in plasticity and 5) the PN in epileptogenesis.

1. The extracellular matrix in the central nervous system

The extracellular space (ECS) is recognized as an important mediator of neuronal plasticity (Berardi et al., 2004). The ECS comprises 40% of the brain volume in the developing brain and accounts for 20% of the brain volume in the adult (Nicholson and Sykova, 1998). While the ECS is devoid of cells, neurons and glia interact with multiple molecular components of the ECS. The ECS regulates diffusion of ions and neurotransmitters (Nicholson and Sykova, 1998), and the viscosity of the ECS has a role in the activation of the presynaptic receptors by regulating neurotransmitter “spillover” in the synapse (Pavlov et al., 2004). Changes in the ECS may affect synaptic efficacy, neuronal excitability, and synapse specificity (Kullmann et al., 1999).

The composition of the ECS provides an extracellular microenvironment containing ECM components (Nicholson and Sykova, 1998). The CNS ECM differs from the ECM of other tissues. The major constituents of a non-CNS ECM are glycosoaminoglycan (GAG) sugars, fibrous proteins (collagen and elastin), and adhesive glycoproteins (laminin and fibronectin). In contrast, the ECM of the CNS contains diminutive amounts of fibrous proteins and considerably more GAGs (Novak and Kaye, 2000). The ECM in the CNS has lower levels of standard ECM constituents such as laminin, collagen, and fibronectin; however, it does contain proteoglycans (PGs), negatively charged heavily glycosylated proteins (Watanabe et al., 1989, Ruoslahti, 1988, Ruoslahti 1989; Yamaguchi 2002; Galtrey and Fawcett 2007).

The structural organization of the CNS ECM revolves around hyaluronan, which is a linear polysaccharide. Hyaluronan is highly expressed in the ECM, and molecules that can bind this matrix element can ultimately organize the extracellular space. PGs are capable of binding hyaluronan and because of this ability they serve as organizational scaffolds within the matrix (Fig. 2). PGs have roles in numerous cellular processes including cell adhesion, receptor binding, growth, migration, barrier formation, and interaction with other ECM molecules (for review see, Ruoslahti 1996; Bignami et al., 1992; Rhodes and Fawcett, 2004). PGs are composed of a protein core with GAG side chains; the length and number of side chains can vary, contributing to the diversity of PGs. Each GAG chain is composed of two alternating monosaccharide units - uronic acid and either N-acetylgalactosamine or N-acetylglucosamine. Interactions of these GAG side chains with other molecules mediate many of the functions carried out by PGs (Bandtlow and Zimmerman, 2000), including their

ability to bind to cell surface receptors such as receptor protein tyrosine phosphatase sigma (RPTP-sigma) (Shen et al., 2009).

Of the PGs, the lectican family is particularly enriched in the brain (for review see, Yamaguchi, 2000). Lecticans, a family of chondroitin sulfate proteoglycans (CSPGs), have a large globular N-terminal capable of binding hyaluronan, and a C-terminal domain that can bind other ECM and cell-surface molecules (Fig. 2). This unique ability of lecticans to bind hyaluronan, the backbone of the CNS ECM, with other ECM or cell-surface molecules allows them to serve as a molecular bridge between ECM and cells and gives them the role of organizers of the extracellular space. Lecticans are known to inhibit neurite outgrowth and migration in two ways. First, they act as a physical barrier. Second they create a cell-signaling barrier where they act as axon growth inhibitory ligands, which activate the Rho-family GTPases and disrupt the actin cytoskeleton (Sandvig et al., 2004). In mammals there are four lecticans including aggrecan (Doege et al., 1987), versican (Zimmermann and Ruoslahti, 1989), neurocan (Rauch et al., 1992) and brevican (Jaworski et al., 1994; Yamada et al., 1994). All lecticans share a great deal of amino acid homology and differ primarily by the number of chondriotin sulfate (CS) side chains attached to the protein backbone, with brevican having 1–3 CS chains and aggrecan having ~100 CS chains (Yamaguchi 2000; Viapiano and Matthews, 2006). The fact that all lectican members are expressed in the CNS and two, neurocan and brevican, are expressed exclusively in the CNS attests to their uniquely important role in the nervous system. Interestingly, in the adult brain all lecticans are capable of surrounding the cell body and proximal neurites of a subset of neurons in a mesh-like structure containing holes at sites of synaptic contacts forming a structure called the PN (Hockfield and McKay, 1983).

2. The perineuronal net component of the extracellular matrix

One way the ECM likely plays a role in the regulation of plasticity is through a unique structure called the PN. The PN is a complex extracellular structure whose very existence was questioned for a number of years. The PN ignited much debate when initially described, by Camillo Golgi in 1893, as ‘a thin envelope, with reticular or continuous shape involving not only the cell bodies but also their branches’ (for review see Celio et al., 1998; Spreafico et al., 1999). In 1897, Santiago Ramón y Cajal argued that the PN was an artifact of coagulated pericellular fluids. This claim impeded further research in the field, and interest in the PN was not resurrected until the 1980s. Equipped with new histological techniques the PN was confirmed to be a real structure (Fig. 1).

A variety of techniques have been used to visualize the PN including monoclonal and polyclonal antibodies to CSPGs (Hockfield and McKay, 1983; Kosaka et al., 1989; Guimaraes et al., 1990; Wintergerst et al., 1996; Lander et al., 1997) as well as immunodetection with lectins, which are capable of binding the sugar N-acetylgalactosamine component of CS chains (Hartig et al., 1992; Brauer et al., 1993; Brückner et al., 1993; Brückner et al., 1996). Today, we know that the PN surrounds synapses on the cell body, proximal dendrites, and the axon hillock of a subset of neurons, and therefore is prominently positioned to influence synaptic development and stabilization (Hockfield et al., 1990; Frischknect et al., 2009; Pyka et al., 2011; for review see Wang and Fawcett, 2012). PNs have apertures at points of synaptic contact (Brückner et al., 1993) and because of this expression pattern it is believed that they block the formation of new synapses (Celio and Blümcke, 1994; Pyka et al., 2011).

While the complex molecular interactions and functions of the PN are still being investigated, the main components of the PN are hyaluronan, hyaluronan synthases (HASs), CSPGs - primarily lecticans, link proteins (HAPLNs), and tenascin-R (TN-R) (Fig. 2)

(Koppe et al., 1997; Yamaguchi 2000; Carulli et al., 2007; Galtrey et al., 2008; Giamanco et al., 2010; Kwok et al., 2010, 2011). Hyaluronan is synthesized and docked to the membrane by HASs, which are located in the plasma membrane (Kwok et al., 2010). *In situ* hybridization has shown that one of three HASs are expressed in almost all cells bearing a PN in the cerebellum and the spinal cord (Carulli et al., 2006, 2007; Galtry et al., 2008). Lecticans, a critical component of the PN, are capable of binding hyaluronan and other ECM or cell-surface molecules. HAPLNs stabilize interactions between hyaluronan backbone of the ECM and lecticans. *In situ* and immunohistochemistry studies have found HAPLNs to be exclusively expressed by PN-bearing neurons (Rauch, 2004; Carulli et al., 2006; Galtrey et al., 2008). *In vivo* loss of HAPLN1 causes decreased PN production (Carulli et al., 2010). Additionally, *in vitro* co-expression of aggrecan, HAPLN1, and HAS3 was necessary for the formation of compact PNs (Kwok et al., 2010). Tenascins are a family of extracellular matrix glycoproteins with four members, TN-C, -R, -W, and -X. Of the four family members TN-R is highly expressed in the nervous system (for review see Tucker and Chiquet-Ehrismann 2009). TN-R forms a trimer and the G3 domain of lecticans binds to the fibronectin III repeats in the TN-R trimer (Hagihara et al., 1999; Lundell et al., 2004). Immunohistochemistry shows colocalization of TN-R and PNs (Hagihara et al., 1999; Weber et al., 1999; Carulli et al., 2006), and TN-R knockout mice have abnormal PNs (Weber et al., 1999). One trimer can bind up to three lectican molecules, which strengthens the PN structural integrity.

3. PN expression during development is activity-dependent

Activity plays a critical role in the development of mature synaptic connections that ultimately define the functional neural network. PNs are first detected postnatally and reach adult levels within weeks of initial expression (Hockfield and McKay 1983; Sur et al., 1988; Kalb and Hockfield, 1988). Furthermore, PN expression has been shown to be activity-dependent in the visual (Sur et al., 1988; Guimaraes et al., 1990; Kind et al., 1995; Lander et al., 1997), motor (Kalb and Hockfield, 1988, 1990a,b), and somatosensory systems (McRae et al., 2007), as well as in the pallial (cortical) song nuclei (Balmer et al., 2009).

Visual System

In the visual system, sensory experiences have been shown to shape cortical circuits during development (Berardi et al., 2003). The role of the PN in activity-dependent plasticity has been most prominently explored using visual deprivation paradigms (Sur et al., 1988; Zaremba et al., 1989; Guimaraes et al., 1990; Hockfield et al., 1990; Pizzorusso et al., 2002, 2006; Kind et al., 1995, 2012). In the visual system expression of PNs coincides with the maturation of the CNS (Sur et al., 1988; Guimaraes et al., 1990). In the cat, sensory deprivation early in development leads to inappropriate synapse stabilization, and decreased PNs in the visual system (Sur et al., 1988; Guimaraes et al., 1990; Kind et al., 1995; Lander et al., 1997; Kind et al., 1995, 2012) but sensory deprivation in the adult had no effect on PN expression (Sur et al., 1988).

Motor Systems

Perineuronal nets surround all the sciatic motor neurons in the spinal cord of the adult hamster (Kalb and Hockfield 1988, 1990b). Neonatal disruption of neuronal activity by either sciatic nerve crush or sciatic nerve lesions suppressed PN immunoreactivity. The same manipulations in adults did not alter PN expression. Furthermore, deafferenting of large-diameter primary afferents by dorsal rhizotomy in neonatal animals led to decreased PN expression around the sciatic nerves but had no effect in the adult (Kalb and Hockfield 1990a).

Somatosensory System

PNs are expressed postnatally in the rodent barrel cortex and sensory deprivation in the form of unilateral whisker trimming significantly reduced PNs in the deprived barrel cortex. The same manipulations done in adults however, did not alter PN expression levels implying that the development of the PN requires neuronal activity, but this activity is not required for the maintenance of the PN (McRae et al., 2007).

Pallial Nuclei

Birdsong learning in the zebra finch takes place during a critical period similar to that of human speech development and there are many similarities between human speech and birdsong (Doupe and Kuhl, 1999). PNs develop postnatally in the seven song nuclei investigated byBalmer et al. (2009). They also found that the percentage of PV interneurons with a PN predicts song maturity. Additionally, there was a decrease in PV and PN expression following isolation from a songbird tutor.

Summary

These studies suggest that PN expression is not dictated simply by neuronal activity, but rather its expression is dependent on neuronal activity during a circumscribed developmental period. Furthermore, once established ongoing physiologic activity is not required to maintain the PN. Taken together these studies suggest an imperative role for neuronal stimulation, during a critical developmental window, in the expression of the PN. The mechanisms underlying the ability of activity to regulate the expression of the PN remain unclear. The imperviousness of the postnatal PNs to altered neuronal activity suggests the PN is well suited to stabilize synapses in the mature animal (Zaremba et al., 1989).

4. PN expression reduces plasticity

During development, the extracellular environment is quite soluble due, in part, to expression to high amounts of hyaluronan, which because of its ability to interact with and organize water provides large hydrated spaces optimal for axonal migration and cell motility. In the adult hyaloronan is expressed at lower levels and is more insoluble. The insolubility of hyaluronan in adults is likely due to the presence of interactions with the lecticans through their hyaluronan-binding domain. These interactions form insoluble aggregates within the extracellular space. These insoluble aggregates ultimately seem to play an important role in the decreased plasticity and motility found in the mature nervous system (Rauch, 2004). This change in the extracellular space is likely mediated by the shift in lectican expression from neurocan and V1 versican in the immature brain to aggrecan, V2 versican, and brevican in the mature brain (Yamaguchi, 1996; Milev et al., 1998; Viapiano et al., 2003; Rauch, 2004).

4.1 Perineuronal nets appear late in postnatal development

The appearance of the PN in cortex, spinal cord, and hippocampus is relatively late during postnatal development and not likely involved in early developmental processes such as cell migration, synaptogenesis or process elongation (Hockfiled and McKay, 1983; Sur et al., 1988; Kalb and Hockfield, 1988; Lurie et al., 1997; Balmer et al., 2009; McRae et al., 2010). It is of interest that PNs first appear around the close of the critical period for multiple cortical areas. The critical period is a time during development where environmental input has the strongest influence on neuronal characteristics, anatomy, and physiology. In particular neuronal stimulation during the critical period is necessary for stabilizing appropriate synaptic connections in order to create a properly functioning CNS (for review see, Mennerick and Zorumski et al., 2000; Zito et al., 2002; Goda and Davis, 2003).

PNs are believed to have a role in decreasing plasticity partially because of their correlative expression (Sur et al., 1988; Zaremba et al., 1989; Hockfield et al., 1990) with the close of the critical period (Sur et al., 1988; Guimaraes et al., 1990; Hockfield et al., 1990; Pizzorusso et al., 2002, 2006). In fact the timing of PN appearance corresponds almost perfectly to close of the critical period and other indicators that a neuron has acquired mature neuronal properties (Kalb and Hockfiled, 1988; Sur et al, 1988; Hockfield et al., 1990; Lander et al., 1997; Lurie et al., 1997; Pizzorusso et al., 2002). Once the mature sets of synapses are ensheathed by a PN, they are stabilized and subject to little reorganization in the adult (Sur et al, 1988; Kalb and Hockfield, 1990a; Hockfield et al., 1990; Pizzourusso et. al., 2002; McRae et al., 2007).

4.2 Degrading the perineuronal net in the visual system

In attempt to gain a better understanding of potential roles for the PNs in decreased plasticity, PN components have been enzymatically degraded. Chondroitinase ABC (ChABC), a bacterial enzyme isolated from *Proteus vulgaris*, catalyzes the removal of CS glycosoaminoglycan side chains in the PN as well as other extracellular CS containing molecules (Yamagata et al., 1968; Bukalo et al., 2001) and ChABC treatment has been used as a means of degrading the PNs (Brückner et al., 1998; Bukalo et al., 2001; Bradbury et al., 2002; Pizzorusso et al., 2002, 2006; Corvetti and Rossi, 2005; Bowes et el., 2011) (Fig. 2). Hyaluronidase is an endogenous enzyme, which cleaves hyaluronan and is also known to degrade the PN (Miyata et al., 2005) (Fig. 2).

Utilizing ChABC, the role of ECM in visual system plasticity was eloquently addressed. Monocular deprivation in a young animal during the critical period leads to a shift in ocular dominance favoring the non-deprived eye; however, in the adult, due to the less plastic nature of the brain, this shift does not occur. Pizzorusso and colleagues (2002) were able to directly demonstrate a correlation between PNs and the close of the critical period by using ChABC to enzymatically degrade the PN in adult rats. This treatment ultimately led to the loss of PNs in the visual cortex. Remarkably, after treatment with ChABC they reinstated ocular dominance plasticity in monocular deprived adult animals. By removing the PN, they restored synaptic plasticity normally seen only during the critical period. This suggests that the ECM of the adult exerts a powerful inhibitory control on ocular dominance plasticity. Usually when rodents experience monocular deprivation during the critical period, there is minimal cortical response to stimulation of the deprived eye in adulthood. However, when ChABC was used in combination with reverse lid suturing (the previously deprived eye is opened and the non-deprived eye is sutured) there was total recovery of visual acuity and spine density (Pizzorusso et al., 2006). These works demonstrated that the mature ECM is inhibitory for activity-dependent plasticity and the CSPGs presumably in the PN have a role in the decreased plasticity.

4.3 Degrading the perineuronal net in the fear conditioning

PN expression is associated with the close of the critical period in other regions of the CNS including the amygdala. Gogolla and colleagues (2009) demonstrate that the PN is required for the permanence of fear memory. In adult mice fear conditioning (where a tone is paired with a painful footshock) produces a long lasting memory that can be temporarily inhibited with extinction training, but is ultimately resistant to erasure. However, in young animals the strength of the memory is reduced without extinction training and seven days of extinction training induces erasure of the conditioned memory. The appearance of PNs in the amygdala coincides with the protection of fear memories seen in adults. After degrading PNs with ChABC in the amygdala of adult mice the fear memory was extinguished following extinction training similar to what was observed in the young animal. Therefore, degrading the CSPGs, presumably in the PN, reinstated plasticity in fear memory permanence.

4.4 Degrading the perineuronal net and synapse stabilization

The PN is believed to have a role in synapse stability. In hippocampal neurons co-cultured with astrocytes the appearance of the PN was concurrent with the appearance of synapses. Enzymatic degradation of the PN, using ChABC or hyaluronidase lead to increased number of synaptic puncta on the neurons (Pyka et al., 2011). Direct evidence for the role of the PN on synapse stability comes from work done by Frischknect and colleagues (2009) where they used dissociated hippocampal neurons and showed that the diffusion constant of the AMPA receptor subunit GluR1 and the surface area explored by AMPA receptors increased following hyaluronidase treatment. This work suggests a prominent role for the PN in synapse stabilization.

4.5 Degrading the Glial Scar and CNS injury

Reactive gliosis following a CNS injury results in a glial scar rich in CSPGs (McKeon et al., 1999). The upregulation of CSPGs following injury creates a barrier to regeneration while simultaneously restricting plasticity (for review see Fawcett and Ascher, 1999). This upregulation of CSPGs exerts an inhibitory effect on axonal growth of dorsal root ganglion (DRG) neurons in the spinal cord (Smith-Thomas et al., 1995). The inhibitory role of CSPGs has been largely attributed to negatively charged CS side chains, which create a large repellant barrier. ChABC removes the CS side chains, which removes the inhibitory effect on DRG growth and supports regeneration of DRG axons (Zuo et al., 1998). ChABC promotes plasticity in the injured CNS, primarily by providing a permissive environment for the sprouting of damaged axons and dendrites.Lin et al. (2008) found that a single ChABC treatment within 24 hours of a nigrostriatal brain lesion, maintained low CS levels throughout the 28 day testing period compared with the sham treated animals' 4-fold increase in CS seven days after injury. ChABC treatment has also been shown to enhance axonal regeneration along with locomotor and proprioceptive recovery following acute cervical dorsal column crush (Bradbury et al., 2002). Following thoracic spinal cord hemisection, repeated intraspinal ChABC injections improved recovery of locomotor behavior, and hindlimb motion in cats as well as enhanced axonal growth (Tester and Howland 2008; Jefferson et al., 2011). In the squirrel monkey 11–12 weeks after a dorsal column lesion at the cervical spine level (C5/C6) the preserved afferents from D1 (the thumb) activated a larger receptive field in the primary somatosensory cortex in ChABC treated animals, indicating that the loss of CSPGs promoted enhanced axonal sprouting (Bowes et al., 2012). The presence of CSPGs inhibits axonal growth; use of ChABC degrades the matrix and increases the permissiveness for regeneration in the injured CNS.

4.6 Confounds of enzymatic degradation

The ChABC studies are pivotal in our understanding of the role that PN plays in the developing and mature CNS, however, there are complications with the specificity of enzymatic degradation of the PN. ChABC is used because it has the ability to enzymatically degrade the CS side chains in the PN (Bukalo et al., 2001). However, CS side chains are not found exclusively in the PN; rather, they are distributed throughout the extracellular space. Hyaluronidase randomly cleaves glycosidic bonds in hyaluronan (Cramer et al., 1994). Hyaluronan is the major constituent of the ECM and hyaluronidase treatment likely impacts the PN and other components of the ECM. As a result, ChABC and hyaluronidase treatments may broadly disturb the extracellular milieu.

The extent to which ChABC and hyaluronidase treatments alter other ECM components remains unclear. In addition to degrading CS side chains ChABC also degrades dermatan sulfate side chains of proteoglycans, and hyaluronan, leaving a protein core, carbohydrate stubs (Caterson, 1985, Brückner et al, 1998; Fox and Caterson, 2002; Galtrey et al., 2007). Hyaluronidase is known to cleave the glycosidic bonds of hyaluronan, but it also cleaves

these bonds in chondroitin and CSs (for review see Girish and Kemparaju, 2007) and has been shown to lead to degradation of the PN (Miyata et al., 2005). Both enzymes induce long-lasting changes in the ECM, including but not limited to a transient loss in the appearance of PN. ChABC and hyaluronidase treatments have been important for dissecting out the role of the ECM and PN in neuronal function, they do however, lack specificity. While future studies utilizing targeted gene deletion may be more specific, interpreting results from these studies may be difficult due to the complex interactions between the components of the PN and the other ECM components.

5. Epileptogenesis and the ECM

Early in development, synapses are established and then go through a period of activity-dependent modification where some synapses are stabilized and others are abolished. The structural plasticity of the CNS is predominant during development. The mature ECM is inhibitory for activity-dependent plasticity, however injury of adult tissue may reactivate mechanisms that were operating during development. A prolonged seizure or status epilepticus (SE) leads to synaptic rearrangements in the hippocampus, including axonal sprouting and increased dendritic spine number and length, presumably via enhanced plasticity (Suzuki et al., 1997). These changes require a permissive extracellular environment, indicating that alterations in the ECM likely contribute to the plasticity observed during epileptogenesis.

5.1 Decreases in inhibition and the progression of epilepsy

One theory of why seizures occur is an imbalance between excitation and inhibition. Following SE there is persistently abnormal inhibition due to altered GABA receptor subunit expression (Brooks-Kayal et al., 1998; Zhang et al., 2004a, Gorter et al., 2006, Raol et al., 2006; Zhang et al., 2007). Alternatively faulty GABAergic circuitry may have a role in initiating and ultimately maintaining the seizure prone condition of the brain. Because the PN has been shown to primarily envelope the cell surface of GABAergic nonpyramidal cells, alterations in its expression could influence GABAergic circuitry (Brückner et al., 1993; Celio and Chiquet-Ehrismann, 1993; Wintergerst et al., 1996; Schuppel et al., 2002). Inhibitory basket cells (the primary cell with a PN within the hippocampus) in the dentate region of the hippocampus display reduced synaptic input and output following pilocarpine induced SE (Zhang and Buckmaster 2009). Based on the role of the PN in synaptic stability and its location around GABAergic interneurons this structure may contribute to the progression of epilepsy.

5.2 Changes in the extracellular matrix and the perineuronal net in several epilepsy models

Neurocan and phosphacan are the predominant CSPGs present in the developing hippocampus and both have been found in PNs. Their expression has been linked to sculpting of axonal path finding, cell adhesion, elongation, process outgrowth, branching, and synapse formation and plasticity (Engel et al., 1996; Meyer-Puttlitz et al., 1996; Margolis et al., 1996; Margolis and Margolis, 1997; Garwood et al., 2001; Zhou et al., 2001; Okamoto et al., 2001; Faissner et al., 2006). Neurocan is expressed around the developing mossy fiber tract and has been proposed to act as a barrier for directing mossy fiber extension (Seki and Rutishauser, 1998; Okamoto et al., 2001). Neurocan expression peaks during development with little expression in the adult (Yamaguchi 1996); however, following SE it is re-expressed in the adult hippocampus (Kurazono et al., 2001; Matsui et al., 2002; Heck et al., 2004). Ihara's spontaneous epileptic rats exhibit a decrease in neurocan expression at 2 to 3 weeks of age compared to Sprague Dawleys, followed by a reappearance of hippocampal neurocan at 8 months (Kurazono et al., 2001). Seven days after a single unilateral intrahippocampal injection of domoate, a glutamate agonist used to

induce SE, there was an increase in neurocan in the dentate gyrus of the hippocampus (Heck et al., 2004). The abnormal re-expression of neurocan is a candidate for contributing to mossy fiber sprouting and new synapse formation in the dentate granule layer seen in Ihara's spontaneous epileptic rats and following SE.

Phosphacan has been shown to function as a barrier, as well as promote neurite outgrowth in the hippocampus (Wilson and Snow, 2000; Garwood et al., 2001). Its role in epileptogenesis is unclear as there are conflicting results on its expression.Heck et al. (2004) found an increase in phosphacan expression in the dentate gyrus 14 days after SE induction with domoate. In contrast,Okamoto et al. (2003) found that kanic acid induced SE, led to a decrease in phosphacan protein as early as 24 hours post-SE, and a decrease in phosphacan-expressing PNs surrounding PV cells in the hippocampus 1 week post-SE, with little change in the number of PV cells present. In a subset of rats, the decrease in phosphacan containing PNs was ameliorated 8 weeks after the insult. One possible explanation for the opposing results is that Heck et al., (2004) used an antibody that recognized all isoforms of phosphacan, while Okamoto et al., (2003) used an antibody that recognized the core glycoprotein of phosphacan. The studies also used different species and different means of SE induction. Taken together these studies demonstrate that neurocan and phosphocan expression is altered with seizure activity but more work needs to be done to understand the significance of the changes.

Phosphacan and neurocan are not only found within the PN, they are both expressed throughout the ECM neuropil. While all CSPGs family members can be found in PNs, aggrecan is unique because, unlike the other CSPGs and lecticans, in the CNS it is found exclusively in the PNs (Matthews et al., 2002; Galtrey et al., 2008; Morawski et al., 2012). Aggrecan expressing PNs develop postnatally in the hippocampus primarily around PV interneurons (McRae et al., 2010, 2012). Inducing SE with kainic acid early in development, prior to the expression of the PN led to accelerated expression of aggrecan positive PNs. This increase in aggrecan was transient and was attenuated by postnatal day 21. Similar to Okamoto's work there was no change in PV expression (McRae et. al., 2010). It is possible that this premature expression of PNs could influence synaptic plasticity but further study is required. SE induction early in life, leads to less neuronal injury, increased seizure susceptibility, less substantial mossy fiber sprouting, and milder epilepsy (Jensen et al., 1992; Dube et al., 2000; Barum et al., 2002; Zhang et al., 2004a,b).

In contrast, following SE adult rodents have severe neuronal injury, cell loss, enhanced mossy fiber sprouting, and epilepsy. SE induced in the adult, after the PN has matured, resulted in a persistent decrease in aggrecan containing PNs at one week persisting up to two months after SE (McRae et al., 2012). Similar to Okamoto's work there was no decrease in PV expression, in fact there was an increase at one week. Thus a lack of PV cells did not contribute to the decrease in aggrecan containing PNs in the hippocampus. Loss of the PN correlated with a decrease in *aggrecan* mRNA two months after SE induction, implying mechanisms other than decreased transcription may contribute to the changes in the PN seen one week after SE. In addition to a decrease in the total number of aggrecan expressing PNs there was also PNs with poor structural integrity (degraded PNs) (Fig. 3), a phenotype not described in the epileptic hippocampus previously (McRae et al., 2012).

5.3 PN support structures in epileptogenesis

One explanation for the loss of PNs and the appearance of degraded PNs in the hippocampus following SE could be changes in PN support structures. HAPLN1 and HAS3 immunohistochemistry decreased prior to changes in aggrecan (McRae et al., 2012). Since aggrecan containing PNs develop in hippocampal cultures over a time course similar to what is seen *in vivo* we treated mature cultures with KCL and found that aggrecan was attenuated

in the PN, with HAPLN1 and HAS3 expression decreasing prior to the loss of aggrecan in the PN (McRae et al., 2012). HAPLN1 and HAS3 both have a role in binding of aggrecan with hyaluronan. In both human TLE and animal models of TLE, hyaluronan increased (Perosa et al., 2002a,b Bausch, 2006). The increase in hyaluronan and the decreases in aggrecan, HAPLN1, and HAS3 would likely result in an increase in unbound hyaluronan in the extracellular environment. Overall the changes in the extracellular environment described to date following SE would likely be supportive of increased neurite outgrowth and synaptic plasticity.

In the mature CNS, epilepsy leads to changes in the extracellular space, such as increased neurocan, altered phosphocan, and decreased aggrecan expression, making it similar to the permissive environment of the immature CNS. In the adult SE has a deleterious effect on aggrecan expressing PNs. One of the primary functions of the PN is to bind hyaluronan in the extracellular space (Fig. 2). Decreased PG expression, resulting in less binding of excess hyaluronan, may contribute to enhanced network plasticity and promote epileptogenesis.

5.4 Degradation of the perinerunal net by endogeneous proteases in epilepsy

Another explanation for the loss of PNs and the appearance of degraded PNs in the hippocampus could be enzymatic degradation of the PN. It is appears that proteases capable of degrading lecticans are altered by seizure activity. Two candidates are A Disintegrin and Metalloproteinase with Thrombospondin Motifs (ADAMTSs) and Matrix Metalloproteinases (MMPs). After SE there is evidence of PNs with poor structural integrity, which is likely the result of degradation in addition to the loss of HAPLN1 and HAS3 (Fig. 3). ADAMTS4/5 have been show to cleave various lecticans in the CNS. High levels of *ADAMTS4* mRNA expression have been localized to various CNS areas including the hippocampus (Yuan et al., 2002). Kainic acid-induced seizures produced a dramatic elevation in *ADAMTS4* transcript that correlated with an increase in a cleavage product of brevican, a member of the lectican family, in the hippocampus (Yuan et al., 2002).

MMPs are zinc-dependent cell membrane bound or secreted protease, which are important for ECM remodeling throughout the body (Sternlicht and Werb, 2001). MMPs have also been shown to cleave lecticans and participate in various CNS pathologies (Gottschall and Deb, 1996; Yong et al., 1998; Miwa et al., 2008). MMP-9 has been shown to have a prominent role in epileptogenesis. Wilczynski and colleagues (2008) showed that MMP-9 knockout mice took longer to kindle and had less severe seizures following pentylenetetrazole (PTZ) kindling induced epilepsy. They also found that transgenic mice over-expressing MMP-9 had an increased susceptibility to PTZ kindling. In addition, MMP-9 protein and cleavage activity was upregulated near hippocampal synapses following SE induced with kainic acid. MMP-9 expression increased 24 hours after SE onset, peaked at 72 hours, and remained elevated 7 days after the insult. MMP-9 deficiency attenuated seizure-induced dendritic spine pruning after kainic acid induced SE. MMP-9 may also plays a role in PN destruction in epileptogensis.

Tissue-type plasminogen activator (tPA), a serine protease expressed at low levels in the CNS. Interestingly, CSPGs are a target of tPA (Wu et al., 2000) and tPA activation leads to the site specific degradation of lecticans through the activation of MMPs (Baricos et al., 1995; Nagase, 1997). Synthesis of tPA is increased following events that require synaptic plasticity or excessive neuronal activity such as motor learning, long-term potentiation, kindling, and seizures (Qian et al., 1993; Carroll et al., 1994; Seeds et al., 1995; Yepes et al., 2002).

Tissue-type plasminogen activator deficient mice have reduced mossy fiber sprouting and delayed seizure progression (Wu et al., 2000; Yepes et al., 2002). Seizures lead to an up

regulation in tPA activity, which can directly degrade CSPGs and activate MMPs, and indirectly degrade CSPGs. Simultaneously, increased levels of ADAMTS may be secreted and lead to further degradation of lecticans. Further work is needed to understand the relationship between these proteases and PN stability in epileptogenesis.

There are many questions that need to be explored to better understand the role of the ECM and PN in the progression of epilepsy. Are other lecticans being cleaved by proteases following SE? An increase in *ADAMTS4* mRNA and brevican cleavage products following SE were discussed above but ADAMTSs are also called aggrecanases for their high affinity to cleave aggrecan, suggesting a possible second target. Are changes in the electrophysiology of PV expressing interneurons following SE related to alterations in the PN? Future studies will be needed to further elucidate the mechanisms for altered PN expression and the consequences of PN loss on PV interneuron synaptic plasticity following SE. These and other studies will be needed to determine if the PN and ECM are therapeutic targets for the prevention of epilepsy.

6. Summary

Over the course of development the CNS changes from an environment that is conducive to neuronal plasticity to one in which synapse formation is restricted. The expression of the PN appears primarily around inhibitory interneurons late in postnatal life, its expression is activity-dependent and its presence leads to reduced plasticity. SE leads to increased hyaluronan, aberrant lectican expression, and decreased PN support structures, as well as increased protease activity against ECM and PN components all of which likely contribute to free hyaluronan. An increase in unbound hyaluronan in the ECM, may leave the system more malleable and susceptible to increased neurite outgrowth and synaptic plasticity after SE. Elucidating the effects of SE on the ECM and the PN will contribute to our understanding of how the extracellular environment contributes to epileptogenesis and may provide novel therapeutic targets to prevent epilepsy.

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Highlights

- Composition of the extracellular matrix of the central nervous system.
- Specialized perineuronal net component of matrix found around interneurons.
- During development neuronal activity is required for perineuronal net expression.
- Perineuronal net leads to decreased plasticity in adult central nervous system.
- Extracellular matrix and perineuronal net are altered during epileptogenesis.

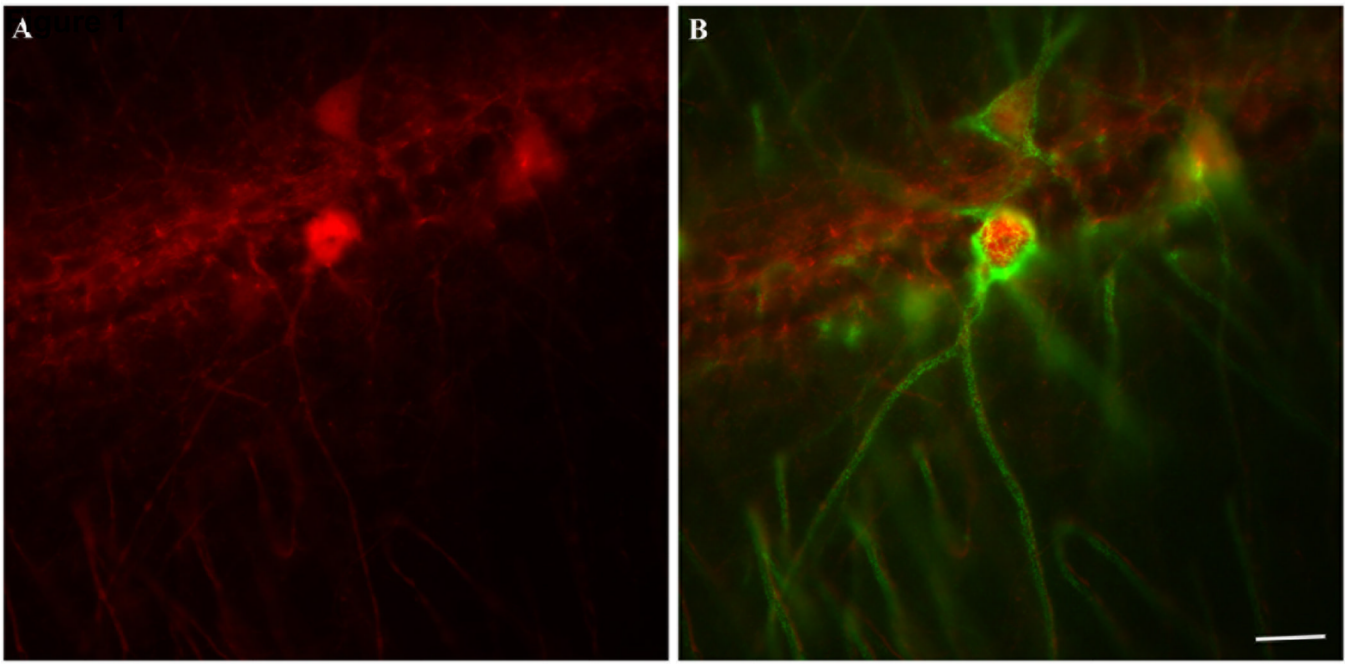

Figure 1. Immunohistochemical staining of the perineuronal net in the CNS

A) Parvalbumin immunostaining labels inhibitory interneurons in the rat hippocampus (red). B) Cat-315 (green) an antibody that detects the aggrecan component of the perineuronal net surrounds the parvalbumin expressing cells and extends down the proximal appendedages. Scale bar 20 μm.

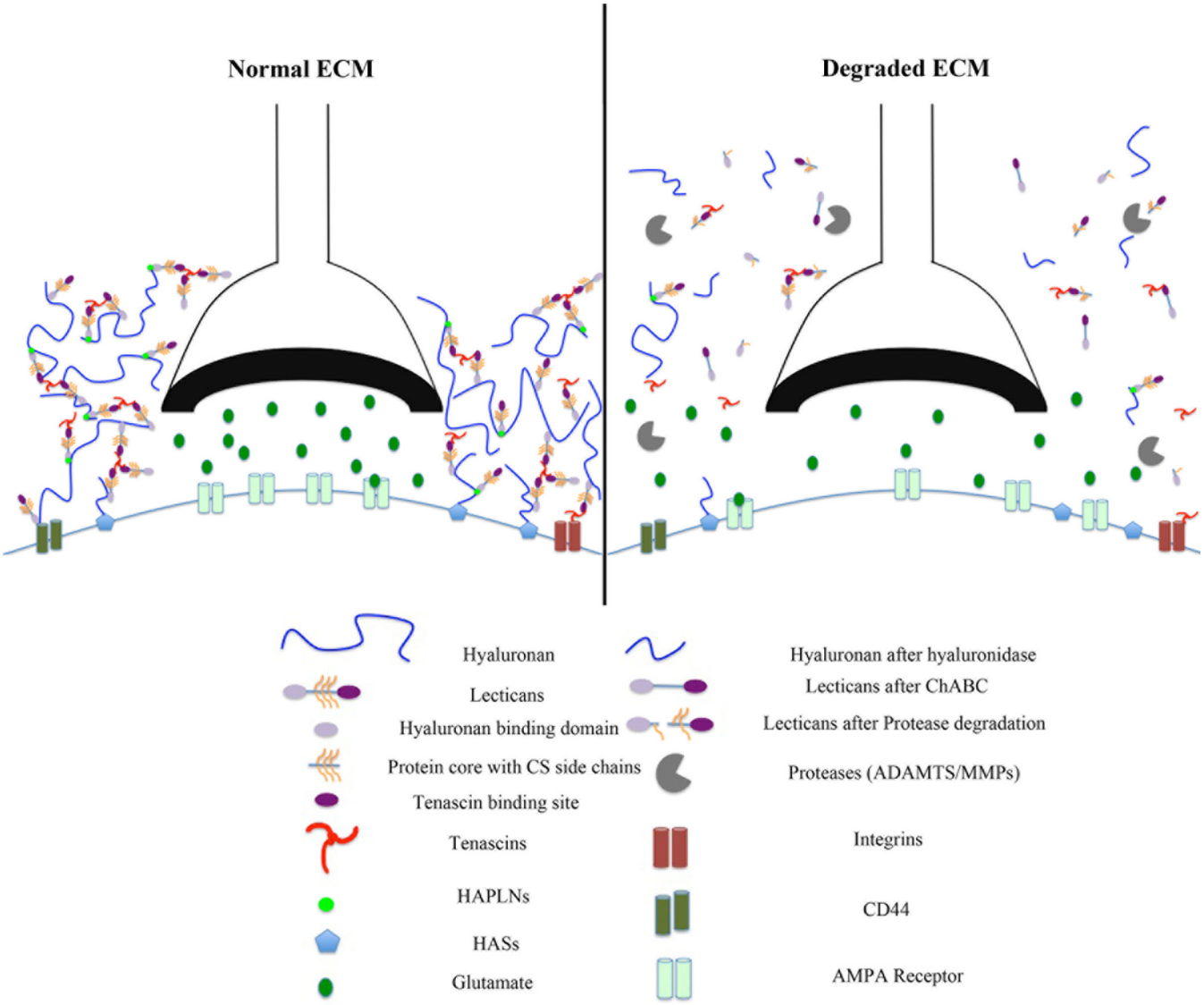

Figure 2. Schematic representation of the perineuronal net of the central nervous system

Under normal conditions the extracellular matrix forms a specialized structure surrounding a subset of neurons. The perineuronal net forms a dense structure around synapses. It is composed of multiple components including hyaluronan, which is synthesized by hyaluronan synthases (HASs) and docked by HASs as well as CD44, a receptor for hyaluronan. Lecticans bind hyaluronan through their hyaluronan binding domain at their N-terminal. At the C-terminal lecticans can bind tenascins or other extracellular or cell surface molecules. Link proteins (HAPLNs) stabilize the interactions between hyaluronan and lecticans. After degradation of the extracellular matrix through enzymes such as chondroinase ABC, hyaluronidase, or increased protease activity, the perineuronal net component deteriorates and there is the potential for extrasynaptic movement of receptors and neurotransmitters into the extrasynaptic space.

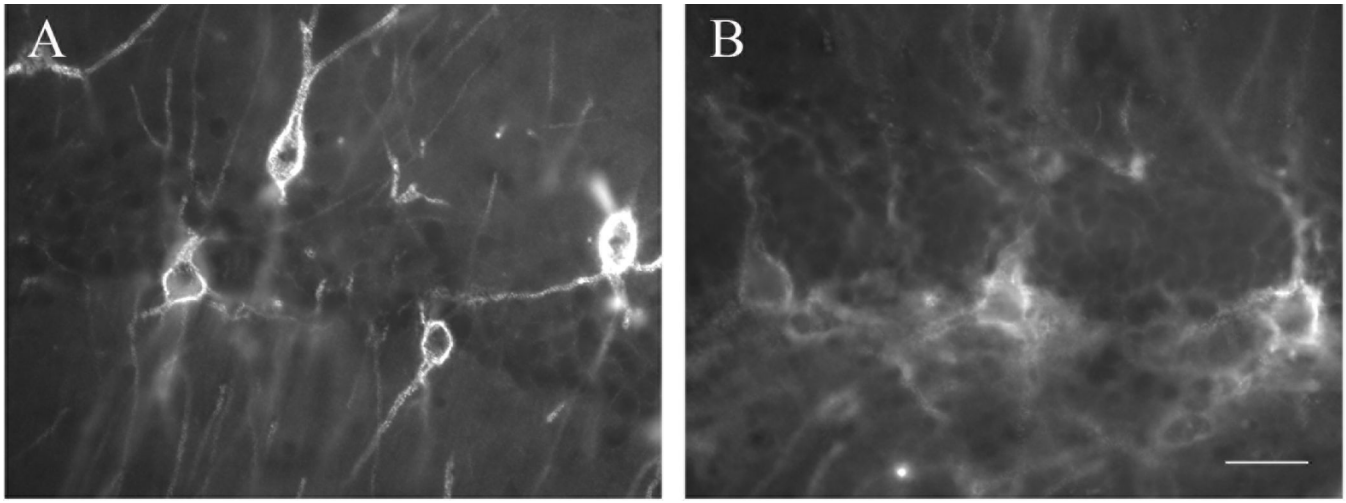

Figure 3. The perineuronal after status epilepticus

A) The aggrecan component of the perineuronal net detected with Cat-315 is located in a compact sheath around the cells in the dentate gyrus of control animals. B) Two months post-SE there is the appearance of degraded perineuronal nets with compromised structural integrity. Scale bar, 40 μm.