Chapter 7

Neuronal Plasticity in the Juvenile and Adult Brain Regulated by the Extracellular Matrix

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Abstract

In brains of higher vertebrates, the delicate balance of structural remodeling and stabilization of neuronal networks changes over the life-span. While the juvenile brain is characterized by high structural plasticity, it is more restricted in the adult. During brain maturation, the occurrence of the extracellular matrix (ECM) is a critical step to restrict the potential for neuronal remodeling and regeneration, but providing structural tenacity. How this putative limitation of adult neuronal plasticity might impact on learning-related plasticity, lifelong memory reformation, and higher cognitive functions is subject of current research. Here, we summarize recent evidence that recognizes the ECM and its activity-dependent modulation as a key regulator of learning-related plasticity in the adult brain. We will first outline molecular concepts of enzymatic ECM modulation and its impact on synaptic plasticity mechanisms. Thereafter, the ECM's role in converting juvenile to adult plasticity will be explained by several key studies in wild-type and genetic knockout animals. Finally, current research evidences the impact of ECM dynamics in different brain areas including neocortex on learning-related plasticity in the adult brain impacting on lifelong learning and memory. Experimental modulation of the ECM in local neuronal circuits further opens short-term windows of activity-dependent reorganization. Malfunctions of the ECM might contribute to a variety of neurological disorders. Therefore, experimental ECM modulation might not only promote complex forms of learning and cognitive flexible adaptation of valuable behavioral options, but has further implications for guided neuroplasticity with regenerative and therapeutic potential.

Keywords: Learning, Plasticity, Memory, Cortex, Protein turnover

1. Introduction

In the brains of higher vertebrates, both neurons and glial cells produce and secrete the molecules that accumulate and form the extracellular matrix (ECM). In the nineteenth century, pioneers

of brain cell biology including Camillo Golgi and Santiago Ramon y Cajal have discovered a mesh-like structure surrounding the most neurons and synapses in the adult brain [1]. This extracellular scaffold has been seen initially as a key component for ensuring the structural stability of the respective tissue [2]. Later research evidenced that the ECM has multiple functions including regulation of cell adhesion, cell-to-cell communication, cell differentiation, cellular compartmentalization, and several forms of neuronal plasticity [3]. During brain maturation, the ECM undergoes profound changes. In late embryonic phases, the ECM is composed of particular proteoglycans and glycoproteins like neurocan and tenascin-C, which are downregulated during adulthood. Other components like the glycoprotein tenascin-R and chondroitin sulfate (CS) proteoglycans (CSPGs), such as brevican and aggrecan, are the main molecular components of the adult brain ECM. One of the most important structural components in the adult ECM is the unbranched polysaccharide hyaluronic acid (HA). HA forms the backbone that structurally orchestrates the enmeshment of all other ECM components. Interestingly, it has been shown that this developmental shift of the juvenile and adult forms of the ECM coincides with the closure of the so-called critical periods during brain maturation of respective brain regions. This led to the hypothesis that the brain's ECM is involved in regulating the switch from juvenile to adult plasticity by structural tenacity restricting the potential for neuronal reorganization [4]. Thereby, the brain has evolved mechanisms that guarantee structural stability of the neuronal networks established during experience-dependent learning. This brain function is fundamental for strengthening neuronal connections and their respective forms of processing impacting on long-term memory storage and recall. Nevertheless, current research has shown that dynamic adaptations of the ECM can alter several forms of synaptic plasticity and thereby regulate flexible learning and memory organization in the adult brain.

2. The structural foundation of the ECM in the vertebrate's brain

Components of the ECM in the mature brain are interlinked in a complex netlike architecture [5]. The linear HA backbone binds and coordinates proteoglycans especially of the lectican family that are cross-linked by glycoproteins such as the tenascins. Thus, this form of ECM is referred to as HA-based ECM. It is rich in the glycosaminoglycan CS attached to CSPGs of the lectican family, as for instance brevican and aggrecan [6]. The main carrier of CS within the ECM is aggrecan with its multiple attachment sites, while Brevican is a part-time proteoglycan existing glycoprotein and proteoglycan [7,8]. Brevican and aggrecan both bind to the ECM glycoprotein tenascin-R (**Figure 1A**). Other so-called cartilage link proteins form a complex with N-terminal domains of the lecticans and HA and thereby contribute to the ECM stability [6]. A large variety of other components including reelin, laminins, thrombospondins, heparin-sulfate proteoglycans, guidance molecules, and even transcription factors are incorporated in the complex ECM structure (**Figure 1A**). This form of the homogenous HA-based ECM loosely enwraps cell bodies, dendritic, and synaptic structures of most neurons. Further, the mature brain contains a specialized glycosaminoglycan-rich ECM structure around synapses and somata of a small proportion of neurons. This more rigid form is called the perineuronal nets (PNNs), which are especially rich in aggrecan and CS. Such PNNs are most abundant on

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GABAergic interneurons expressing the calcium buffer protein parvalbumin (PV; **Figure 1B**). Recent evidence shows that PNNs are highly heterogeneous and can be found on various types of neurons throughout the CNS. For the formation of WFA-positive PNNs, the cartilage link protein Crtl1/Hapln1 has been identified as a key regulator [9].

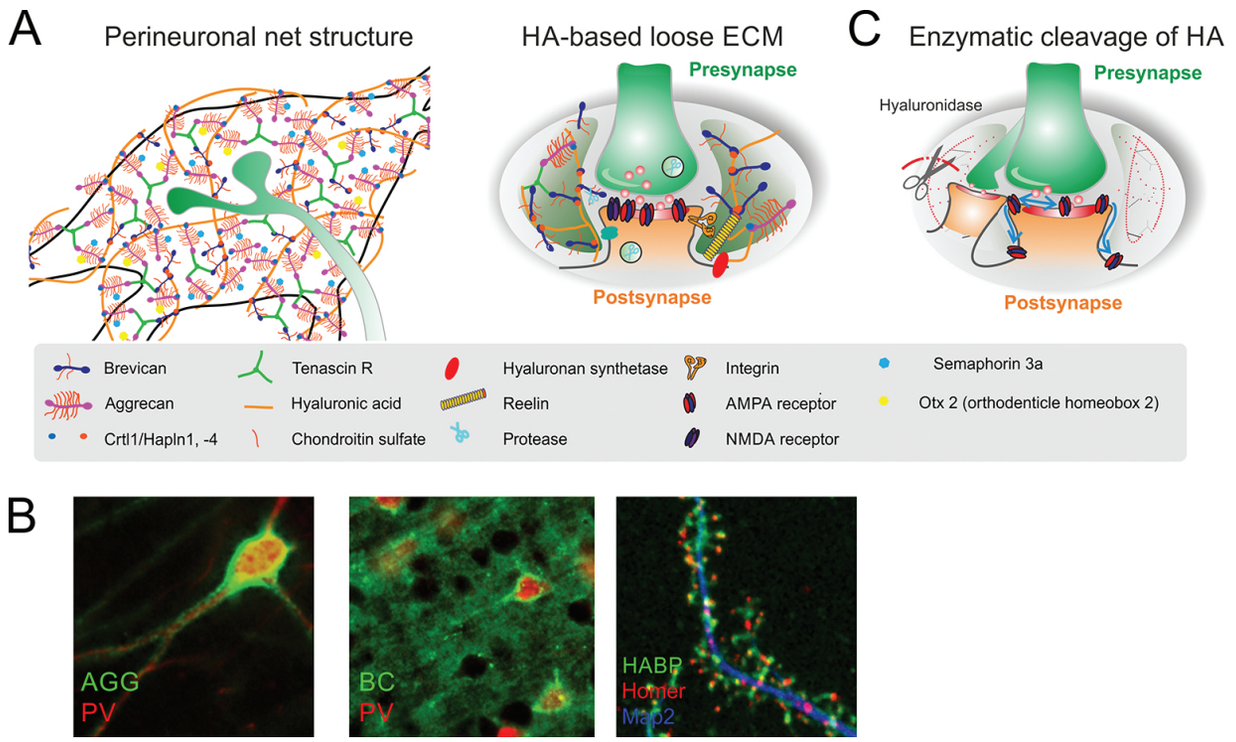

Figure 1. Cell types and their specific forms of the hyaluronan-based ECM. (**A**) The mature ECM is based on the backbone of hyaluronic acid (orange). Schematized are the specialized form of the PNN (*left*) and the loose ECM (*right*) around synaptic contacts. Densely packed PNNs are rich in CS and aggrecan. Small signaling molecules such as semaphorin 3a or Oxt2 are bound to CS within the PNN and mediate several functions or regulate gene expression. The loose ECM around excitatory, spiny synapses is rich in brevican and contains only little aggrecan and is thus less rich in CS. ECM proteins (e.g., reelin) signal through their receptors (e.g., integrins) and regulate several cellular processes including trafficking of glutamate receptors (see C). ECM function is modulated by proteases (scissors) that may free synapses by removing ECM or unmask signaling molecules. (**B**) *Left*, Parvalbumine positive interneuron (PV, red) with typical PNN stained for aggrecan (AGG, green). *Middle*, Dendritic spines and synapses of excitatory neurons (red) are also surrounded by brevican (BC, green), although it is less specific for PNN's. *Right*, Dissociated cortical neuron stained with the dendritic marker Map2 (blue) and Homer (red) to stain excitatory synapses and hyaluronic acid binding protein (HABP, green) to label extracellular matrix. Note the loose appearance around dendrites, spines, and synapses. (**C**) Enzymatic weakening of the ECM by glycosidases (e.g., hyaluronidase, see scissors) changes the micromillieu around synapses by for instance increased lateral diffusion and synaptic exchange of AMPA receptors (blue arrows). Further, removal of stabilizing cues facilitates structural plasticity such as *de-novo* formation of new synapses and synaptic scaling. Modified from Ref. [22].

The structural foundation of the mature ECM allows regulating several functions beyond mechanical stability of neuronal networks. In the juvenile brain, the ECM regulates neuro- and gliogenesis, cell migration, axonal pathfinding, and synaptogenesis. Adult forms of the HA-based ECM are in the service of multiple functions including regulation of synaptogenesis and synaptic plasticity, compartmentalization of the neuronal surface, neuroprotection, regulation of ion homeostasis, and neuron–glia interactions. The remainder of this review will consider

several ECM-based mechanisms for regulating synaptic plasticity and its effects on brain development and adult learning behavior.

3. ECM-guided switch from juvenile to adult synaptic plasticity

Early in life, high structural plasticity allows profound shaping of brain circuits by experience. Such critical periods in the juvenile brain are limited by the occurrence of the ECM implementing adult brain plasticity modes. For instance, in wild-type animals, dark rearing delays not only the critical periods of developmental plasticity in visual cortex of rodents, but also the formation of PNNs. During the development, the cartilage link protein Crtl1/Hapln1 is organizing the formation of PNNs. Crtl1/Hapln1 k.o. mice do not develop normal PNN structures in the visual cortex. These mice show juvenile forms of ocular dominance (OD) plasticity and sensitivity of the visual system to deprivation throughout the life-span [9].

The seminal study by Pizzorusso et al. [10] has elucidated the ECM as the regulatory switch between juvenile and adult plasticity. The authors combined monocular deprivation with injection of the ECM-cleaving enzyme chondroitinase ABC (chABC) into visual cortex of adult rats (see also **Figure 1C**). The local weakening of the ECM "re-juvenated" the visual cortex and restored the critical period form of OD plasticity (**Figure 2**). By the same manipulation Pizzorusso and colleagues restored in a follow-up study, the visual acuity in adult animals grown up with long-term monocular deprivation [11]. Similarly, application of the serine protease tissue-type plasminogen activator (tPA) into the visual cortex can prolong or reactivate critical periods of OD plasticity in visual cortex [12] based on increased structural remodeling [13].

Later studies identified the regulatory role of the ECM in other forms of developmental plasticity during brain maturation of different vertebrate species. For instance, birdsong learning in the zebra finch occurs during a sensitive period similar to the language development in humans. It has been shown that with the end of this critical period PNNs around PV-positive neurons emerge in brain areas that are dedicated to singing [14]. In another set of experiments, Gogolla et al. [15] shown that the maturing ECM in the amygdala essentially makes fear memories erasure resistant in adult animals. In rats not older than 3–4 weeks, a conditioned fear memory trace can be erased permanently by extinction, that is, the presentation of the conditioned stimulus without the aversive stimulus. However, after this period extinction only attenuates the fear response, but it reinstates instantaneously if the aversive stimulus is presented again. Hence, a permanent loss of the fear memory is only found before the ECM in the amygdala is formed and is preserving established fear memories. Gogolla and colleagues now attenuated the ECM in the amygdala by chABC injections in adult rats. This led to a complete erasure of the fear response after an extinction phase even if the aversive stimulus is presented to these animals again. In addition, the early preweaning environment impacts on rodent ECM maturation in a functional manner. Improved performance in water maze learning in the adult age after early postnatal-enriched housing has been correlated with increased PNN formation in the striatum reflecting functional shaping of neuronal circuits involved in motor learning [16].

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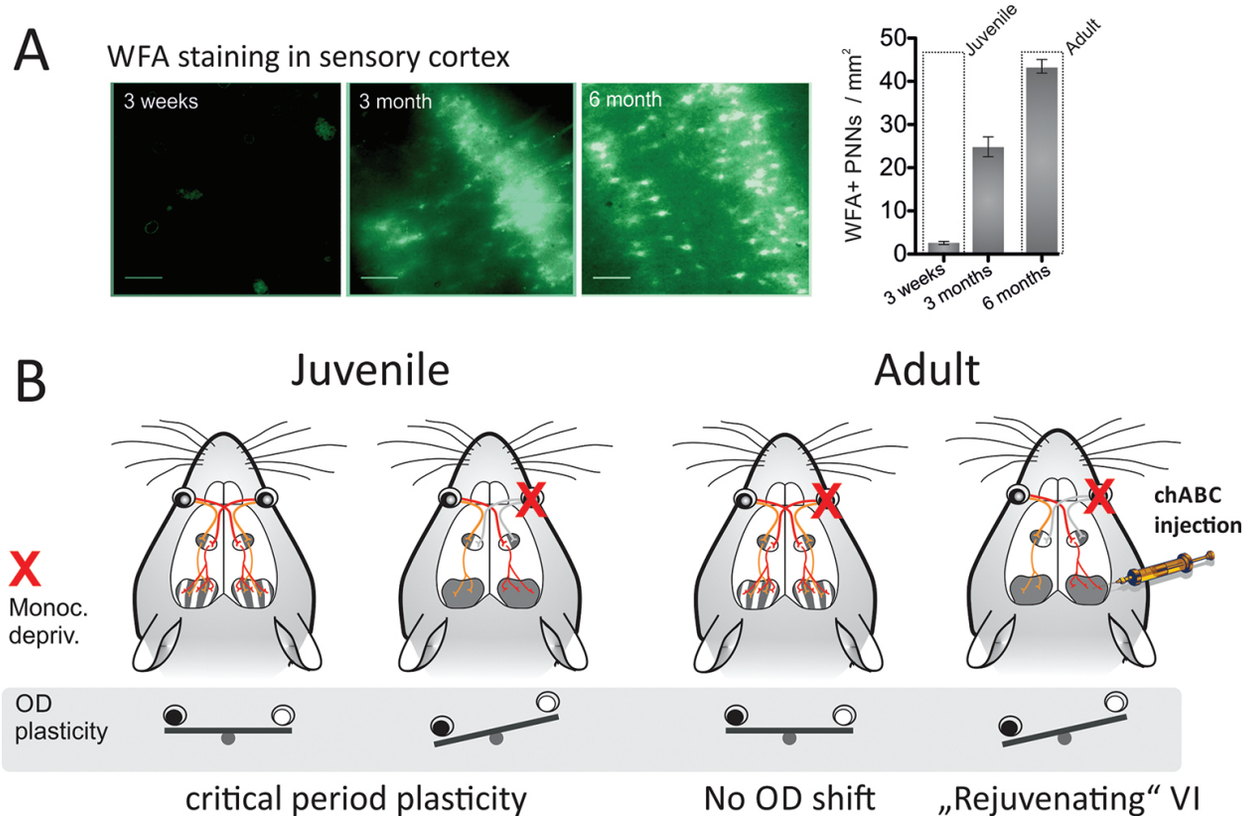

Figure 2. The emerging ECM during brain maturation and its regulatory role for juvenile brain plasticity (**A**) *Wisteria floribunda* (WFA) fluorescein staining against sugar chains of the CSPGs in rodent sensory cortex shows that the diffuse HA-based ECM and perineuronal nets around cells emerge with brain maturation (≥3 month), but were abundant in juvenile state (3 weeks). Modified from Ref. [42]. (**B**) Pizzorusso et al. [10] demonstrated the inhibitory role of the developmental maturation of the ECM in visual cortex of rats for early experience-dependent plasticity. In juvenile rats, monocular deprivation (MD) leads to an ocular dominance shift (left). After the critical period, MD alone did not cause such a shift in the adult. In this study, the authors found that weakening of the ECM by chABC treatment in visual cortex reactivated OD plasticity shifting toward the nondeprived eye.

The emerging ECM in the developing brain seems to be involved in a complex molecular machinery organizing the maturation of neuronal networks. For instance, in mouse visual cortex, it has been shown that critical periods are initiated, maintained, and closed by the action of the orthodenticle homeobox 2 homeoprotein (Otx2) on PV+-GABAergic interneurons [17]. Interestingly, Otx2 contains a glycosaminoglycan binding sequence that mediates its allocation to the PNNs by specific binding to CS D and E. Thereby, a constant level of Otx2 in PV+-neurons keeps a mature, consolidated, and persistent PNN-state in the adult brain. Hydrolysis of the PNNs by chABC reduces the amount of endogenous Otx2 in PV cells mediating the reopening of OD plasticity in adult mice [18].

The reviewed studies in this paragraph have shown how the change of the juvenile form of the ECM to a more rigid adult ECM mediates the different modes of neuronal plasticity during brain development. Why has this restriction of adult reorganizational and regenerative plasticity only evolved in higher vertebrates? The evolutionary benefit may be to preserve the costly acquired hardwired connections during early life experience, which are fundamental

for rapid experience-based behavioral adaptations of higher vertebrates [4]. Nonetheless, the adult, healthy brain retains a remarkable capability of plastic reorganization that is essential for constantly adapting to our ever-changing environment.

4. The hyaluronic acid-based ECM regulates adult synaptic plasticity

The functional mechanisms by which the HA-based ECM implements adult forms of brain plasticity including classical (Hebbian) and homeostatic plasticity are still elusive (for an overview see [3]). Research investigating knockout models lacking particular components of the ECM have provided major insights into the impact of the ECM on adult synaptic plasticity. For instance, different mouse models deficient in specific matrix components, as tenascin-R, brevican, or neurocan, all showed impaired forms of hippocampal long-term potentiation (LTP). However, during the brain development of k.o.-models compensatory mechanisms might mimic the deficit of a particular matrix component and hence limit the significance of the findings. Therefore, another experimental strategy is to use acute enzymatic weakening of the ECM based on the local application of different matrix-degrading enzymes (**Figure 1C**). For instance, it has been shown that treatment with chABC impaired theta-burst-induced LTP in CA1–CA3 pyramidal synapses in hippocampal slices [19]. It has been suggested that this is due to an increased excitability of GABAergic perisomatic interneurons [3]. Similar findings have been found with Injection of the hyaluronidase (HYase) from *Streptomyces hyalurolyticus* [20]. In contrast to other hyaluronidases, this enzyme is highly specific to HA and does not digest CS. The phenotype in these experiments could be rescued by perfusion with HA. It has been suggested that this is due to the fact that HA directly regulates L-type voltage-gated calcium channels (L-VDCC; Cav1.2) of CA1 neurons and thus postsynaptic Ca^{2+} entry and hippocampal-dependent forms of learning [3,20]. In a recent study, we have described a molecular mechanism by which acute ECM removal was altering short-term-dependent forms of synaptic plasticity [21]. We measured paired pulse ratios in dissociated hippocampal cultures by cell-attached recordings. We found the typical robust paired pulse depression (PPD) under control conditions. Interestingly, digestion of the ECM by infusion of HYase prevented cells from expressing PPD. We further found increased lateral diffusion of extrasynaptic AMPA receptors after ECM digestion as key correlate of this effect [21]. This results in a higher exchange between synaptic desensitized receptors with extrasynaptic naïve ones, which quickly replenishes the pool of excitable receptors in the active zone of a synapse (**Figure 1C**). In such conditions, synapses are able to follow higher firing frequencies. Blockade of lateral diffusion of AMPA receptors by cross-linking with antibodies, on the other hand, led to stronger PPD caused by accumulated desensitized synaptic AMPA receptors [22]. Similarly, mobility of other receptor types can be modulated by specific proteolytic enzymes. For instance, MMP9 increases the mobility of NMDA-type, but not AMPA receptors. These results have shown that the perisynaptic ECM forms surface compartments that act as diffusion barrier for membrane proteins such as AMPA receptors. Modulation of lateral receptor diffusion provides a novel mechanism of short-term plasticity due to a changed extracellular micromillieu at individual synapses. Interestingly, lack of the major hyaluronan synthetase

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HAS3 in the hippocampus did not lead to a striking morphological change in the ECM. However, the extracellular space was reduced, which resulted in a more dense packing of cells and lower diffusion of soluble molecules in the CA1 *stratum pyramidale* [23]. Further, these animals were prone to epileptic seizures which underlines the importance of the regulatory function of the HA-based ECM that is required for instance for volume transmission and ion homeostasis.

Weakening of the ECM and the corresponding synaptic networks might further promote architectural remodeling and even increased spine motility. In *in vitro* hippocampal cultures, microinjections of chABC treatment increased the motility of local spines and induced spine remodeling in a β1-integrin-dependent manner [24].

5. ECM proteolysis and the generation of synaptic signaling molecules

Endogenous ECM-modulating enzymes regulate synaptic function in the juvenile and adult brain [25,26]. Such enzymes can exert their function by either altering the extracellular milieu via digestion of the ECM or by generating proteolytic fragments that may act as signaling molecules. An important group of such enzymes are the metalloproteases of the ADAMTS-family (a disintegrin and metalloproteinase with thrombospondin motifs). Within this family, ADAMTS-4/-5 are particularly interesting, as they are known for their ability to digest aggrecan and brevican. Therefore, they have been termed previously aggrecanase-1/-2. The current terminology, however, better reflects the ability of these enzymes to digest all members of the lectican family. Interestingly, their activity is increased after epileptic seizures and regulates homeostatic plasticity [27]. However, their impact on synaptic plasticity remains elusive and is subject of current research. The best-studied extracellular protease is the matrix metalloprotease 9 (MMP9). The activity-dependent expression of MMP9 influences synaptic plasticity by regulating spine enlargement and synaptic potentiation [25]. Recently, a molecular signaling cascade regulating synaptic plasticity has been identified based on the MMP-9-dependent cleavage of neuroligin-1 [28]. This study has demonstrated that focal activation of a single spine by glutamate uncaging is sufficient to cleave neuroligin-1. Moreover, the activity of MMP9 has been shown to be NMDA-receptor dependent and hence implemented locally input-specific forms of synaptic plasticity. Thereby, extracellular MMP-9 triggers a specific retrograde regulation of presynaptic efficacy by targeting postsynaptic neuroligin-1 [26,28]. Similarly, the brain-specific serine protease neurotrypsin is regulated in an activity-dependent manner and requires concomitant activation of the postsynaptic neuron [29]. Proteolytic cleavage of agrin by neurotrypsin unmasks a signaling molecule harboring a single laminin G3 domain. This 22 kDa molecule can further regulate spine morphology and de-novo synapse generation. Together, this suggests that proteolysis of components of the ECM by exoenzymes not only modify the structural rigidity, but also activates instructive signal molecules that locally modulate synaptic functions [25]. This may temporally restore local divisions of "juvenile" environments as a major constituent of the balance between plasticity and tenacity in the mature brain.

We have shown that the ECM in the adult brain is a plastic structural scaffold shaped by network activity. Depending on the current activity level, the ECM can incorporate secreted components or release signaling messengers by proteolytic cleavage. Cleaved products can trigger signaling through diverse ECM receptors and modulate the activities of transmitter receptor, ion channels, or integrin signaling impacting on plastic shaping of individual synapses.

6. Role of the ECM in control of adult learning behavior and cognitive flexibility

Experimental weakening of the ECM by local injection of matrix-digesting enzymes can promote functional neurorehabilitation in the injured brain. This has been related mostly to injuries on the level of the peripheral nervous system and spinal cord [4,30,31]. Experience-driven plasticity does, however, not only lead sensory development or neuronal rehabilitation, but is also indispensable during learning, memory formation, and re-consolidation throughout life. The question now arises how forms of ECM-dependent plasticity in the adult brain might govern learning-related plasticity, lifelong memory reformation, and the organization of cognitively flexible behavior. In this respect, several studies have investigated the involvement of ECM functions in memory storage in adult animals. This has been characterized the best for long-term plasticity in the hippocampus and fear memory in the amygdala. However, available evidence is controversial about how ECM functions may impact on learning and memory processes.

For instance, it has been reported that tenascin-R knockout mice show normal hippocampus-dependent spatial memory acquisition in a Water maze. In subsequent reversal learning though animals showed more vulnerable spatial long-term memory yielding enhanced relearning performance due to less conflicting past and actual learning contingencies [32]. Another study found an already impaired acquisition of hippocampus-dependent contextual memory in same knockout mice [33]. Injection of chABC in the bilateral striatum, however, has been related to an improvement of water maze acquisition learning, while the recall of the learned values was unaffected [34].

In addition to deficits in matrix components, studies also found effects of deficits in exoproteases modeling the ECM. Loss of MMP9 activity has been associated with impaired hippocampal-dependent learning and amygdala-dependent learning. This is in line with findings of wild-type mice trained in an inhibitory avoidance (IA) learning paradigm [35,36]. Hippocampal LTP has been related to increased levels of MMP3 and MMP9. Both proteases were upregulated for at least ~48 h promoting local plastic synaptic environments underlying the learning performance. Intra-hippocampal injections of MMP9 blockers completely abrogated memory for the IA response when tested days later. Comparably, hippocampal MMP3 and MMP9 were found to be increased during water maze acquisition learning in a NMDA-dependent manner. Hippocampal injection of the broad-spectrum MMP9 inhibitor FN-439

also prevented elevated MMP9 levels, altered hippocampal LTP, and prevented spatial acquisition learning [35].

Similarly, spatial training in a water maze in wild-type rats has been found to correlate with increased levels of hippocampal brevican and versican in the membrane fraction [37]. These findings indicate that hippocampal-dependent learning induced a period of intrinsic activity-induced focal MMP-mediated proteolysis driving long-lasting synaptic modifications underlying learning and memory consolidation. Effects of changes in the ECM on initial learning are, however, still unclear [25,33].

More recently, insights into the impact of the ECM onto behavior came from studies using experimental, enzymatic, and local weakening of the ECM. The study of Gogolla et al. manipulating adult fear extinction suggested that memory acquisition differs in juvenile and adult brains due to changes of the mature ECM functions. The authors further argued that intra-amygdala injections of chABC in adult rats had no effect on acquisition learning of fear, but only on extinction, reinstatement, and renewal of the fear memory [15]. A further study showed that intra-hippocampal and prefrontal injection of chABC and HYase in mice impair long-term trace contextual fear conditioning [38]. This finding has been related to the impairment in the L-VDCC-dependent component of hippocampal LTP by cleaved extracellular HA [20].

In addition to spatial memory, another set of studies examined the function of the ECM in memory consolidation of drug seeking. A recent study showed that intracerebral injection of FN-439 impaired the acquisition of a cocaine-induced conditioned place preference (CPP) of rats. FN-439 injection 30 min prior to cocaine memory re-activation further attenuated the reinstatement of CPP in extinguished animals. The study further showed that intra-amygdala injections of chABC during active extinction of cocaine-induced CPP prevented its subsequent priming-induced reinstatement. ChABC injections alone had no effect on the retention, retrieval, or relearning of CPP [39]. Similarly, enzymatic weakening of PNNs in the prelimbic cortex or in the amygdala of adult rats impaired the acquisition and reconsolidation of drug-induced memories [40,41].

With respect to cognitively flexible adaptation of behavior, we have recently shown that weakening of the ECM in auditory cortex promotes complex forms of cortex-dependent re-learning in the Mongolian gerbil [42]. In our experiments, we trained animals on frequency-modulated tone discrimination based on the rising or falling modulation direction in a go-/nogo-task. Such auditory learning is known to depend on learning-induced plastic reorganization of neuronal circuits in the auditory cortex. After acquiring robust discrimination of the stimulus contingencies, the animals were trained to reverse their choice. We found that ECM weakening by local HYase injection in bilateral auditory cortex accelerated the demanding relearning performance (**Figure 3**). Specifically, animals had to inhibit the obsolete initial behavioral strategy and then establish its successful reversal. Importantly, attenuation of the ECM did neither affect the acquisition learning nor erased already established, learned memory traces (**Figure 3B**). That means attenuation of the ECM in sensory cortex of these animals promoted the flexible adaptation of the effectively appropriate strategy during cortex-dependent learning behavior that bases on "reprogramming" previously acquired auditory

memories. The ECM reconstitutes after several days to weeks limiting again the promoting effects onto cognitive flexibility (**Figure 3A**). A comparable finding investigated log-term object recognition memory in knockout mice of the link protein Crtl1/Hapln1—a key molecule for stabilization of PNNs. The Ctrl1/Hapln1 knockout mice have attenuated PNNs in the perirhinal cortex. Long-term object recognition memory, a task depending on perirhinal cortex, was enhanced in these mice. Local injection of chABC in wild-type mice had the same memory-prolonging effect in the object recognition task, but also attenuated over time [43]. In this study, the attenuation of the PNNs was accompanied by enhanced perirhinal LTD, which is thought to be the major synaptic mechanism underlying object recognition memory.

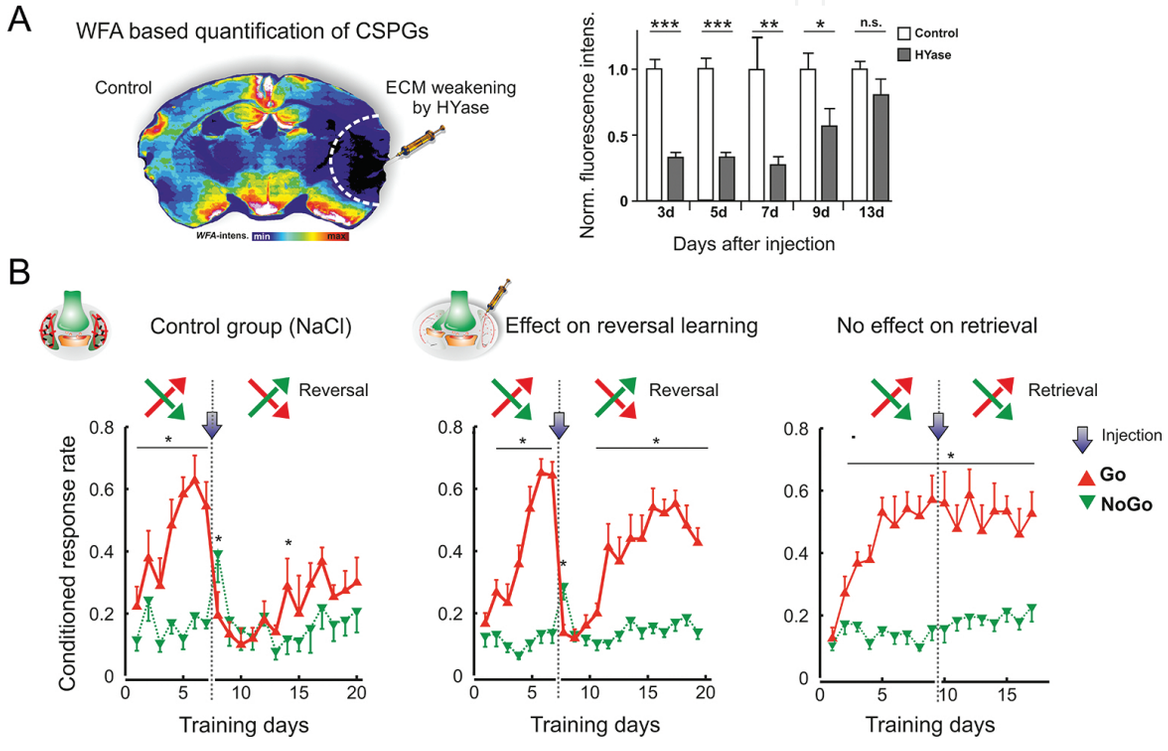

Figure 3. Local enzymatic weakening of the ECM in auditory cortex of Mongolian gerbils enhanced the cognitive flexibility in a relearning paradigm. (**A**) *Right*, Quantification of ECM weakening after local injection of HYase in unilateral auditory cortex of Mongolian gerbils (right) compared to control (left) based on WFA staining. *Left*, HYase injection significantly weakened the ECM for about 1 week and reconstituted fully after 2 weeks. (**B**) Mongolian gerbils were trained in a two-compartmental go/no-go Shuttle-box in order to discriminate two frequency-modulated sounds (modulation direction indicated by rising and falling arrows). Gerbils showed successful acquisition depending on the contingency of the stimuli as a go-stimulus (red) or Nogo-stimulus (green). In two groups, the contingency was reversed after seven training days (*left and middle*). Conditioned response rates were strongly reduced in both experimental groups indicating the active inhibition of the previously established discrimination strategy. HYase-treated animals were significantly better in correcting the behavioral strategy and successfully relearn the task (middle). Interestingly, HYase treatment did not interfere with the recall of already established cortex-dependent auditory memories (*right*). Modified from Ref. [42].

The both last-mentioned studies therefore promote the view that the perineuronal ECM in the adult brain actively organizes the balance between memory stability and flexibility. Cortical attenuation of the ECM in the mature brain might hence promote the cognitive flexibility that can build on learned behaviors and allows for an enhanced activity-dependent memory re-

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organization (see also Ref. [44]). And regeneration of the ECM gradually restores normal, restrictive adult plasticity levels. Generally, all studies summarized in this review emphasized that the increased experience-based plasticity by acute, enzymatic PNN diminution is activity-dependent and the rather inconspicuous effect of mere ECM attenuation in general. Mechanistically, enzymatic ECM degradation might facilitate the rearrangement of functional network connectivity by a shift in the balance between excitation and inhibition leading to destabilized existing patterns of neuronal network interactions [45].

7. Impact of the ECM in old age on memory function and cognitive integrity

With respect to age, the importance to provide profound tenacity to conserve experience-based memories might increase over the life span. Deficits or malfunctions of several ECM molecules or ECM-chopping enzymes can affect cognitive and psychological conditions. For instance, in humans neurotrypsin has been identified as essential component for cognitive functions. Deficits in the neurotrypsin genotype have been correlated with severe mental retardation [46]. Further, Cichon et al. [47] reported a genetic variation of neurocan as susceptibility factor for bipolar disorders. With relation to ageing, hippocampal ECM levels have been suggested to show an age-dependent increase conquering age-related cognitive decline. In this line, the Alzheimer's disease (AD) mouse model APP/PS1 showed a significant upregulation of several matrix components correlating with impairments in hippocampal LTP and contextual memory [48]. Intra-hippocampal injections of chABC restored both [48] suggesting an important, but yet elusive role for the ECM in early memory impairment in AD, as the mere correlative findings about ECM alterations in dementia are highly controversial and are far from conclusive [33]. That these data might have relevant impacts for human AD is indicated by findings of correlating HA levels in the cerebrospinal fluid of female AD patients and particular AD-related biomarkers [49]. Further, MMP9 levels have been found to be increased in Alzheimer patients [50] and to cleave the amyloid beta peptide leading to AD-typical neuritic plaques [51]. Its role in Aβ-induced cognitive decline is however elusive [52].

8. Outlook

We have summarized recent evidence showing that experimental modulation of the ECM promotes "windows of opportunities" with an increase in learning-related plasticity yielding cognitively flexible adaptation of learned behaviors and the underlying memories. How the ECM, in addition, impacts onto several mental disorders that generally develop after the closure of major critical periods for higher brain functions, as for instance affective disorders or schizophrenia, are exciting new research directions. We are envisaging future challenges in developing new tools for guided neuroplasticity with therapeutic potential for memory disorders, stroke, or neuroprosthetic applications based on ECM manipulations.

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