# The dual role of the extracellular matrix in synaptic plasticity and homeostasis

*Alexander Dityatev*, Melitta Schachner‡§ and Peter Sonderegger‖*

Abstract | Recent studies have deepened our understanding of multiple mechanisms by which extracellular matrix (ECM) molecules regulate various aspects of synaptic plasticity and have strengthened a link between the ECM and learning and memory. New findings also support the view that the ECM is important for homeostatic processes, such as scaling of synaptic responses, metaplasticity and stabilization of synaptic connectivity. Activity-dependent modification of the ECM affects the formation of dendritic filopodia and the growth of dendritic spines. Thus, the ECM has a dual role as a promoter of structural and functional plasticity and as a degradable stabilizer of neural microcircuits. Both of these aspects are likely to be important for mental health.

NPxY motif
A peptide motif with the amino acid sequence Asn–Pro–any amino acid–Tyr, which is important for protein–protein interactions.

**Department of Neuroscience and Brain Technologies, Italian Institute of Technology, via Morego 30, Genova, 16163 Italy.*
*‡Zentrum fuer Molekulare Neurobiologie Hamburg, Falkenried 94, D-20251 Hamburg, Germany.*
*§Keck Center for Collaborative Neuroscience and Department of Cell Biology and Neuroscience, Rutgers University, Piscataway, New Jersey 08854, USA.*
*‖Department of Biochemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.*
*Correspondence to A.D.*
*e-mail:*
*alexander.dityatev@iit.it*
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In the brain, extracellular matrix (ECM) molecules are synthesized by neurons, glia and non-neural cells and are secreted into the extracellular space. Here, they associate with cell surface receptors and form heterogeneous aggregates that regulate diverse cell functions. Early studies highlighted the role of numerous ECM molecules in long-term potentiation (LTP) and long-term depression (LTD) at excitatory glutamatergic synapses in the hippocampus. These ECM molecules included Reelin, tenascin R (TNR), tenascin C (TNC), chondroitin sulphate proteoglycans (CSPGs) and laminins, and the major ECM receptors, integrins (reviewed in REF. 1). The list of ECM molecules with synaptic function continues to grow. In this Review, we focus on selected ECM molecules and ECM receptors the contribution of which to various aspects of synaptic plasticity is most well characterized. We also discuss the role of ECM molecules in homeostatic processes. Finally, we highlight the link between the ECM and learning and memory, and its implications for mental disorders.

## ECM and induction of synaptic plasticity

Activity-dependent changes in many types of synapses occur when the concentration of $Ca^{2+}$ inside the postsynaptic cell exceeds a critical threshold. This triggers activation of several $Ca^{2+}$-sensitive enzymes — such as protein kinases and factors controlling activity of small GTPases — that mediate the early phase (induction) of synaptic plasticity. The major sources of $Ca^{2+}$ influx in postsynaptic excitatory neurons are NMDA (*N*-methyl-D-aspartate) receptors and voltage-dependent $Ca^{2+}$ channels[2,3]. The activities of these molecules, and hence $Ca^{2+}$ homeostasis and synaptic plasticity, are influenced by several ECM molecules, as discussed below.

***Reelin-dependent regulation of NMDA receptors.*** A prototypic example of an ECM molecule that regulates NMDA receptors is Reelin (reviewed in REF. 4), which is encoded by the *RELN* gene. Reelin is well known for its involvement in neuronal migration and, in particular, in controlling the order of cerebellar and cerebral cortical layers during development[5]. Recent data also suggest its importance for maintaining cortical organization in the adult CNS[6]. The binding of Reelin to the lipoprotein receptors very-low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor type 2 (APOER2) results in activation of the cytoplasmic adaptor protein disabled 1 (DAB1), which interacts with NPxY motifs in the cytoplasmic tails of both receptors (FIG. 1). Analysis of knock-in mice, in which this motif in APOER2 has been mutated to disrupt the APOER2–DAB1 interaction, shows that the NPxY motif is indispensable for normal neuronal migration and positioning during development, and for the induction of LTP in the hippocampal CA1 region in the adult[7]. However, exogenously added Reelin rescues LTP in this mutant, an effect that was not observed in the hippocampus of *APOER2*-deficient mice[8]. This suggests that some LTP-related signalling that is mediated by Reelin binding to

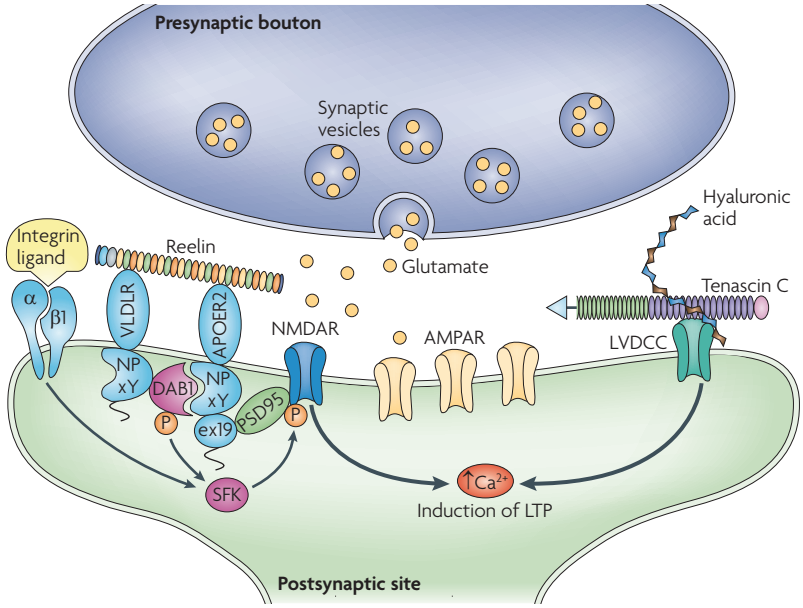


Figure 1 | **Role of the ECM in induction of synaptic plasticity.** Reelin signals through its receptors, very-low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor type 2 (APOER2), which interact with adaptor protein disabled 1 (DAB1) through the NPxY motifs in the cytoplasmic domain of both receptors. The major structural feature of Reelin is a series of eight repeats, each containing two related subdomains, A (shown in yellow) and B (shown in orange), that are separated by epidermal growth factor-like motifs (shown in green)[5]. Reelin also contains a unique Reelin domain (shown in grey) and a region with similarity to F-spondin (shown in turquoise). Clustering of Reelin receptors leads to clustering of DAB1, activation of Src family tyrosine kinases (SFKs) and tyrosine phosphorylation of NMDARs (*N*-methyl-D-aspartate receptors) that increases receptor activity. A protein domain of APOER2 encoded by exon 19 (ex19) mediates association of APOER2 with the NMDAR complex. Similarly, SFKs mediate signalling between integrins and NMDARs. Hyaluronic acid and tenascin C support the activity of neuronal L-type voltage-dependent $Ca^{2+}$ channels (LVDCCs). Hyaluronic acid is a large, negatively charged, non-branched polymer composed of repeated disaccharides of glucuronic acid (shown in blue) and *N*-acetylglucosamine (shown in brown). Tenascin C consists of the tenascin assembly domain (shown as a light blue triangle), an array of epidermal growth factor-like motifs (shown in green), fibronectin type III domains (shown in purple) and the terminal fibrinogen globe (shown in pink)[118]. $Ca^{2+}$ influx through NMDARs and LVDCCs induces long-term potentiation (LTP). AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; PSD95, postsynaptic density protein 95.

Sensorimotor gating of the startle reflex
Inhibition of the startle reflex by a weak 'prepulse' stimulus that occurs 30–500 ms before the startling stimulus.

Reversal learning
The form of learning in which an organism shifts its response from a stimulus that is no longer rewarded to a previously unrewarded one.

Dendritic spine
A small protrusion of the dendritic membrane that represents the postsynaptic component of the majority of the excitatory synapses in the CNS.

APOER2 occurs independently of the APOER2–DAB1 binding site, possibly involving interactions with other adaptor proteins. Clustering of DAB1 through clustering of Reelin receptors activates Src family tyrosine kinases (SFKs). This promotes phosphorylation of DAB1 and of GluN2 subunits of NMDA receptors, resulting in potentiation of NMDA receptor-mediated $Ca^{2+}$ influx. Phosphorylated DAB1 further activates phosphatidylinositol 3-kinase (PI3K). This increases cell surface expression of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors after application of exogenous Reelin in hippocampal slices and cultures[9], but it remains to be studied whether endogenous Reelin contributes to the increase in synaptic expression of AMPA receptors during LTP via the DAB1–PI3K pathway. APOER2 associates with postsynaptic density protein 95 (PSD95), an abundant scaffolding protein in the postsynaptic density, through a domain encoded by exon 19. This interaction is crucial for the coupling of the Reelin signalling complex to NMDA receptors: mice lacking this exon in APOER2 do not increase LTP and NMDA receptor-mediated currents after application of Reelin, and perform poorly in contextual fear conditioning and water maze tasks[10].

Changes in Reelin signalling could be involved in schizophrenia. The disease is associated with hypermethylation of the *RELN* promoter and lower levels of Reelin expression[11]. There is also a strong association between *RELN* variants and neuropsychological traits that are related to verbal and visual working memory and executive functioning in patients with schizophrenia[12]. In agreement with these findings, Reelin-haploinsufficient mice show numerous abnormalities in excitatory synaptic transmission and plasticity, GABA (γ-aminobutyric acid)-ergic transmission, sensorimotor gating of the startle reflex, contextual fear conditioning and reversal learning[13–15], which strongly overlap with the 'schizophrenic phenotype'. By contrast, overexpression of Reelin in transgenic mice increases the number of presynaptic contacts in the hippocampus and leads to hypertrophy of dendritic spines. Moreover, it potentiates synaptic responses in area CA1 of alert behaving mice after high-frequency stimulation of CA3 axons or following an associative learning task[16].

A recent study suggests a possible link between Reelin signalling and Alzheimer's disease[17] — a neurodegenerative disease that is associated with accumulation of amyloid-β plaques. In mouse hippocampal slices, application of oligomeric amyloid-β at concentrations that are found in patients with Alzheimer's disease led to impaired LTP, and this was prevented by co-application of Reelin. This rescue of LTP required APOER2-dependent activation of SFKs. In summary, these findings implicate the Reelin signalling pathway in the pathogenesis of neuropsychiatric and neurodegenerative syndromes in humans and point to the therapeutic potential of manipulating this pathway.

***Integrin-dependent regulation of NMDA receptors.*** The major receptors for ECM molecules are integrins that are heterodimeric integral membrane proteins composed of an α-chain and a β-chain. Similarly to Reelin, ECM ligands of integrins might support the induction of LTP through the modulation of NMDA receptors (FIG. 1). Activation of integrin receptors with synthetic Arg–Gly–Asp peptides (RGD peptides) or an endogenous integrin ligand, fibronectin, caused a robust increase in tyrosine phosphorylation of focal adhesion kinase, proline rich tyrosine kinase 2 and SFKs. Increases in the phosphorylation level of these proteins were blocked with neutralizing antibodies to β1-containing integrins. Both the RGD peptide and fibronectin caused rapid SFK-dependent increases in tyrosine phosphorylation of the NMDA receptor subunits GluN2A and GluN2B. These molecules also caused a prominent increase in the amplitude and duration of NMDA receptor-mediated synaptic currents in an SFK-dependent manner[18] (FIG. 1). However, these data should be viewed with caution as RGD peptides can directly

potentiate NMDA receptors, presumably by acting at the glycine-binding site[19]. It will be important to verify this mechanism in mice that are deficient in β1 integrins by showing that the level of tyrosine phosphorylation of NMDA receptors and the magnitude of NMDA receptor-mediated currents is unchanged by RGD peptides, and that deficient LTP and working memory in these mice can be rescued through pharmacological potentiation of NMDA receptors.

***Regulation of L-type $Ca^{2+}$ channels.*** In addition to NMDA receptors, L-type voltage-dependent $Ca^{2+}$ channels (LVDCCs) also contribute to the increase in postsynaptic $Ca^{2+}$ concentration, particularly during strong or repetitive high-frequency stimulations, and therefore promote the induction of some forms of LTP[3]. The importance of ECM molecules for the amplification of LVDCC activity in neurons was originally found using mice deficient in the ECM molecule TNC[20]. This glycoprotein is most prominently expressed during development of the nervous system, and its expression is upregulated following synaptic stimulation[1]. Mice deficient in TNC show impairment in several forms of synaptic plasticity that are known to involve LVDCCs[20]. Injection of TNC fibronectin repeats 6–8 into hippocampal slices impairs LTP in area CA1 (REF. 21). Recent data show that LVDCC activity and LTP are regulated by hyaluronic acid[22] (FIG. 1), a key ECM glycosaminoglycan that is synthesized in neural cells by a class of integral membrane proteins. It is extruded through the cell membrane into the extracellular space, where it serves as a scaffold for the assembly of the ECM.

Although evidence for a direct physical interaction between LVDCCs and TNC or hyaluronic acid is lacking, both ECM molecules are expressed perisynaptically in close proximity to LVDCC-immunopositive dendritic domains and interact with CSPGs[23]. It is therefore possible that these ECM molecules are constituents of a functional complex. Consistent with this notion, LTP at CA3–CA1 synapses is similarly impaired by genetic ablation of TNC, removal of hyaluronic acid by the exogenous, highly specific enzyme hyaluronidase or by inhibiting LVDCCs. The deficit in synaptic plasticity after hyaluronidase treatment is rescued by re-introduction of hyaluronic acid or by pharmacological potentiation of LVDCCs. Removal of hyaluronic acid reduces $Ca^{2+}$ transients in dendritic spines of hippocampal pyramidal neurons owing to impaired activity of LVDCCs (FIG. 1), prevents somatic translocation of the phosphorylated extracellular signal-regulated kinase1 (ERK1) and ERK2 in area CA1, prevents activation of the cyclic AMP-responsive element-binding protein (CREB) and reduces hippocampus-mediated contextual fear conditioning[22].

Recordings in a heterologous expression system (Chinese hamster ovary cells) demonstrated that hyaluronic acid potentiates the activity of $Ca_v1.2$ but not the $Ca_v1.3$ α1 subunit of LVDCCs[22]. This is consistent with the predominant expression of the $Ca_v1.2$ subunit in the hippocampus and its reported role in LTP and phosphorylation of ERK1, ERK2 and CREB[24]. As other ECM components, such as heparin, laminin, fibronectin and retinoschisin, also potentiate the activity of LVDCCs in different cell types[25–28], this seems to be a common signalling mechanism by which ECM molecules modulate $Ca^{2+}$ signalling.

The relationship between LVDCCs and the ECM can be bidirectional: the circadian rhythm of retinoschisin secretion in the retina is regulated by LVDCCs and, in a positive-feedback loop, secreted retinoschisin modulates the circadian rhythm of LVDCC currents and synaptic transmission between photoreceptor and bipolar cells[26]. This provides a local mechanism that supports circadian oscillations in the retina and thereby allows the visual system to accommodate daily changes in ambient illumination over 10–12 orders of magnitude. Another example of the bidirectional relationship between LVDCCs and the ECM is the formation of hyaluronic acid-rich perineuronal nets associated with hippocampal perisomatic interneurons. This process requires the activity of LVDCCs, and the perineuronal nets in turn modulate the firing threshold of the cells on which they are formed[29]. As perineuronal nets surround GABAergic perisomatic contacts on these cells and LVDCC activity regulates the turnover of synaptic GABA type A ($GABA_A$) receptors[30], it will be important to investigate whether hyaluronic acid also modulates synaptic transmission and/or plasticity of these synapses through the regulation of LVDCCs.

## ECM and consolidation of synaptic plasticity

The consolidation phase of synaptic plasticity follows the induction phase when the activation of $Ca^{2+}$-dependent enzymes is sufficiently strong to promote changes of the actin cytoskeleton, local protein synthesis and synaptic adhesion, which are thought to support stabilization of new synaptic configurations. Regulation of actin pools within dendritic spines modulates spine size and enlargement, organization of the postsynaptic density, receptor trafficking and localization of the translational machinery[31]. Synaptic consolidation is known to depend on actin, owing to its characteristic sensitivity to 5-Hz stimulation and adenosine, which can block actin polymerization.

***Integrins and actin polymerization.*** Integrin-mediated regulation of actin polymerization and stabilization of newly formed actin filaments has been proposed to play a crucial part in consolidation of LTP[32] (FIG. 2). In support of this hypothesis, function-blocking antibodies against β1 integrins suppress the formation of fibrous (F)-actin in dendritic spines and the stabilization of LTP. Actin polymerization during consolidation of LTP is controlled by the RhoA–Rho-associated protein kinase (ROCK)–cofilin signalling pathway, whereas signalling via Ras-related C3 botulinum toxin substrate 1 (RAC1)–p21-activated kinase (PAK) stabilizes newly formed filaments[33] (FIG. 2). During development, α5 integrin signalling regulates spine morphogenesis and synapse formation by a mechanism that is dependent on SFKs, the small GTPase RAC1 and the signalling adaptor GIT1 (REF. 34).

It remains to be elucidated exactly when, how and which integrin heterodimers regulate signalling via

RGD peptide
A peptide containing a motif with the amino acid sequence Arg–Gly–Asp. Such motifs in extracellular matrix proteins are important activators of integrin signalling.

Perineuronal nets
Aggregates of extracellular matrix molecules that embed cell bodies, axon initial segments and proximal dendrites of a subset of neurons in a mesh-like structure.

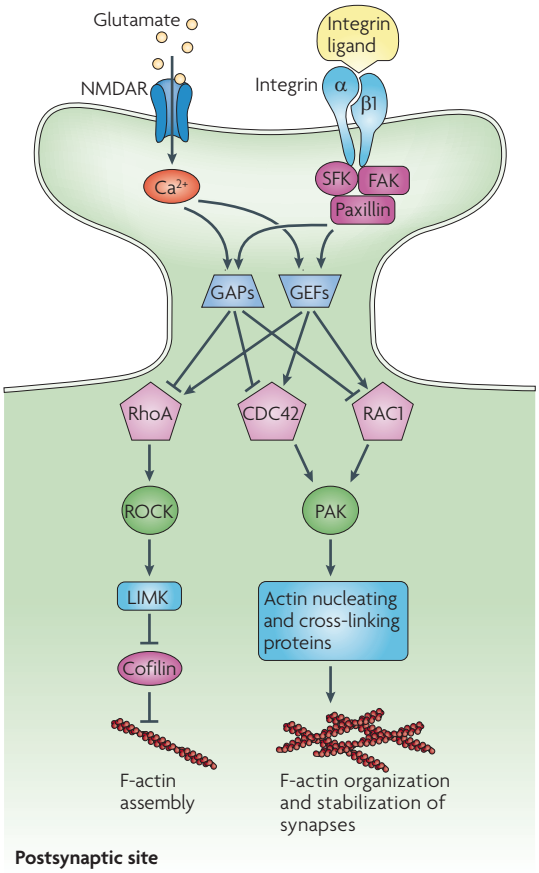


Figure 2 | **NMDA receptor and integrin co-signalling regulates cytoskeletal dynamics during stabilization of long-term potentiation.** NMDARs (*N*-methyl-D-aspartate receptors) and integrins activate the small GTPases RhoA, Ras-related C3 botulinum toxin substrate 1 (RAC1) and cell division cycle 42 (CDC42) through distinct but partially overlapping sets of GDP–GTP exchange factors (GEFs). They can also inhibit activities of these GTPases through sets of GTPase-activating proteins (GAPs). Synaptic activity-triggered signalling from RhoA to cofilin rapidly (within ~2 minutes) leads to fibrous (F)-actin assembly. Parallel activation of p21-activated kinase (PAK) by RAC1 and CDC42 influences later stages (after >10 minutes) of long-term potentiation (LTP) consolidation through modification of proteins involved in higher-order organization of actin in dendritic spines[33]. FAK, focal adhesion kinase; LIMK, LIM domain kinase; ROCK, Rho-associated protein kinase; SFK, Src family tyrosine kinase.

Focal adhesion
A large, dynamic protein complex through which the actin cytoskeleton of a cell connects to the extracellular matrix through integrins, providing a cell anchor and a sensor of extracellular signals.

Membrane ruffles
Processes that are formed by the movement of lamellipodia in the dynamic process of folding back onto the cell body from which they have extended.

RhoA and RAC1 GTPases during and after induction of LTP in the adult CNS. However, some insight is provided by the observed role of integrins in early development. For several cell types, the initial cell attachment and spreading over a substrate is accompanied by reduced activity of the GTPase RhoA and simultaneous activation of the GTPases RAC1 and cell division cycle 42 (CDC42), which suppresses actomyosin contractility and enhances actin-mediated protrusion of neural processes. Integrins activate RAC1 and CDC42 by activating the focal adhesion kinase (FAK)–Src-mediated tyrosine phosphorylation of breast cancer anti-oestrogen resistance protein 1 (also known as p130CAS)[35]. The FAK–Src complex also phosphorylates paxillin, which recruits ADP-ribosylation factor (ARF)–GTPase-activating protein (GAP). Phosphorylated paxillin also recruits the GDP–GTP exchange factor (GEF) for CDC42 and RAC1, Rho guanine nucleotide exchange factor 7 (ARHGEF7; also known as β-PIX). ARHGEF7 recruits and activates RAC1 through a direct interaction within focal adhesions and membrane ruffles[36]. α5β1 integrins stimulate the SFK-mediated tyrosine phosphorylation of p190RhoGAP[37], which leads to maturation of dendritic spines and to synapse and dendrite stability in the postnatal mouse hippocampus[38]. Thus, integrin signalling through SFKs regulates the localization and activity of GEFs and GAPs to coordinate actin cytoskeleton stabilization with membrane protrusion and therefore enlargement of the surface membrane.

***Genetic analysis of integrin functions in mice.*** As integrins have important roles during development within and outside the nervous system, identification of integrins involved in LTP and other synaptic functions in the adult CNS using conventional knockout mice is problematic. In the past, researchers have therefore mostly relied on the use of function-blocking integrin-specific antibodies. However, mice with conditional ablation of the β1 integrin gene in forebrain excitatory neurons were recently generated, and these enable genetic analysis of β1 integrin-dependent synaptic functions. Postnatal deletion of β1 integrin (driven by the α-$Ca^{2+}$/calmodulin-dependent protein kinase II promoter) resulted in impaired LTP but normal synaptic responses to paired-pulse or high-frequency stimulation. By contrast, embryonic deletion of β1 integrin (driven by the empty spiracles homologue 1 promoter) impaired both LTP and synaptic responses to high-frequency stimulation[39,40]. Thus, β1 integrins are essential for LTP in the adult and are crucial for maturation of the readily releasable pool of synaptic vesicles during early development[39,40]. The defects in LTP in the mutant mice closely resemble those observed in previous pharmacological studies[41]. In addition, mice conditionally deficient in α3 or α8 integrins in excitatory neurons of the postnatal forebrain show impaired LTP at CA3–CA1 synapses[42,43]. As β1 integrin forms ECM receptors in association with α3 or α8 integrins, these data indicate an important role for α3β1 and α8β1 integrins in synaptic plasticity. The relevance of these findings is further underscored by studies in mice that are conditionally deficient in α3 or β1 integrins: they have selective deficits in hippocampus-dependent working memory but interestingly not in spatial learning in the water maze paradigm. This new generation of integrin-deficient mice will be useful in further dissecting integrin signalling during LTP and working memory formation.

## ECM and homeostasis

In addition to synaptic plasticity, which can be rapidly induced in response to sensory stimuli, homeostatic forms of plasticity operate on a slower timescale and help to preserve neural cells by preventing pathological hypo- or hyper-excitation of neurons, which can

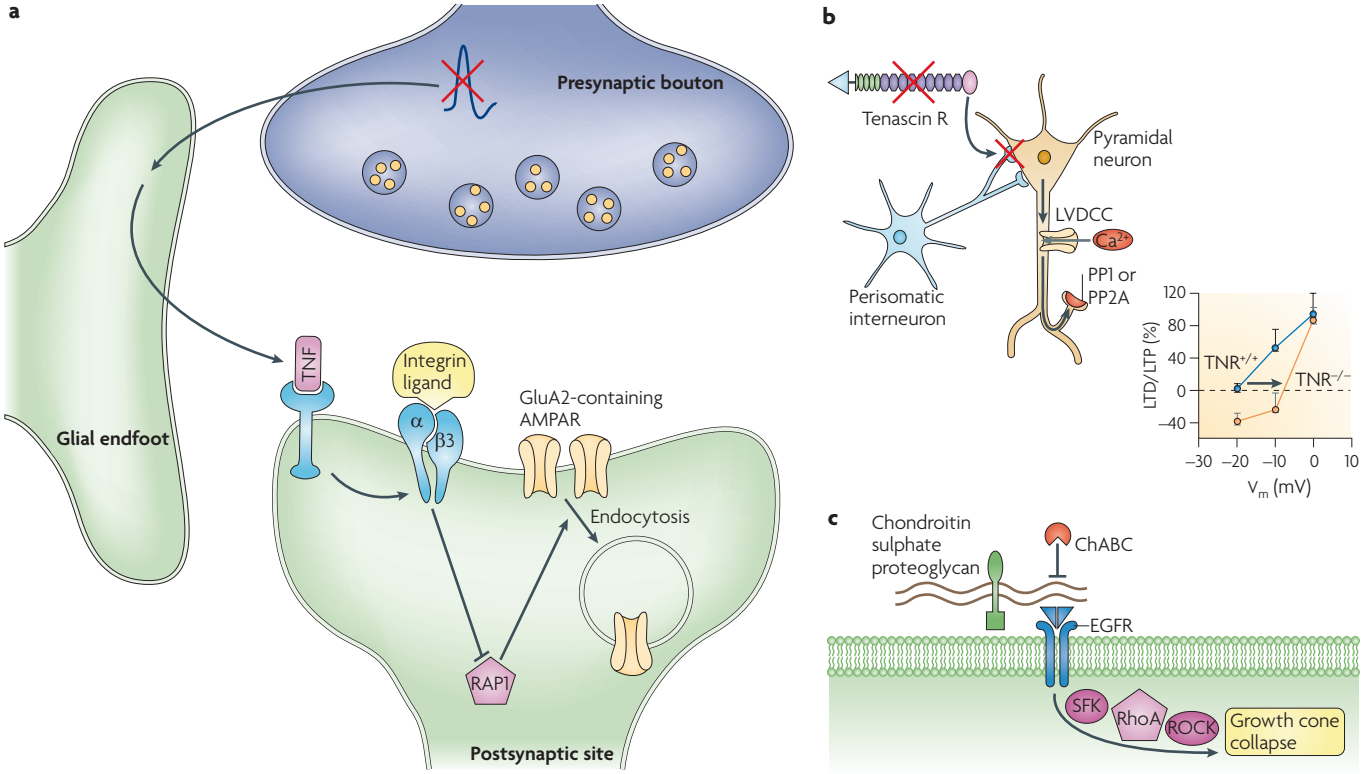


Figure 3 | **Role of the ECM in homeostatic plasticity. a** | Integrins are involved in homeostatic synaptic scaling. Pharmacological blockade of action potentials leads to secretion of tumour necrosis factor (TNF) from glial cells, presumably owing to a reduction in glutamate release. This increases expression of β3 integrins at the postsynaptic cell surface through an unknown mechanism. Integrins inhibit the small GTPase Ras-related protein 1 (RAP1), which normally stimulates the endocytic removal of GluA2-containing AMPARs (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors) from the cell surface. As a net outcome, synaptic expression of AMPARs is increased, resulting in larger excitatory postsynaptic currents. **b** | Tenascin R (TNR) contributes to the ability of a synapse to undergo plastic changes (metaplasticity). Like tenascin C (TNC), it consists of the tenascin assembly domain (shown as a light blue triangle), an array of epidermal growth factor-like motifs (shown in green), fibronectin type III domains (shown in purple) and the terminal fibrinogen globe (shown in pink)[118]. Genetic ablation of tenascin R in mice results in a loss of perisomatic inhibitory synapses on pyramidal cells in hippocampal area CA1. This disinhibition of neurons increases the activities of L-type voltage-dependent $Ca^{2+}$ channels (LVDCCs) and serine/threonine protein phosphatase 1 (PP1) and/or PP2A, which results in metaplastic changes, such as a shift in the threshold for induction of long-term potentiation (LTP) by +10 mV. **c** | Chondroitin sulphate proteoglycans (CSPGs; protein backbone shown in light green and chondroitin sulphate chains in brown) induce growth cone collapse and repulsion of axons and thus inhibit structural plasticity. One of the identified mechanisms involves the epidermal growth factor receptor (EGFR)–RhoA–Rho-associated protein kinase (ROCK) signalling pathway. Treatment with chondroitinase ABC (ChABC) or inhibitors of EGFRs, RhoA or ROCK prevents the repulsion of axons by CSPGs, facilitates regeneration in the spinal cord and re-opens the critical window for ocular dominance plasticity in the visual cortex. LTD, long-term depression; SFK, Src family tyrosine kinase; $V_m$, membrane potential.

lead to neural dysfunction and cell death. For example, homeostatic regulation of synaptic strength, termed synaptic scaling, allows neurons to maintain their firing rate within a certain range despite perturbations (such as changes in sensory inputs) that are imposed on a network[44,45].

***Synaptic scaling.*** Adjustment of synaptic strength by synaptic scaling can be modelled *in vitro*: in response to prolonged blockade of action potentials by tetrodotoxin, all excitatory synapses on pyramidal cells are equally 'scaled up' due to the increase of postsynaptic AMPA receptor density, as detected by analysis of the amplitude distribution of miniature excitatory postsynaptic currents (mEPSCs)[44,45]. The mechanism underlying synaptic scaling involves glia-derived tumour necrosis factor (TNF)[46,47], which upregulates the expression of β3 integrins at the postsynaptic cell surface (FIG. 3a). Pharmacological disruption of integrin–ligand binding rapidly and reversibly reduces mEPSC amplitudes by increasing endocytosis of the AMPA receptor subunit GluA2 through activation of Ras-related protein 1 (RAP1). The effect is selective for the GluA2 over the GluA1 subunit, and NMDA receptor-mediated currents are not affected. Previous studies revealed that RAP1-mediated phosphorylation of GluA2 depends on p38 mitogen-activated protein kinase (p38MAPK) and some of its downstream kinases, such as MAPK-interacting kinase and/or MAP kinase-activated protein kinase 5 (also known as PRAK). The precise molecular

target of RAP1–p38MAPK remains undefined, but Ser880 of GluA2 is a likely candidate[48]. Phosphorylation of GluA2 at Ser880 disrupts its interaction with glutamate receptor-interacting protein (GRIP)–AMPA-binding protein (ABP) but not with PKC-interacting protein 1 (PICK1), which reduces the recycling of AMPA receptors and thus depresses synaptic transmission. Cultures from β3 integrin-deficient mice do not undergo the homeostatic up-scaling in mEPSC amplitudes in response to prolonged tetrodotoxin incubation that is seen in wild-type slices[19]. Therefore, it seems that upregulation of β3 integrins in response to blockade of neuronal firing inhibits RAP1-mediated endocytosis of AMPA receptors and thereby elevates synaptic expression of AMPA receptors. The binding partners of β3 integrins, which trigger homeostatic plasticity, are currently not known. However, the ECM is a reasonably stable structure that is well suited to act as a long-term modulator of synaptic activity, and ECM ligands of β3 integrins (for example, tenascins and thrombospondins) are already known to participate in synaptic functions. Such ECM molecules are therefore good candidates for long-term adjustments of synaptic strength through β3 integrin signalling. It will be important to determine which ECM molecules signal through β3 integrins in excitatory neurons, and to verify whether the associated homeostatic mechanisms are active in the adult CNS.

A recent study demonstrates that the homeostatic synaptic scaling also occurs in inhibitory interneurons[49], identified by their expression of parvalbumin. Excitatory synapses onto these interneurons accumulate high levels of the neuronal activity-regulated pentraxin (NARP). This ECM molecule can bind to and induce clustering of AMPA receptors on inhibitory interneurons (reviewed in REFS 50,51). Increasing network activity results in a homeostatic increase of excitatory synaptic input onto parvalbumin-expressing interneurons that increases the inhibitory drive to excitatory neurons[49]. This drive is impaired in cultured neurons lacking NARP and in brain slices from NARP-deficient mice. Together, these data indicate that activity-dependent recruitment of ECM molecules promotes synaptic expression of AMPA receptors on inhibitory interneurons to rebalance the network excitation–inhibition ratio and thus avoid hyper-excitability.

***Metaplasticity.*** Not only the strength of the synapses but also their predisposition to undergo plastic changes is dependent on previous changes in synaptic strength. This potential for activity-dependent adjustment of the mechanisms underlying synaptic plasticity is termed metaplasticity[52]. It is commonly manifested as a homeostatic increase or decrease of the threshold stimulation that is necessary for the induction of LTP when the basal excitatory activity is elevated or reduced, respectively. As basal excitatory activity is under the control of GABAergic inhibition, it is noteworthy that the ECM glycoprotein TNR regulates the efficacy of GABA release and the number of perisomatic inhibitory contacts in the CA1 region of the hippocampus[53–55]. TNR is expressed exclusively in the CNS by oligodendrocytes, Purkinje cells, motor neurons and subpopulations of interneurons (reviewed in REF. 1). Mice deficient in TNR show an abnormal structure of perineuronal nets, impaired perisomatic GABAergic inhibition and increased basal excitatory transmission[55,56]. Consistent with the expected metaplastic changes due to a reduction in perisomatic inhibition, the threshold for the induction of LTP, measured as a membrane potential at which a postsynaptic cell must be held during presynaptic stimulation to enable induction of LTP, was increased by 10 mV in TNR-deficient mice (FIG. 3b). This threshold and levels of LTP induced by theta burst stimulation were restored in TNR-deficient mice by pretreatment of hippocampal slices with the $GABA_A$ receptor agonist zolpidem, indicating that disinhibition is a cause of metaplastic synaptic changes in these mice[57]. This link between reduced perisomatic inhibition and impaired synaptic plasticity may be of clinical relevance as these are two important hallmarks of schizophrenia.

The mechanisms downstream of impaired GABAergic inhibition that lead to metaplastic changes involve elevated activities of LVDCCs and serine/threonine protein phosphatase 1 and/or 2A (FIG. 3b). This was suggested by experiments in which pretreatment of hippocampal slices from TNR-deficient mice with inhibitors of LVDCCs and phosphatases restored normal levels of LTP in area CA1. This observation is in agreement with previous studies that implicated phosphatases in metaplastic changes following low-frequency stimulation[58]. Also, loss of $Ca^{2+}$ entry via LVDCCs during prolonged blockade of AMPA receptors (that is, a condition opposite to disinhibited synaptic transmission in TNR-deficient mice) triggers homeostatic synaptic modifications, such as an increased pool size and turnover rate of presynaptic vesicles, as well as an enhanced postsynaptic contribution of $Ca^{2+}$-permeant homomeric GluA1 receptors[59]. As $Ca^{2+}$-permeant GluA1 receptors may facilitate induction of LTP, these changes can be viewed as both homeostatic and metaplastic.

TNR-deficient mice have also been used to show that a deficiency in an ECM molecule can induce brain subregion-specific synaptic abnormalities: in these mice, whereas perisomatic innervation of principal neurons by GABAergic interneurons is reduced in area CA1, it is normal in area CA3 and increased in the dentate gyrus. *In vivo*, this abnormality in the dentate gyrus leads to a $GABA_A$ receptor-dependent reduction in LTP and an enhanced excitability of granule cells following tetanic stimulation of entorhinal cortex axons projecting to the dentate gyrus[60]. Behaviourally, TNR-deficient mice show enhanced reversal learning, improved working memory and enhanced reactivity to novelty than wild-type littermates. Faster reversal learning rates correlate with increased ratios of parvalbumin-positive interneurons to granule cells and increased densities of parvalbumin-positive terminals on somata of granule cells, which indicate increased perisomatic GABAergic inhibition in the dentate gyrus[60]. These data demonstrate that modification of the ECM by ablation of TNR leads to a new structural and functional design of the dentate gyrus,

Theta burst stimulation
Several bursts of high-frequency (for example, 100 Hz) stimulation, which are delivered at 5 Hz to mimic the hippocampal theta rhythm that is thought to be important for learning and memory.

Granule cell
A tiny neuron found in specific brain areas, including the dentate gyrus, where it is the principle excitatory neuron.

with enhanced GABAergic innervation and altered plasticity, that promotes working memory and reversal learning. Enhanced inhibition in the dentate gyrus probably increases the signal-to-noise ratio by suppressing the background firing of neurons and/or by increasing the activity-dependent disinhibition of neurons that encode task-relevant features of the environment. Such changes therefore improve the filtering of sensory information and allow the discrimination of biologically relevant features[60].

***Synaptic stabilization.*** The 'critical period' of early postnatal development is characterized by an increased sensitivity to particular patterns of environmental stimuli. Experiences at this time induce the encoding and stabilization of functional microcircuits in the brain. If the organism does not receive the appropriate stimulus during this critical period, it may be difficult or even impossible to develop the appropriate functions later in life. Once formed, functional microcircuits must be stabilized to maintain performance and support vital functions.

It is thought that a chondroitin sulphate-rich ECM serves as an inhibitory 'barrier' that restrains structural plasticity in the mature visual cortex[61]. This is illustrated by the paradigm of monocular deprivation, which impairs visual cortical responses to the deprived eye and affects axonal morphology and dendritic spine density during a critical postnatal period. The end of this critical period coincides with maturation of CSPG-rich perineuronal nets and inhibitory intracortical circuitry. Injection of chondroitinase ABC (ChABC), a bacterial enzyme that degrades chondroitin sulphates, into the visual cortex at the end of the critical period reactivates sensitivity to monocular deprivation[61]. Moreover, ChABC injection into the adult rat visual cortex enables structural and functional recovery from early monocular deprivation[62], suggesting a potential therapeutic strategy to treat patients with reduced visual acuity (amblyopia).

Consistent with the proposed barrier function of the ECM against structural plasticity in the visual cortex, monocular deprivation in young mice increases the activity of tissue plasminogen activator (TPA), which degrades ECM molecules. The resultant loss of dendritic spines is prevented by ablation of TPA, supporting the importance of ECM remodelling in ocular dominance plasticity[63]. Furthermore, deficiency in the Nogo 66 receptor (also known as Reticulon 4 receptor), which transduces myelin-derived inhibitory signals to neurons, prolongs the critical period[64].

However, the molecular mechanisms mediating the effects of CSPGs on ocular dominance plasticity have not been fully elucidated. A complicating issue is that CSPGs consist of a large variety of core proteins that are covalently linked to chondroitin sulphate glycosaminoglycans with a complex pattern of sulphate groups, which are important determinants of CSPG function[65]. Also, the core ECM CSPGs, such as aggrecan, versican, neurocan, brevican and phosphacan, are composed of multiple domains with multiple signalling functions[65]. As chondroitin sulphates and other growth inhibitors signal through epidermal growth factor receptor (EGFR), RhoA and its effector ROCK[66], ChABC treatment may lead to inhibition of this signalling pathway and thus promote structural plasticity (FIG. 3c). Conversely, ChABC treatment increases the expression of the phosphorylated, active form of ERK1 (REF. 67), a kinase involved in excitatory synaptic plasticity. Also, acute ChABC treatment elevates the intrinsic excitability of perisomatic hippocampal interneurons[29], and recent data indicate the importance of an inhibition–excitation balance in setting the time window for the critical period[68,69]. An attractive hypothesis is that slow homeostatic mechanisms to counteract an increased excitability of GABAergic interneurons after ChABC treatment could lead to a reduced excitatory input to these cells and/or to a reduced inhibitory input to principal cells.

CSPGs also play a part in determining the stability of emotional memories. In the basolateral amygdala, formation of perineuronal nets marks the end of a developmental period during which fear memories can be erased through extinction training (that is, by repetitive presentation of a conditioned stimulus without unconditioned reinforcement). This phenomenon coincides with the ability to form contextualized fear memories and extinction memories[70]. Injection of ChABC renders subsequently acquired fear memories susceptible to ablation, although it neither strengthens new inhibitory learning during extinction training nor impairs memory reconsolidation. Because ChABC is effective if applied before but not after conditioning, it is possible that the state of fear memories acquired after ChABC treatment fundamentally differs from the state of memories acquired under normal conditions. ChABC treatment abolishes LTP of monosynaptic thalamolateral amygdala excitatory inputs and reduces synaptic plasticity at glutamatergic inputs onto local feedforward interneurons (that is, LTP of disynaptic inhibitory inputs)[70]. These changes in synaptic plasticity in response to ChABC may represent a synaptic correlate of 'erasable' (that is, less widely encoded and less redundant) memory.

These data are consistent with earlier studies showing impaired LTP and LTD after ChABC treatment of hippocampal slices[71]. They are also in agreement with studies in mice deficient in the CSPGs brevican or neurocan, which show impaired early and late LTP, respectively[72,73]. As ChABC elevates excitability of perisomatic hippocampal interneurons[29], it seems likely that the increase in GABAergic transmission in acutely ChABC-treated slices inhibits the induction of LTP, similar to the situation in the dentate gyrus of mice deficient in TNR[60]. In summary, removal of CSPGs may shift the developmental status of several brain regions to a more immature and thus unstable and more plastic state.

## Regulation of ECM molecule functions

The transient nature of many ECM functions suggests a key role for the regulation of individual ECM components, either by controlling their expression or by post-translational modifications. Important post-translational ECM modifiers include extracellular

Extinction memory
The memory that is formed when an animal learns that a conditioned stimulus no longer predicts a harmful stimulus.

Memory reconsolidation
The process in which previously consolidated memories are recalled and then actively consolidated, leading to their preservation.

Early and late LTP
Long-term potentiation (LTP) may be subdivided into an early phase, which lasts up to 3 hours, and a later phase, which follows the early one and may last days to months. The extension of early into late LTP requires gene transcription and protein synthesis.

Dendritic filopodium
A thin and 'headless' membrane protrusion that is not strictly part of a synapse but serves as a precursor of new dendritic spines during activity-dependent synaptogenesis.

proteases, such as the matrix metalloproteinases (MMPs)[74]. Their proteolytic activity on ECM components was mainly thought to mediate ECM inactivation and/or degradation. However, recent observations on the proteolytic activities of MMP9 and the serine proteinase neurotrypsin have refuelled the idea that their activity on ECM molecules may activate previously cryptic functional epitopes, which could have a role in structural plasticity[75] (FIG. 4).

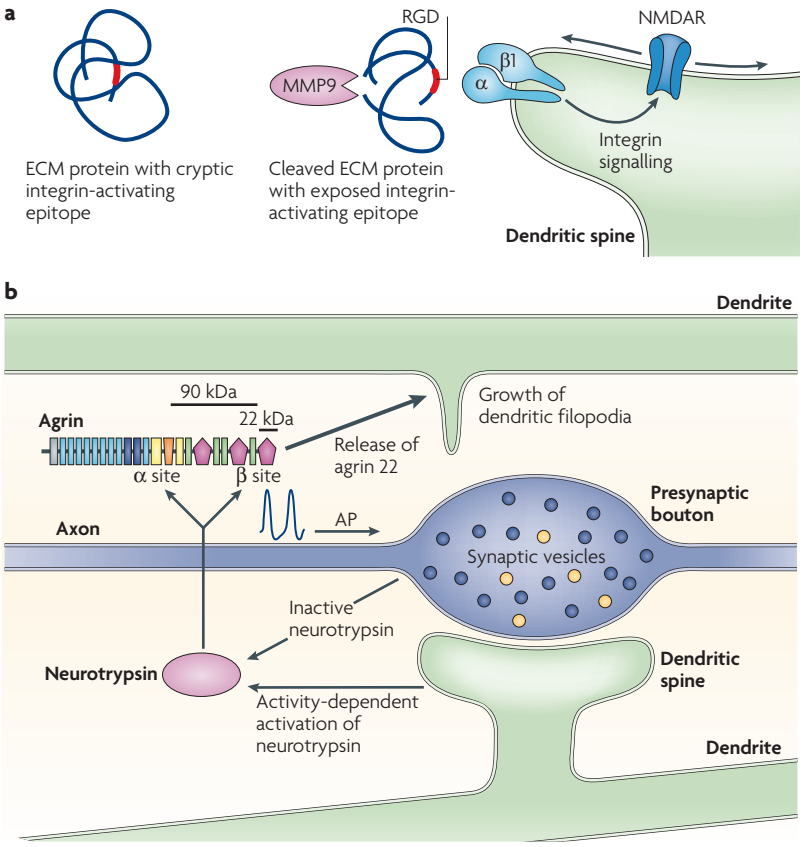


Figure 4 | **Proteolytic unmasking of ECM-resident signalling functions.** **a** | Unmasking of previously unrecognizable receptor-binding sites may result from conformational changes due to proteolytic cleavage within a domain of an extracellular matrix (ECM) molecule. Different molecular effects resulting from proteolytic cleavage of an ECM protein by matrix metalloproteinase 9 (MMP9) may lead to the exposure of a previously hidden, or 'cryptic', binding site for integrin receptors, such as an Arg–Gly–Asp (RGD)-containing binding site for a β1 integrin receptor. Integrin signalling increases the lateral mobility of NMDARs (*N*-methyl-D-aspartate receptors) through an unknown mechanism. Receptor diffusion between synaptic and extrasynaptic domains might be a mechanism for the regulation of synapse maturation and plasticity[119,120]. **b** | Unmasking of a cryptic ECM-resident signal may also result from the proteolytic separation of one or multiple domains from the parent ECM molecule. The neuronal serine protease neurotrypsin is stored in presynaptic terminals and secreted in an inactive form in association with presynaptic action potential (AP) firing. Its activation requires an NMDAR-dependent postsynaptic process. Activated neurotrypsin cleaves agrin and yields a carboxy-terminal 22-kDa fragment (agrin 22), which is essential for the formation of dendritic filopodia[96]. The domain structure of agrin is illustrated: a transmembrane segment that is present in transmembrane isoforms (shown in grey); cysteine-rich repeats that are similar to follistatin (shown in light blue); laminin epidermal growth factor (EGF)-like domains (shown in dark blue); serine/threonine-rich regions (shown in yellow); sperm protein, enterokinase and agrin domain (shown in orange); EGF-like domains (shown in green); and laminin globular domains (shown in pink). The neurotrypsin-dependent α- and β-cleavage sites are indicated together with the C-terminal 90-kDa and 22-kDa fragments (shown by black bars), which result from the cleavage of agrin.

***MMP9.*** MMPs are crucial effectors in the development and remodelling of various tissues[74]. In the brain, MMPs act on various ECM components — including laminin, TNR and brevican — as well as membrane proteins and pro-forms of cytokines and growth factors (reviewed in REF. 76).

MMP9 is the best-characterized MMP family member involved in activity-dependent structural and functional changes at CNS synapses. Increased neuronal activity enhances MMP9 expression in various experimental paradigms, from the cellular level to animal models of learning and memory[77–80]. These effects are inhibited by blocking NMDA receptors or MMP9 expression or activity[78,79], demonstrating the essential role of MMP9 in establishing and maintaining LTP and learning. Comparative studies showed that LTP induced by tetanic stimulation is occluded by LTP induced by MMP9 (REF. 81) and that the establishment of MMP9-induced LTP was occluded by LTP induced by tetanic and theta burst stimulation[81,82]. This indicates that MMP9-induced synaptic potentiation involves the same molecular components as electrically induced forms of LTP.

A possible mechanism by which MMP9 could regulate ECM function is by stimulating synaptic receptor signalling. Recent studies linked several MMP9-dependent synaptic effects to signalling through β1 integrins and exposure of a cryptic RGD motif. The resultant β1 integrin signals increased the surface diffusion of NMDA receptors through an unknown mechanism[83] (BOX 1; FIG. 4a). A function-blocking antibody against β1 integrins (and integrins α3, α5 and αV) inhibited MMP9-induced synaptic potentiation[79,82] and spine expansion[82]. MMP9-induced LTP in hippocampal slices was also abrogated by pre-incubation with a synthetic RGD peptide[79]. Applied alone, RGD-containing peptides elicit a slowly developing synaptic potentiation in hippocampal slices[84]. Together, these findings provide further evidence linking MMP activity to integrin signalling[79].

Owing to the large number of MMP9 target proteins, not all effects of MMP9 activity will converge on integrin signalling. However, the principle of proteolytic activation of cryptic ECM-resident signals may also apply to other targets of MMP9. For example, intercellular adhesion molecule 5 (ICAM5; also known as telencephalin) was recently found to be proteolytically cleaved and shed from hippocampal neurons upon activation of NMDA and AMPA receptors by a mechanism that is abolished by inhibition of MMP9 and MMP2 (REF. 85). ICAM5 is mainly located in dendritic filopodia and immature thin spines, and its deficiency caused retraction of the thin spine heads in response to NMDA receptor stimulation. Therefore, it was suggested that cleavage of ICAM5 by MMP9 and MMP2 and the resultant shedding of its ectodomain are essential for ICAM5-mediated development of dendritic spines. The receptor for ICAM5 and the molecular mechanisms linking the solubilized ICAM5 ectodomain to the growth of dendritic spines remain to be determined.

Box 1 | **Exploration of receptor surface trafficking using single particle tags**

The methods for studying the mobility of receptors that reside in synaptic or extrasynaptic surface membranes have recently been expanded from bulk measurements, such as fluorescence recovery after photobleaching (FRAP), to single-molecule techniques[107]. To label single molecules, a single dye or a single particle may be used. Single particle tracking with quantum dots (Qdots) is the preferred method owing to several advantages, including their superior brightness, a larger absorption cross section allowing excitation with a mercury lamp and superior photostability. Qdots are nanometre-sized semiconductor fluorescent particles that provide long-lasting fluorescence emission. They can be tracked by, for example, monitoring the trajectory of a marker that is attached to the diffusing molecule. The migratory characteristics of the diffusing molecule are then derived by statistical analysis of the trajectory of the single particle.

Single Qdots coupled to an antibody against the GluN1 subunit of the NMDA (*N*-methyl-D-aspartate) receptor were used to track the effect of matrix metalloproteinase 9 (MMP9) on receptor diffusion in cultured rat hippocampal neurons. These studies indicated that MMP9 enhances the surface diffusion of NMDA receptors in the absence of proteolytic cleavage of the receptors[83]. The mobility-promoting effect of MMP9 was selective for NMDA receptors. Immunostaining for brevican or visualization of hyaluronic acid with fluorescent hyaluronic acid-binding protein indicated that enhancement of NMDA receptor mobility by MMP9 was not accompanied by extensive degradation of extracellular matrix components. Application of MMP9 altered the time course, but not the amplitude, of NMDA-induced currents in cultured hippocampal neurons[108], through a proteolytic target that remains unknown. Previous reports indicate that neither the GluN1 nor the GluN2A subunits of the NMDA receptor are cleaved by MMP9 and that the MMP9 effects on NMDA receptor kinetics are reversible after washout of MMP9. Therefore, an indirect effect of MMP9 — for example, the proteolytic activation of a cryptic ligand for the receptor, such as β1 integrin — was proposed.

***Neurotrypsin.*** The neural serine proteinase neurotrypsin has recently been recognized as an essential mediator in the activation of an ECM-derived signalling process that promotes the growth of dendritic filopodia in association with synaptic LTP. Neurotrypsin is predominantly expressed in neurons of the cerebral cortex, the hippocampus and the amygdala[86,87]. It is essential for higher brain functions in humans, as a deletion in the coding region that results in a truncated protein without the catalytic domain causes a severe form of mental retardation[88].

The sole target so far identified for neurotrypsin is agrin, a heparan sulphate proteoglycan widely expressed in the nervous system and many extraneural organs[89]. Agrin plays an essential part in the development and maintenance of the neuromuscular junction[90,91]. Here, the agrin signal is mediated by muscle-specific receptor tyrosine kinase (MUSK)[92] and its co-receptor low-density lipoprotein receptor-related protein 4 (LRP4)[93,94]. By contrast, in the CNS, agrin binds to and inhibits the α3 subtype of the $(Na^+ + K^+)$ATPase[95]. Several recent studies suggest a role for agrin in the formation and/or maintenance of central synapses[96,97].

Neurotrypsin cleaves agrin at two homologous, highly conserved sites, releasing a 90-kDa (agrin 90) and a 22-kDa (agrin 22) 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[98,99]. Several lines of evidence from enzymological studies indicate that neurotrypsin is highly specific for agrin[98,99].

In the adult CNS, neurotrypsin is found in presynaptic boutons, in particular around the presynaptic membrane in the region lining the synaptic cleft[88,100]. Studies with fluorescently tagged neurotrypsin (BOX 2) in cultured hippocampal neurons demonstrated that neural activity regulates the recruitment and exocytosis of neurotrypsin at synapses[101].

Agrin cleavage requires not only neurotrypsin exocytosis after presynaptic depolarization but also the concomitant activation of the postsynaptic neuron. This was discovered when the NMDA receptor antagonist MK801 was found to block agrin cleavage associated with LTP, without affecting neurotrypsin secretion from presynaptic terminals[102]. This indicates that neurotrypsin is secreted from presynaptic terminals in an inactive form and that an NMDA receptor-dependent postsynaptic mechanism is required for its activation in the extracellular space. These results qualify neurotrypsin-dependent agrin cleavage as a coincidence detector for correlated activity of the presynaptic and postsynaptic neuron, with a possible involvement in Hebbian learning.

Recently, activity-dependent exocytosis of neurotrypsin from presynaptic terminals and cleavage of agrin were found to be crucial for the formation of dendritic filopodia in the context of NMDA receptor-dependent plasticity[102], which is thought to promote experience-dependent structural plasticity through the formation of new synapses[103]. In neurotrypsin-deficient mice, there was no activity-dependent generation of dendritic filopodia (BOX 2). However, filopodia formation was completely restored by exogenous administration of agrin 22. This effect of agrin 22 suggests an important role for the neurotrypsin–agrin system in the structural reorganization of synaptic circuits during neural development and for experience-related structural plasticity in the adult (FIG. 4b), which is consistent with the observation that loss of neurotrypsin's catalytic domain causes severe mental retardation[88].

## Conclusions and perspectives

During development and in the adult, the ECM provides multiple cues that promote synaptic plasticity on the one hand, and maintain the homeostasis of neural circuitries on the other. Transgenic ablation and/or enzymatic degradation of ECM molecules predominantly reduce LTP and learning and memory, but may reinstate developmental forms of plasticity. In the healthy brain, the balance between plasticity-promoting and plasticity-inhibiting cues can be locally modified by

Hebbian learning
The strengthening of synaptic connections when the presynaptic and the postsynaptic neuron are active simultaneously, which is often summarized as 'cells that fire together, wire together'.

Box 2 | **Imaging techniques for ECM research**

**Visualization of protein secretion with pHluorin**

To test for regulated exocytosis from presynaptic nerve endings, the protein of interest may be tagged with pHluorin, a pH-sensitive variant of green fluorescent protein (GFP)[109,110]. The pH dependence of this molecule means that it can only be detected when localized in a compartment with neutral pH (such as the cytoplasm or extracellular space) and remains undetected when contained in secretory vesicles with acidic pH. For example, the fusion of neurotrypsin with pHluorin enabled discrimination between intracellular and extracellular pools of neurotrypsin and monitoring of secretion of neurotrypsin after synaptic activation[101,102].

**Light-microscopic characterization and quantification of dendritic spines and filopodia**

To visualize dendritic spines and filopodia in hippocampal slices, a transgenic mouse line expressing a reporter GFP in sparse neurons[111,112] is crossed with the mouse line of interest — for example, a mouse line lacking a gene with presumed involvement in synapse formation or synaptic plasticity. The numbers of spines and filopodia are counted in reconstructed three-dimensional images of dendrites by inspection from all directions over a length of 30–40 μm[102] and characterized according to morphological criteria[113].

**High-resolution fluorescent *in situ* zymography**

Fluorescent *in situ* zymography allows enzymatic activity in tissue sections and cell preparations to be visualized and quantified using substrates that show altered fluorescent characteristics after an enzymatic reaction. For the detection of metalloproteinase-dependent reactions, dye-quenched gelatin is the preferred substrate. In this preparation, the fluorophore molecules are so tightly packed that they quench one another. Following proteolytic cleavage of the gelatin, the quenching is removed and the product becomes fluorescent[114]. To allow progress of the enzymatic reaction, *in situ* zymography is typically applied to unfixed tissue and therefore has limited resolution. However, a recently developed approach combining alcohol fixation and polyester wax embedding has increased the resolution of this technique to close to that of the light microscope[115]. The method successfully demonstrated the co-localization of MMP9 activity with glutamatergic synapses.

**Visualization of ECM-dependent intracellular signalling**

For analysis of extracellular matrix (ECM)-dependent signalling, the probes for high-resolution live-cell imaging of activated Src kinases or small GTPases are indispensible[116,117]. Available probes are based on the principle of fluorescence resonance energy transfer (FRET) — a process by which a fluorophore in an excited state (the donor) transfers its energy to a neighbouring fluorophore (the acceptor), causing the acceptor to emit fluorescence at its characteristic wavelength.

activity-dependent secretion and proteolytic cleavage of ECM molecules. Proteolytic ECM modifications underlie functional and structural synaptic plasticity, including the enlargement of dendritic spines and the growth of filopodia resulting in new spines. Important directions for future investigations relate to the mechanisms by which remodelling of the ECM governs structural and functional synaptic plasticity. The availability of high-resolution imaging techniques (BOX 2) will be instrumental in visualizing the sites of ECM remodelling and associated synaptic changes in excitatory and inhibitory synapses, as well as the involvement of glial cells.

The importance of the ECM for both experience-induced plasticity and homeostatic maintenance suggests that it could have a pivotal role in the pathogenesis of neurological and neuropsychiatric disorders. Indeed, emerging data support the view that ECM aberrations are likely to contribute to imbalanced synaptic function in epilepsy[104], Alzheimer's disease[4] and other neurodegenerative disorders[105], mental retardation[88] and schizophrenia[11,106]. Extensive data demonstrate that aberrant remodelling of the ECM contributes to tumour formation, abnormal migration and differentiation of stem cells during development and in the adult, and impaired regeneration after injury of the central and peripheral nervous systems[65]. The development of molecular and cellular tools that target synaptic ECM molecules and interactions with their receptors is likely to stimulate further analysis of the normal and pathological mechanisms in synaptic functions that are mediated by the ECM.


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### Competing interests statement
The authors declare no competing financial interests.

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