Chapter 7
The Brain's Extracellular Matrix and Its Role in Synaptic Plasticity

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Abstract The extracellular matrix (ECM) of the brain has important roles in regulating synaptic function and plasticity. A juvenile ECM supports the wiring of neuronal networks, synaptogenesis, and synaptic maturation. The closure of critical periods for experience-dependent shaping of neuronal circuits coincides with the implementation of a mature form of ECM that is characterized by highly elaborate hyaluronan-based structures, the perineuronal nets (PNN), and PNN-like perisynaptic ECM specializations. In this chapter, we will focus on some recently reported aspects of ECM functions in brain plasticity. These include (a) the discovery that the ECM can act as a passive diffusion barrier for cell surface molecules including neurotransmitter receptors and in this way compartmentalize cell surfaces, (b) the specific functions of ECM components in actively regulating synaptic plasticity and homeostasis, and (c) the shaping processes of the ECM by extracellular proteases and in turn the activation particular signaling pathways.

Keywords Cell surface molecules • Extracellular matrix • Perineuronal nets • Proteases • Synaptic plasticity

7.1 Introduction

The extracellular matrix (ECM) wrapping neural cells in the brain is produced by both neurons and glial cells. During postnatal maturation of neuronal circuits, this originally rather diffuse ECM condenses into a netlike structure around a subclass of neurons. These structures are termed perineuronal nets (PNN) and have been discovered by the pioneers of brain cell biology including Camillo Golgi and Santiago Ramon y Cajal (for review, see Celio et al. 1998). Nonetheless, the

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M.R. Kreutz and C. Sala (eds.), *Synaptic Plasticity*,
Advances in Experimental Medicine and Biology 970,
DOI 10.1007/978-3-7091-0932-8_7,

occurrence of an ECM in the brain has only been generally accepted in the 1970s (for review, see Zimmermann and Dours-Zimmermann 2008). The unbranched polysaccharide hyaluronic acid is the core component of the ECM in the brain. It acts as a kind of backbone to recruit proteoglycans and glycoproteins into ECM structures (Bandtlow and Zimmermann 2000; Rauch 2004; Frischknecht and Seidenbecher 2008). Major components of the hyaluronic acid–based ECM are chondroitin sulfate proteoglycans (CSPGs) of the lectican family (also named hyalecticans), tenascins, and so-called link proteins (Bandtlow and Zimmermann 2000; Yamaguchi 2000; Rauch 2004). In addition, a variety of other glycoproteins and proteoglycans contribute to the brain's ECM. These include laminins, pentraxins, pleiotrophin/HB-GAM, phosphocan, reelin, thrombospondins, and the heparan-sulfate proteoglycan (HSPG) agrin or cell surface-bound HSPGs of the syndecan and glypican families. Moreover, matrix-shaping enzymes, like proteases and hyaluronidases, are found in the brain ECM (Bandtlow and Zimmermann 2000; Dityatev and Schachner 2003; Christopherson et al. 2005; Dityatev and Fellin 2008; Frischknecht and Seidenbecher 2008).

During CNS development, the ECM undergoes significant changes. Initially, a juvenile ECM is synthesized during late embryonic and early postnatal development of the mammalian brain. The lectican neurocan and tenascin-C are prominent constituents of this juvenile matrix (Carulli et al. 2007). The adult ECM is characterized by downregulation of these components and the upregulation of other components including tenascin-R, brevican, aggrecan (Fig. 7.1), or particular versican isoforms (Milev et al. 1998; Carulli et al. 2007; Zimmermann and Dours-Zimmermann 2008; Carulli et al. 2010). A systematic biochemical and immunohistochemical investigation revealed differentially extractable ECM fractions from the adult brain (Deepa et al. 2006). Most of the material is loosely associated with

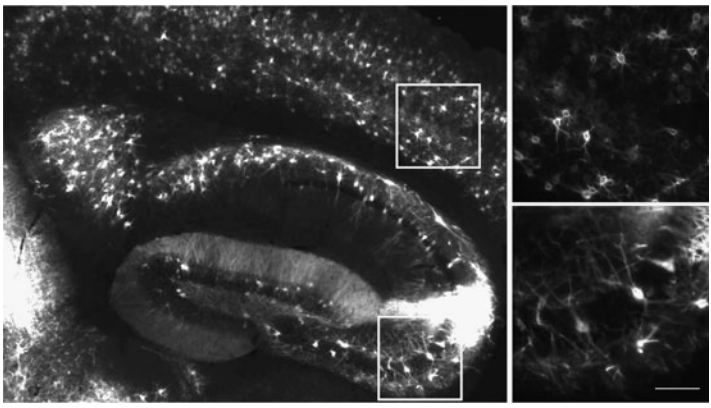

Fig. 7.1 Aggrecan is a major component of perineuronal nets (*PNN*). Staining of brain sections from 6-month-old mice with anti-aggrecan antibodies identifies numerous interneurons in the hippocampus and cortex surrounded by a PNN. Higher magnifications of cortical and hippocampal CA3 areas are shown in the upper and the lower box, respectively (scale bar: 100 μm). Of note, the massive aggrecan immunoreactivity of the CA2 region in the hippocampus

brain membranes. Nonionic detergents and salt can solubilize another fraction of ECM material that is thought to be more tightly associated with neural cell membranes. A final fraction comprising roughly a quarter of the CSPG material can only be extracted with urea. This fraction includes aggrecan, versican V2, neurocan, brevican, as well as phosphacan, and is not present in the young brain before closure of the critical period in the visual cortex (Deepa et al. 2006). The fraction is thought to represent cartilage-like ECM material forming the PNN (Fawcett 2009a). The PNN-like material can be removed from brain tissue entirely with the hyaluronic acid hydrolyzing enzyme hyaluronidase and partly with chondroitinase ABC, an enzyme that removes glycosaminoglycan chains from CSPGs (Deepa et al. 2006).

PNN are most prominent on GABAergic interneurons expressing the calcium buffer protein parvalbumin (Celio et al. 1998; Hartig et al. 1999). However, recent studies revealed that PNN are highly heterogeneous and that they occur on various types of neurons including excitatory principal neurons and inhibitory neurons throughout the CNS (Bruckner et al. 2000; Matthews et al. 2002; Wegner et al. 2003; Alpar et al. 2006). Mouse mutants for brevican, aggrecan, cartilage link protein Crtl1 (Hapln1) and tenascin-R display abnormal PNN indicating the importance of lecticans and tenascins for these ECM specializations (Bruckner et al. 2000; Brakebusch et al. 2002; Giamanco et al. 2010). After prolonged time in culture, PNN-like structures form also in primary neuronal cultures of various CNS areas (Miyata et al. 2005; John et al. 2006; Dityatev et al. 2007). Also here, GABAergic neurons first accumulate ECM material on their surfaces (Dityatev et al. 2007); however, virtually all neurons including their neurites are more or less densely covered within netlike structures after about 3 weeks in culture (John et al. 2006). This hyaluronan-based ECM tightly wraps synapses and is interspersed between neurons and astrocytes. Formation of cartilage-like cell surface structures resembling PNN can be triggered by heterologous overexpression of hyaluronan synthase (HAS), Crtl1, and aggrecan in human embryonic kidney cells (Kwok et al. 2010).

7.2 The Tetrapartite Synapse

The concept of a tripartite synapse implying that not only the canonical pre- and postsynaptic elements of two adjacent neurons but also the endfeet of glial cells contribute to the structure and function of brain synapses has been developed more than a decade ago and is nowadays widely accepted (for a review, see Araque et al. 1999; Haydon 2001; Slezak and Pfrieger 2003; Faissner et al. 2010). The term tetrapartite synapse or "synaptic quadriga" has been coined to indicate that, in addition to these three cellular parts, ECM structures produced by both astrocytes and neurons contribute to the functional synaptic complex (Dityatev et al. 2006; John et al. 2006; Dityatev et al. 2010b). A well-studied example of such a quadriga is the vertebrate neuromuscular junction where nonmyelinating Schwann cells tightly wrap the presynaptic terminal, and a prominent agrin–laminin-based basal

lamina fills the synaptic cleft between the motorneuron ending and the postsynaptic membrane of the muscle (for review, see Sanes and Lichtman 2001; Faissner et al. 2010). At CNS synapses, the synaptic cleft is much less wide as compared to the neuromuscular synapse (20 vs. 50 microns, respectively), and it does not contain a basal lamina. Nonetheless, within the synaptic cleft of excitatory CNS synapses, regularly assembled ECM structures are found, the biochemical identity of which is currently unknown (Zuber et al. 2005). Maybe, like at the neuromuscular junction, an agrin-based ECM is found also in the cleft of central synapses. Consistent with this hypothesis, processing of agrin by the extracellular protease neurotrypsin is involved in the development and plasticity of CNS synapses (Matsumoto-Miyai et al. 2009; see below).

The ECM of the synaptic cleft seems to be clearly distinct from the perisynaptic ECM wrapping CNS synapses. For example, biochemical fractionation has demonstrated that ECM components of the mature brain, like brevican, tightly associate with synaptic protein preparations (Seidenbecher et al. 1995, 2002; Li et al. 2004). However, at the ultrastructural level, brevican immunoreactivity is strictly perisynaptic and not found within the synaptic cleft (Seidenbecher et al. 1997). Brevican is primarily synthesized by astrocytes (Yamada et al. 1997; John et al. 2006) confirming that glia-derived components contribute to the PNN-like ECM tightly associated with the synaptic complex. As will be discussed in detail below, both passive and active functions might be assigned to the perisynaptic ECM. Passive functions include the trapping of trophic factors like neurotrophins, fibroblast growth factors, midkine, or pleiotrophin in the vicinity of their cognate high affinity receptors (Celio and Blumcke 1994; Galtrey and Fawcett 2007; Fawcett 2009a), as well as the formation of diffusion barriers for cell membrane proteins (Frischknecht et al. 2009, see below). Active functions include specific interactions of ECM components with synaptic proteins and pathways as well as the generation of signaling components via proteolysis of ECM components (see below).

Astrocyte-derived ECM components serve important functions already during synaptogenesis and synaptic maturation. For example, thrombospondins (TSPs), oligomeric ECM proteins secreted by astrocytes in the brain, have been identified as a major factor for synapse development inducing synapse formation and maturation of the presynaptic bouton. However, these synapses were postsynaptically silent (Christopherson et al. 2005). Mice lacking both TSP1 and 2 exhibited a significantly decreased number of excitatory synapses in the cerebral cortex (Christopherson et al. 2005). Another member of the thrombospondin family (TSP4) is strongly expressed in adult astrocytes and is a candidate for regulating synaptic plasticity in the CNS (Eroglu 2009). The postsynaptic partner of TSPs is the non-channel-forming $\alpha 2\delta$-1 subunit of voltage-gated calcium channels, which also acts as receptor for the antiepileptic analgesic drug gabapentin. Binding of TSPs to the gabapentin receptor is also required for postsynaptic activation of excitatory synapses (Eroglu et al. 2009). Moreover, the interaction of TSP1 with the postsynaptic cell adhesion molecule neuroligin 1 seems to accelerate the process of synaptogenesis in hippocampal primary cultures (Xu et al. 2010).

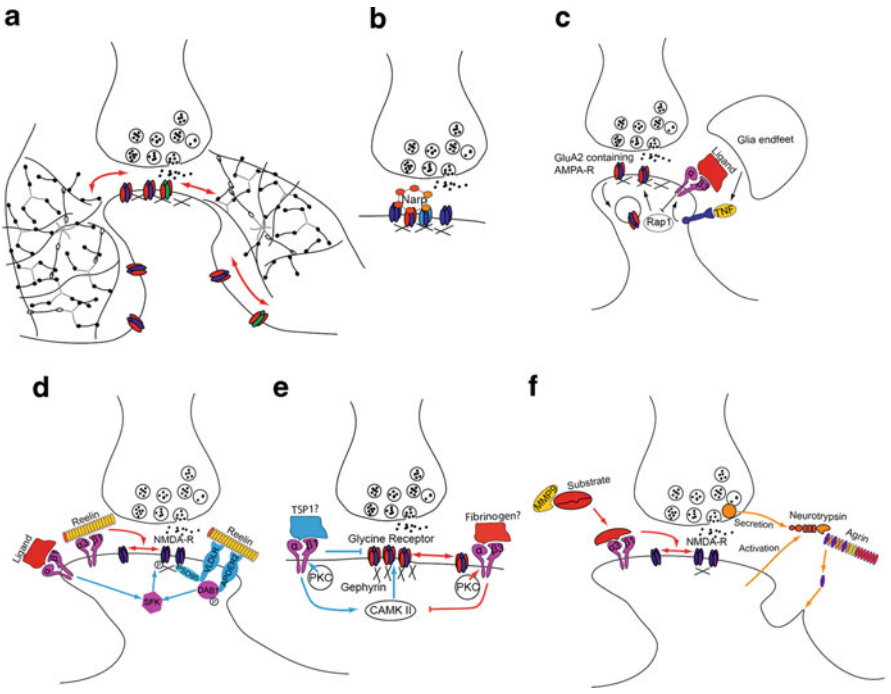

Fig. 7.2 Examples of ECM function in the modulation of synaptic transmission. (**a**) The perisynaptic ECM represents a physical barrier for lateral diffusion of neurotransmitter receptors (*red arrows*). Surface receptors such as AMPA-receptors are limited in lateral diffusion by the ECM, which reduces the exchange of synaptic and extrasynaptic receptors in the adult brain (Frischknecht et al. 2009). (**b**) On parvalbumin-positive inhibitory neurons multimers of the neuronal pentraxins NP1 and narp bind to and cluster AMPA receptors in response to elevated synaptic activity and thus regulate homeostatic scaling (Chang et al. 2010). (**c**) ECM-integrin signaling reduces AMPA receptor internalization and thus contributes to synaptic scaling. Reduced synaptic activity is thought to induce the release of tumor necrosis factor alpha (*TNF*) from glial cells, which in turn enhances surface expression of β3-containing integrins. β3-Integrins inhibit the small GTPase Rap1, which in its active state is responsible for removal of GluA2-containg AMPA receptors from the cell surface (for review, see Pozo and Goda 2010; Dityatev et al. 2010a). (**d**) Reelin signaling alters activity (*blue arrows*) and lateral diffusion of NMDA receptors. Upon binding, reelin clusters its receptors, the very-low-density lipoprotein receptor (*VLDLR*) and the apolipoprotein E receptor type 2 (*ApoER2*), which is in contact with NMDA receptors through binding to the scaffold protein PSD-95. Co-clustering of the intracellular adaptor protein disabled 1 (*DAB1*), which binds to both receptors induces activation Src family tyrosine kinases (*SFK*) and to tyrosine phosphorylation of NMDA receptors leading to increased receptor activity. Similarly, SFK mediate NMDA receptor tyrosine phosphorylation after activation of β1-containing integrins (*blue arrows*). Reelin signaling further enhances lateral diffusion of GluN2B but not GluN2A containing NMDA-receptors and thus may contributes to the decrease of synaptic GluN2B receptors during late development (*red arrows*). Hypothetically, in this case reelin signaling may act through direct binding to α3β1-containing integrins (for review, see Herz and Chen 2006; Dityatev et al. 2010a; Frotscher 2010). (**e**) Integrins control surface trafficking of glycine receptors on spinal cord neurons. β1 – (*blue arrows*) and β3 – (*red arrows*) containing integrins act on glycine receptor diffusion in an antagonistic manner. Both integrins require protein kinase C (*PKC*) for their activity. However, activation of β1-integrins by trombospondin 1 also

At inhibitory synapses of the spinal cord, TSP1 via its integrin β1 receptor slows the mobility of extrasynaptic glycine receptors (GlyR) and stabilizes these receptors in synapses (Charrier et al. 2010, see Fig. 7.2e).

7.3 Formation of the Adult ECM: Switch from Developmental to Mature Forms of Synaptic Plasticity

One of the most striking aspects of adult ECM function is its appearance at the end of the critical period of circuit wiring suggesting that it is involved in the implementation of adult plasticity modes (Mataga et al. 2002; Pizzorusso et al. 2002; Mataga et al. 2004; Oray et al. 2004). This seems to occur at the expense of the regenerative potential of the central nervous system (Fawcett 2009b). Initial findings derive from studies on ocular dominance (OD) plasticity in the visual cortex of cats, which, since its discovery (Wiesel and Hubel 1963), has represented a valuable model for studying experience-dependent plasticity in vivo. In the visual cortex, certain groups of neurons respond preferentially to one but not the other eye. Visual deprivation of one eye during a developmental period, the so-called critical period (postnatal days P21–P25 in rodents), leads to a drastic change in the neuronal circuits in the visual cortex. There the number of neurons responding to the nondeprived eye increases at the expense of those responding to the deprived eye. In contrast to what is observed during the critical period, visual deprivation performed in adult animals results in little or no plasticity (Pizzorusso et al. 2002; Berardi et al. 2003). Experiments by Pizzorusso and colleagues (2002) have demonstrated that removal of the hyaluronan-based ECM from the visual cortex can restore OD plasticity in the adult stage. Injection of chondroitinase ABC into the visual cortex and subsequent monocular deprivation resulted in a shift of ocular dominance. Utilizing this treatment, it was even possible to rescue visual acuity in adult animals reared with one long-term deprived eye and thus suffering from strongly asymmetric ocular dominance (Pizzorusso et al. 2006). Investigations on a mouse mutant for the cartilage link protein 1 (Crtl1) revealed that it does not develop

Fig. 7.2 (continued) activates Ca^{2+}/calmodulin-dependent protein kinase II (*CaMKII*) and reduces glycine receptor diffusion and accumulation of the receptor at the synapse. In contrast, activation of β1-integrins by fibronectin blocks of CaMKII and increases lateral mobility and in turn the loss of receptors from the synaptic membrane (Charrier et al. 2010). (**f**) Effects of proteolytic cleavage of ECM molecules on synaptic function. Cleavage of an unknown substrate in the ECM by MMP9 unmasks an RGD signal for β1-containing integrins. Subsequently GluN2A-containing NMDA receptors exhibit increased lateral diffusion within the neuronal membrane (Michaluk et al. 2009). Neurotrypsin is released from presynaptic vesicles. Activation of neurotrypsin requires concomitant activation of the postsynapse (*orange arrows*). Active neurotrypsin processes agrin and releases a 22-kD stable fragment harboring a single laminin G3 domain. This fragment is able to induce dendritic filopodia that may give rise to new synapses (Frischknecht et al. 2008; Matsumoto-Miyai et al. 2009)

normal PNN, retains juvenile levels of OD plasticity, and the visual acuity remains sensitive to visual deprivation (Carulli et al. 2010). In these mice, also plasticity of Purkinje cell axon terminals in the deep cerebellar nuclei is enhanced (Foscarin et al. 2011). In rodents, formation of PNN in the visual cortex is delayed upon dark rearing (Pizzorusso et al. 2002; Carulli et al. 2010) and is reduced in deep cerebellar nuclei by rearing in enriched environment (Foscarin et al. 2011) suggesting an interplay of external stimuli and the synthesis and/or maintenance of PNN.

Another study by Gogolla and colleagues (2009) suggests that similar mechanisms involving the ECM may make particular memories, in this case fear memories, resistant to erasure. Conditioned fear memories can be erased permanently in young rats while animals older than 3–4 weeks are largely resistant to this fear extinction. Fear extinction in both adult and young rats is amygdala dependent. In this brain structure, PNN develop between postnatal days P16 and P21. After this critical period, fear memory can be reduced by repeated exposure to the conditioned stimulus in the absence of the aversive fear-provoking stimulus. However, in contrast to young animals, fear response is reinstated in adult rats when the aversive stimulus is presented again. Similar to the experiments in the visual cortex, removal of the hyaluronan/CSPG-based ECM achieved a rapid and permanent erasure of newly acquired fear memories. Extinction did not take place when fear experience was made before enzyme application indicating that CSPGs are essential for protecting fear memories from erasure during the acquisition phase (Gogolla et al. 2009; Pizzorusso 2009).

7.4 The Hyaluronic Acid–Based ECM Is Indispensible for Mature Forms of Synaptic Plasticity

In the adult mouse brain, the lack of components of the ECM leads to impaired synaptic plasticity (for a comprehensive review, see Dityatev and Fellin 2008). For instance, TNR-deficient mice exhibit impaired long-term potentiation (LTP) but normal long-term depression (LTD). Further, a lack of TNR leads to disinhibition of the CA1 region of the hippocampus and to shift in the threshold for induction of LTP (Bukalo et al. 2007). Mice lacking the CSPGs brevican or neurocan also show impairments in LTP 30 min and 2 h after induction, respectively (Zhou et al. 2001; Brakebusch et al. 2002). Removal of chondroitin sulfates results in the degradation of perineuronal nets, the increase in excitability of perisomatic interneurons (Dityatev et al. 2007), and in the $GABA_A$ receptor-dependent inhibition of LTP induction (Bukalo et al. 2001). Interestingly, it has recently been suggested that hyaluronic acid is not only a fundamental structural element of the PNN but also has a direct impact on synaptic plasticity (Kochlamazashvili et al. 2010). According to this report, hyaluronic acid binds directly to and modulates L-type voltage-dependent calcium channels (L-VDCC; Cav1.2). Acute enzymatic removal of hyaluronic acid with hyaluronidase reduced nifedipine-sensitive Ca^{2+} currents in

dendrites and spines of hippocampal neurons in slices from 2- to 3-month-old mice. Furthermore, it abolished an L-VDCC-dependent component of LTP at the CA3–CA1 Schaffer collateral synapses in the hippocampus. This deficit was completely rescued by the application of exogenous hyaluronic acid. In a heterologous expression system, exogenous HA rapidly increased currents mediated by Cav1.2, but not Cav1.3 subunit-containing L-VDCCs indicating a direct binding of hyaluronic acid to the channel. At the systemic level, intrahippocampal injection of hyaluronidase impaired contextual fear conditioning. Thus, the perisynaptic extracellular matrix influences use-dependent synaptic plasticity through regulation of dendritic Ca^{2+} channels (Kochlamazashvili et al. 2010).

7.5 Role of ECM in Control of Neurotransmitter Receptors

7.5.1 The ECM as a Passive Diffusion Barrier for Cell Surface Molecules

A large pool of surface molecules is highly mobile within the plasma membrane due to lateral Brownian diffusion (Kusumi et al. 1993; Triller and Choquet 2008). However, most surface molecules are restricted in lateral mobility by obstacles (pickets and corrals) compartmentalizing the cell surface. These obstacles are most likely formed by the underlying cytoskeleton or by rigid membrane structures (Kusumi et al. 1993; Choquet and Triller 2003; Kusumi et al. 2005). Synapses represent one of the most important surface compartments and are the sites of neurotransmitter release and detection for interneuronal communication. Interestingly neurotransmitter receptors, such as AMPA-type and NMDA-type glutamate receptors or $GABA_A$ receptors, not only are present in synaptic areas but are also found extrasynaptically. Lateral diffusion of receptors in extrasynaptic and synaptic domains has been investigated intensely in the past years (Triller and Choquet 2008). In general, diffusion of these receptors is more confined in the synaptic compartment as compared to extrasynaptic areas. However, receptors are steadily exchanging between synaptic and extrasynaptic pools. This steady replacement probably constitutes a fundamental mechanism for the maintenance of synaptic receptor pools as the exchange between cell surface and intracellular receptors through exo- and endocytosis occurs outside the synaptic membrane (Newpher and Ehlers 2008; Petrini et al. 2009). In addition, studies on hippocampal slices and primary hippocampal neurons have revealed that lateral diffusion may account for the exchange of desensitized synaptic AMPA receptors, which emerge during high-frequency firing, for naive extrasynaptic ones (Heine et al. 2008). Blockade of lateral diffusion, e.g., by cross-linking with antibodies, resulted in strong paired-pulse depression (PPD) presumably caused by the accumulation of desensitized receptors under the release site. These results demonstrated that the lateral diffusion

of AMPA receptors was a novel postsynaptic mechanism influencing short-term plasticity of individual synapses.

Interestingly, the diffusion rates of AMPA receptors on dissociated hippocampal neurons decreased during synapse maturation, between the second and third week in vitro (Borgdorff and Choquet 2002). During this time period, a hyaluronan–CSPG-based ECM resembling the perisynaptic netlike ECM of the adult CNS is formed in these cultures (John et al. 2006). Similar to the in vivo situation, the netlike structure divides the neuronal surface into multiple compartments of variable size. These ECM-derived cell surface structures restrict the lateral diffusion of extrasynaptic AMPA receptors (Frischknecht et al. 2009; Fig. 7.2a). Removal of the ECM with the enzyme hyaluronidase increased diffusion rates of extrasynaptic but not of synaptic receptors. The exchange between receptors at synapses and extrasynaptic compartments was also increased. Extrasynaptic diffusion rates after hyaluronidase treatment resembled the "juvenile" situation before the ECM is established in the cultures at day 10 in vitro.

An electrophysiological assessment revealed that removal of ECM from dissociated hippocampal neurons and, under certain conditions, also from hippocampal slices affected short-term synaptic plasticity (Frischknecht et al. 2009; Kochlamazashvili et al. 2010). In the presence of the ECM, PPD seems to be much stronger than after hyaluronidase treatment when basically no PPD was observed. Thus, ECM-derived surface compartments can influence short-term plasticity of neurons by controlling lateral diffusion and thus control the synaptic availability of naive AMPA receptors. It should be noted here that ECM nets are not impermeable barriers for diffusing surface proteins. They rather have to be considered as viscous structures that reduce the surface mobility of proteins through weak, transient interactions, or simply as passive obstacles. Accordingly, the size and shape of the extracellular domains of surface-exposed membrane proteins influence the mobility shift by the ECM (Frischknecht et al. 2009). Along this line, the recent characterization of the full crystal structure of AMPA receptors points to their very large extracellular domain, protruding over 10 nm into the extracellular space (Sobolevsky et al. 2009) and, thus, likely to bump into these ECM components.

7.5.2 Specific Effects of ECM Elements on Neurotransmitter Receptor Regulation and Synaptic Plasticity

In addition, there are ECM components that interact specifically with surface molecules and thereby modify synaptic function. Especially the neuronal activity–regulated pentraxin (Narp), an immediate early gene that is upregulated by neuronal activity, has been implicated in activity-dependent synapse formation and synaptic scaling (O'Brien et al. 1999; Chang et al. 2010). Narp is a secreted calcium-dependent lectin that forms a covalent heteromeric complex with the neuronal pentraxin 1 (NP1) (Tsui et al. 1996; Xu et al. 2003). Narp complexes are

enriched at excitatory synapses especially on parvalbumin (PV)-expressing interneurons; they have been suggested to associate with the GluA4 subunit of AMPA receptors and to regulate their synaptic clustering (O'Brien et al. 1999; Sia et al. 2007; Chang et al. 2010). Digestion of the PNN using chondroitinase ABC abolishes Narp clustering on PV-positive neurons indicating that the formation of Narp clusters depends on hyaluronan/CSPG-based ECM structure (Chang et al. 2010). It has been further reported that presynaptically released Narp is required for homeostatic synaptic scaling in PV cells (Chang et al. 2010; Fig. 7.2b). Increased network activity drives Narp expression, which in turn is required for GluA4 upregulation and enhanced mEPSC amplitudes on PV cells after network silencing using TTX. PV-expressing cells from $Narp^{-/-}$ mice show neither increased GluA4 expression nor any form of synaptic scaling (Chang et al. 2010). It is known that PV-positive cells are key players in the control of network activity (Sohal et al. 2009), and the inhibitory network plays an important role in suppression of seizures. Consistently, $Narp^{-/-}$ mice are more sensitive to kindling-induced seizures (Chang et al. 2010).

It is known for long that integrins as classical ECM receptors are involved in synaptic function and plasticity (e.g. Staubli et al. 1998; Chavis and Westbrook 2001; Dityatev and Schachner 2003). Different integrin heterodimers seem to have differential functions in the induction and maintenance of synaptic plasticity including LTP (Chan et al. 2006; for review, see Dityatev et al. 2010a). One particular example is the antagonistic regulation of synaptic accumulations of GlyRs in rat spinal cord neurons (Charrier et al. 2010). Here, β1-containing integrins seem to increase GlyR and gephyrin clusters in synapses, whereas β3-containing integrins have the opposite effect. Potential ligands are TSP1 for β1-containing integrins and fibrinogen for β3-containing integrins. Signaling occurs both via protein kinase C, what affects GlyR mobility inside and outside of synapses, and CaM kinase II that is activated and inhibited by β1 and β3 integrins, respectively, and has opposing effects on synaptic GlyRs (Fig. 7.2e). Thus, active CaMKII is responsible for keeping GlyRs within the synapse. Integrin signaling cooperates with other signaling pathways to regulate synaptic functions. For instance, β1-integrin signaling seems to interact with reelin-induced signaling pathways to regulate NMDA receptor composition and mobility (see below). Interestingly, matrix metalloproteases, like MMP9, can unmask binding motifs on integrin ligands upon proteolytic cleavage and in this way induce integrin-mediated regulation of NMDA receptors (Wang et al. 2008; Michaluk et al. 2009, see below).

Finally, integrins are also involved in homeostatic processes like synaptic scaling. For example, β3-containing integrin complexes regulate the synaptic availability of GluA2-containing AMPA receptors via the small GTPase Rap1 (Cingolani et al. 2008). It is thought that the upregulation of β3-integrins by tumor necrosis factor-α (TNFα) released from astrocytes is responsible for a reduction of GluA2 endocytosis and accordingly a synaptic upscaling in response to tetrodotoxin-induced suppression of network activity (Pozo and Goda 2010; Fig. 7.2c). Accordingly, Steinmetz and Turrigiano (2010) showed that addition of TNFα to hippocampal cultures leads to increased AMPA amplitude at control

synapses. However, addition of the factor to prescaled cultures had exactly the opposite effect suggesting that TNFα is a critical factor for maintaining synapses in a plastic range within which scaling can be accomplished (Steinmetz and Turrigiano 2010).

Reelin is a 400-kDa ECM protein that is well appreciated for its function during development, where it is involved in controlling the migration and laminar arrangement of neurons in various structures including the neocortex, the hippocampus, the cerebellum, and the spinal cord. This molecule has also been implicated in the maintenance of neuronal networks (Frotscher 2010) and in mechanisms of synaptic plasticity, e.g., by controlling NMDA receptor function (Herz and Chen 2006). Reelin mediates its function in the adult CNS as well as during development by binding to its cell surface receptors the very-low-density lipoprotein receptor (VLDLR) and ApoE2 receptor (ApoE2R) and in turn the downstream adaptor protein Dab1 (see Fig. 7.2d for details on reelin signaling pathways). Reelin signaling eventually stabilizes F-actin via inducing n-cofilin phosphorylation (Chai et al. 2009). Reelin-mediated stabilization of F-actin is not only crucial for directional migration processes during cortex development, but in addition may be essential for the maintenance of the adult brain and thus was hypothesized to act as a mediator between stability and plasticity in the adult brain (Frotscher 2010).

Moreover, the reelin receptors VLDLR and ApoE2 seem to be directly in contact with synaptic NMDA receptors via the membrane-associated guanylate kinase homologue PSD-95, and reelin signaling is closely connected to NMDA receptor signaling and thereby regulates synaptic plasticity (Herz and Chen 2006; Rogers and Weeber 2008). Reelin has also been implicated in the control of the subunit composition of somatic NMDA receptors during hippocampal maturation (Sinagra et al. 2005), and reelin secreted by GABAergic interneurons is responsible for maintaining the adult NMDA receptor composition. Blockade of reelin secretion reversibly increases the fraction of juvenile GluN2B-containing NMDA receptors, and addition of exogenous reelin can rescue this effect (Campo et al. 2009). In addition, reelin controls the surface trafficking of GluN2B-containing NMDA receptors (Fig. 7.2d). As shown by single-particle tracking, inhibition of reelin function reduced the surface mobility of these receptors and increased their synaptic dwell time in an integrin-dependent manner (Groc et al. 2007). β1-containing integrin receptors are supposed to cooperate with ApoE2Rs and/ or VLDLRs in this context.

Reelin has also been discussed as a serine protease that is able to digest fibronectin, laminin, and to a lesser extent also collagen IV. Recently, also Caspr (contactin-associated protein), a molecule known to be required for the formation of axoglial paranodal junctions surrounding the nodes of Ranvier in myelinated axons, has been added as a substrate of reelin (Devanathan et al. 2010). Caspr inhibits neurite outgrowth of cerebellar neurons, and it has been proposed that shedding of Caspr by proteolytic action of reelin counteracts its repulsive function (Devanathan et al. 2010). Further, it was proposed that binding of the prion protein (PrP) to Caspr protects Caspr from proteolysis and thus supports its repulsive function during neurite outgrowth (Devanathan et al. 2010). However, it should be noted that the

protease activity of reelin is controversially discussed in the literature (Kohno and Hattori 2010).

7.6 Proteolysis of the ECM and the Generation of Synaptic Signals

ECM-modulating enzymes with major impact on brain development and synapse function are the large family of the matrix metalloproteases (Ethell and Ethell 2007). Probably, the best-studied member of the MMP family in the nervous system is MMP9. Increased neuronal activity enhances expression of MMP9 and leads to increased proteolysis of β-dystroglycan (Szklarczyk et al. 2002; Michaluk et al. 2007). Depletion of MMP9 results in an impairment of LTP at hippocampal synapses (Nagy et al. 2006). Application of MMP9 to neuronal primary cultures affects lateral diffusion of NMDA receptors without changing the mobility of AMPA receptors or the structure of the hyaluronic acid–based ECM (Michaluk et al. 2009). Rather, extracellular MMP9 proteolysis induced β1-integrin-dependent signaling, which then led to the mobilization of NMDA receptors (Fig. 7.2f). β1-Integrin signaling was also identified as being responsible for MMP9-induced spine enlargement and synaptic potentiation (Wang et al. 2008). To date, the target of MMP9 proteolysis within the ECM that induces integrin signaling remains unknown. Recently, the effect of an enriched environment on synapse morphology, ECM structure, and MMP9 and MMP2 activity in the deep cerebellar nuclei (DCN) of adult mice has been examined (Foscarin et al. 2011). Enriched environment leads to an increased size of Purkinje cell axon termini on DCN neurons. At the same time, a reduction of *Wisteria floribunda* agglutinin (WFA) staining, which labels chondroitin sulfates, and hyaluronic acid labeling was observed indicating a downregulation of PNN. This may happen through a downregulation of components of the PNN after environmental enrichment as it was observed on the level of transcripts for aggrecan and Crtl1. Alternatively, the ECM may be degraded by MMP9 and its close relative MMP2, which exhibited an increased activity after environmental enrichment (Foscarin et al. 2011).

Another matrix metalloprotease, MMP3, processes agrin at the basal lamina of the neuromuscular junction in an activity-dependent manner (Werle and VanSaun 2003). Agrin plays a pivotal role in the development and maintenance of the neuromuscular junction. It induces acetylcholine receptor clustering through the muscle-specific receptor tyrosine kinase (MuSK) and its coreceptor low-density lipoprotein receptor-related protein 4 (LRP4) (Sanes and Lichtman 2001; Dityatev et al. 2010a). It has been suggested that agrin processing by MMP3 is indispensable for normal neuromuscular junction development. In line with this view, MMP3 knockout mice exhibit abnormal neuromuscular junction morphology and acetylcholine receptor distribution (VanSaun et al. 2003).

Also the brain-specific serine protease neurotrypsin has been reported to specifically process agrin (Reif et al. 2008). Actually, neurotrypsin seems to be the sole agrin-cleaving protease in the CNS while, in contrast to MMP3, it is not relevant for agrin cleavage at the neuromuscular junction (Bolliger et al. 2010). Like MMP3, neurotrypsin cleaves agrin at two highly conserved sites releasing a 90-kDa and a 22-kDa fragment. Both agrin fragments are absent in the brain of neurotrypsin-deficient mice indicating that in vivo cleavage of agrin in the brain depends on neurotrypsin (Reif et al. 2007; Reif et al. 2008). The 22-kDa fragment resembles a laminin G3 domain. Indeed, it has been shown that the naturally occurring cleavage product forms a stable, well-folded domain while shorter constructs were aberrantly folded (Tidow et al. 2011). Neurotrypsin has been identified as essential for cognitive functions in the human brain. Deletion mutation in the coding region resulting in a truncated protein without protease domain leads to severe mental retardation (Molinari et al. 2002). Furthermore, neurotrypsin is recruited and released at synapses in an activity-dependent manner (Frischknecht et al. 2008). Activation of neurotrypsin proteolytic activity requires concomitant activation of the postsynaptic neuron (Matsumoto-Miyai et al. 2009). It has been demonstrated that the proteolytic fragment of 22 kDa acquired filopodia-inducing signaling properties in hippocampal slice cultures after induction of synaptic LTP (Matsumoto-Miyai et al. 2009). Similar to MMP9, proteolytic cleavage of ECM components by neurotrypsin unmasks a signaling molecule (Fig. 7.2f), which in turn induces alterations in spine morphology and even the generation of new synapses. These examples suggest that the ECM contains a variety of hidden instructive signals that can be unmasked by specific proteolytic enzymes.

Similar to chondroitinase ABC treatment, the topical application of the serine protease tissue-type plasminogen activator (tPA) can prolong the critical period in the visual cortex (Mataga et al. 2004; Oray et al. 2004). Moreover, tPA expression is significantly increased after monocular deprivation during the critical period of development (Mataga et al. 2002). In line with this observation, tPA knockout mice display reduced OD plasticity during the critical period and repetitive application of tPA to the cortex rescues normal plasticity in mutant mice (Mataga et al. 2002). In adult animals, where no OD plasticity occurs, monocular deprivation leads to no significant increase in tPA activity (Mataga et al. 2004). Application of tPA to cortical slices increases spine motility, and a 2-day period of monocular deprivation, which leads to tPA release, has a similar effect (Mataga et al. 2004). The effect of exogenous tPA is prevented in slice from monocularly deprived animals, suggesting that tPA is the mediator of the increased spine motility after monocular deprivation (Mataga et al. 2004). Moreover, tPA activity is also responsible for a change in spine density that follows monocular deprivation (Oray et al. 2004) with a transient loss of spines in the binocular region of the visual cortex. This effect is absent in the tPA knockout mouse and can be rescued by exogenous application of tPA (Oray et al. 2004). Thus, tPA release after monocular deprivation is developmentally regulated, and by degrading ECM proteins, tPA might be a crucial determinant of experience-dependent plasticity *in vivo*.

7.7 Outlook

After their discovery, PNN have been enigmatic for more than a century. Research activities of the last decade strongly imply that one important function of PNN is the restriction of juvenile plasticity in the adult brain at the end of critical periods. This occurs at the expense of the regenerative potential of the mature vertebrate CNS. Moreover, while PNN are most prominent on a particular class of GABAergic interneurons, PNN-like structures are found throughout the brain where they influence and regulate synaptic plasticity processes. There they have passive and active roles, the molecular mechanisms of which are just about to be discovered. The knowledge of these mechanisms will open new avenues for a better basic understanding of brain function. In longer terms this may also provide opportunities for curative intervention, e.g., by keeping the critical periods for brain wiring open for longer time or even reopening them therapeutically to correct miswiring.

Acknowledgments Work in the authors' laboratory was supported by the Deutsche Forschungsgemeinschaft (GU 230/5-3) and the German Federal Minster for Education and Science BMBF via EraNET NEURON (Moddifsyn).

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