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Synaptic circuit remodelling by matrix metalloproteinases in health and disease

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Abstract

Matrix metalloproteinases (MMPs) are extracellularly acting enzymes that have long been known to have deleterious roles in brain injury and disease. In particular, widespread and protracted MMP activity can contribute to neuronal loss and synaptic dysfunction. However, recent studies show that rapid and focal MMP-mediated proteolysis proactively drives synaptic structural and functional remodelling that is crucial for ongoing cognitive processes. Deficits in synaptic remodelling are associated with psychiatric and neurological disorders, and aberrant MMP expression or function may contribute to the molecular mechanisms underlying these deficits. This Review explores the paradigm shift in our understanding of the contribution of MMPs to normal and abnormal synaptic plasticity and function.

Matrix metalloproteinases (MMPs), which are members of the **metzincin** clan of metalloproteinases, compose a large subgroup of zinc-binding, multi-domain endopeptidases that are present in most tissues of the body. In humans, there are 23 distinct MMPs comprising secreted and transmembrane proteins (BOX 1). Much of their biological activity is exerted extracellularly, where they critically influence cellular behaviour through the targeted degradation or the proteolytic processing of various extracellular matrix (ECM) molecules, peptide growth factors, cytokines, chemokines, cell adhesion molecules, and many other types of receptors and glycoproteins that reside on the cell surface. The collective effects of pericellular MMP-mediated proteolysis on cell behaviour can be permissive (they can degrade chemical or physical barriers) and instructive (they can proactively initiate or terminate signaling cascades through the processing of latent bioactive molecules)^{1,2}. MMP-mediated remodelling of the pericellular microenvironment is therefore essential for many physiological processes. However, MMP activity can also have deleterious effects, such as in cancer, rheumatoid arthritis and other disease states^{1,3}.

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Box 1

Classification and domain structure of the MMPs

Matrix metalloproteinases (MMPs) are multi-domain proteins that are named according to a sequential numbering scheme and organized into subgroups on the basis of common domains, inserts and other motifs and on shared canonical substrate preferences^{1, 136, 137}. In humans, 24 MMP genes encode 23 distinct MMPs (two identical genes located on chromosome 1 encode MMP23). MMPs all possess an amino-terminal signal peptide that targets them to the secretory pathway, an autoinhibitory pro-domain and a catalytic domain (see the figure). Most MMPs also possess a carboxy-terminal hemopexin domain, which is coupled to the catalytic domain by a flexible hinge region and is an important mediator of protein–protein interactions. In cooperation with other exosites, the hemopexin domain contributes to the target specificity of MMP proteolysis by coordinating interactions with substrates. The hemopexin domain can anchor MMPs to other cell-surface proteins, thereby positioning or stabilizing MMPs at the membrane surface, which in turn markedly influences how and when MMPs become activated as well as regulates their accessibility to substrate targets. Additionally, MMPs, through their hemopexin domain, can act as ligands, activating downstream signal cascades by binding to receptors (for example, the low-density lipoprotein receptor-related protein)^{138, 139}.

Most MMPs are secreted into the extracellular environment. However, a minority (seven) of MMPs are membrane-associated, anchored by a type I transmembrane domain (MMP14, MMP15, MMP16 and MMP24), a type II transmembrane domain (MMP23), or a glycosylphosphatidylinositol (GPI) linkage (MMP17 and MMP25). A key feature of all MMPs is that they are synthesized as proteolytically inactive zymogens containing a pro-domain (pro-MMPs). A conserved cysteine in the pro-domain controls proteolytic activity of the enzyme by acting as a fourth zinc-coordinating ligand, rendering the catalytic site masked and inoperative when bound. Activation of MMPs requires unmasking of the catalytic site by disruption of this cysteine–zinc bond (the ‘cysteine switch’)¹⁴⁰. For most MMPs, the cysteine switch occurs extracellularly, either through physical removal of the pro-domain via proteolytic cleavage by other extracellular proteases (for example, other MMPs or serine proteases) or by conformationally destabilizing the pro-domain via modifications of the thiol group on the inhibitory cysteine (for example, via oxidation or S-nitrosylation⁶⁹), which activates the MMP prior to subsequent proteolytic cleavage of the pro-domain. A minority of MMPs, including three secreted MMPs (MMP11, MMP21 and MMP28) and the membrane-associated ones, contain a unique sequence at the C-terminal end of the prodomain that is recognized by the convertase furin. Thus, for most of these MMPs, the cysteine switch can occur intracellularly, allowing the release or insertion of an already proteolytically active MMP into the extracellular environment. Although MMP activity is mostly extracellular, some active MMPs can be found in neuronal or glial nuclei^{141, 142}, where they may have transcription factor-like or DNA repair-like activity^{143, 144}.

MMP activity is terminated in brain mostly by four, small (~22 kD), secreted protein inhibitors called TIMPs (tissue inhibitor of metalloproteinases). The TIMPs bind

reversibly to MMPs in a 1:1 stoichiometric ratio, indicating that the balance of MMP:TIMP levels determines overall proteolytic activity. Each of the four TIMP proteins displays preferential MMP-binding specificities, and also possess biological activity independent of their role in regulating MMP activity.

In the CNS, MMPs are synthesized and secreted by neurons and glia and, historically, are associated with roles in early CNS morphogenesis^{4, 5} and protracted pathophysiological cellular remodelling that is triggered by injury or disease (BOX 2). Recent studies, however, show that rapid and focal MMP-mediated proteolysis proactively drives structural and functional synaptic circuit remodelling that is critical for ongoing cognitive processes.

Box 2

Well-established roles of MMPs in CNS injury and disease

Excessive and protracted matrix metalloproteinase (MMP)-mediated proteolysis is a major pathological component of many CNS injuries and diseases such as neuroinflammation, ischaemia and hypoxia, demyelination, traumatic brain and spinal cord injuries, and neuronal degeneration^{5, 129, 145}. A cascade of aberrant MMP activity is often initiated by MMP-mediated proteolytic degradation of the blood–brain barrier (BBB), where, for example, an ischaemic episode triggers upregulation of MMP2, MMP3, MMP7, MMP9 and MMP14 by endothelial cells, pericytes, leukocytes, and other components of the neurovascular epithelium. The MMP-mediated breakdown of the BBB, which occurs over the course of 24–48 h, allows large numbers of inflammatory and other cell types to invade the brain parenchyma, further stimulating excessive MMP expression and secretion by microglia, astrocytes and neurons. MMPs are activated by each other as well as by free radicals (such as nitric oxide), which are produced by hypoxia, thereby exacerbating a cascade of excessive MMP activity. In time, excessive MMP activity can trigger neuronal apoptosis and synapse loss. Such a cascade of excessive and essentially unregulated MMP activity within the brain parenchyma can last weeks, depending on the injury or particular insult. After this protracted destructive phase, further MMP expression can contribute to subsequent reparative processes of cell replacement, remyelination, and re-establishment of connectivity and neurovascular integrity.

Synaptic circuit remodelling, which includes structural alterations in the number or the morphology of **dendritic spines** and their synapses coupled with functional changes in the strength of synaptic signalling, occurs throughout life in naturally occurring contexts (for example, in learning^{6–9}). It has become clear, largely based on studies of experimentally induced forms of synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD), that synaptic remodelling depends crucially on a variety of ECM proteins and their receptors, cell adhesion molecules, growth factors and other targets of MMP proteolysis^{10, 11}. These findings have led to a fresh outlook on the role, timecourse of activation, and regulation of MMPs in the fine-scale modulation of adaptive, non-pathophysiological synaptic function and plasticity.

The goal of this Review is to capture our rapidly evolving understanding of where, when and how MMPs contribute to normal synaptic functional and structural remodelling, and to speculate on the impact of perturbation of these processes in diseases that are characterized by synaptic plasticity deficits].

Expression and localization in brain

Most MMPs are synthesized and secreted as inactive pro-enzymes (zymogens) that are converted to proteolytically active molecules following several regulatory steps (BOX 1). As regulation of MMP activity can occur at the levels of transcription, translation and post-

translation¹, the levels of pro- and active forms of MMP and MMP proteolytic activity cannot always be inferred from MMP mRNA levels.

Biochemical experiments have shown that mRNA levels of MMP2, MMP3, MMP8, MMP9, MMP11, MMP12, MMP13, MMP14, MMP15 and MMP24 are low under basal conditions in adult human and rodent brains, but are much higher during early postnatal development¹²⁻¹⁷. The mRNA levels of other MMPs lie below detectable limits or have not been examined.

For some MMPs, their low transcript levels in total brain samples belie regionally higher levels of *MMP* mRNA expression and higher constitutive levels of protein. In adult mice and rats, elevated levels of constitutive *Mmp9* and *Mmp24* mRNA expression are found in the hippocampus compared with other brain regions¹⁶⁻¹⁹. Moreover, western blot analyses using MMP-specific antisera that recognize both the inactive pro-forms and the active forms of the enzymes, which can be distinguished by differences between the two forms in their molecular mass (the pro-form is heavier than the active form), revealed that hippocampal lysates contain high levels of the pro-forms of MMP2, MMP3, MMP9 and MMP24, but lower levels of the active forms of these MMPs^{16, 18-24}. High-resolution microscopy of immunolabelled hippocampal tissue sections showed that MMP9 is concentrated throughout the synaptic neuropil in discrete puncta that colocalize with presynaptic and postsynaptic molecular markers (FIG. 1A), in non-synaptic dendritic regions and in some glial processes^{18, 19, 21, 25}. Similarly, MMP24 has a punctate, synaptic-like distribution in perfused tissue sections of adult mouse hippocampus and cerebellum¹⁶. Moreover, it colocalizes with synaptic molecular markers in cultured rat hippocampal neurons and is enriched in a synaptosome fraction prepared from adult rat forebrain^{23, 26}. The cellular or synaptic distributions of MMP2 and MMP3 within specific brain regions are currently unknown.

A key question raised by the data above is how much of the MMP protein is proteolytically active under basal conditions? This is a question that cannot be determined definitively by immunocytochemistry, western blotting or — for the gelatinases (that is, MMP2 and MMP9) — *in gel* gelatin-substrate zymography (BOX 3). However, the combination of *in vivo* or *in situ* gelatin-substrate zymography (BOX 3) with immunolocalization has been used to infer the status of endogenous MMP9-mediated proteolytic activity within the synaptic neuropil^{18, 27}. Through these combined approaches, a small number of the MMP9-immunopositive puncta colocalize with discrete hotspots of gelatinolytic activity that are located along hippocampal dendrites and dendritic spines. This pattern is consistent with the idea that much of the MMP9 protein detected immunocytochemically under basal conditions is in the inactive pro-form. It should be noted that these data do not rule out the possibility that other proteases that are capable of cleaving gelatin also contribute to the gelatinolytic activity observed in these experiments^{21, 25}. In any event, such focal gelatinolytic puncta colocalize with molecular markers of excitatory synapses, including AMPA- and NMDA-type glutamate receptors (AMPA- and NMDARs), postsynaptic scaffolding and cytoskeletal proteins, and presynaptic vesicle proteins^{18, 25}, but they seem to be largely absent from GABAergic (inhibitory) synapses²⁵. In the case of MMP24, western blotting and cell-surface biotinylation assays involving cultured rat cortical neurons together indicate

that although both pro- and active forms reach the cell surface, the inactive pro-form is more abundant²³. The active form of MMP24 is only visualized on the cell surface when proteolytic activity is blocked by the broad-spectrum MMP inhibitor GM6001 (also known as Ilomastat). This suggests that the duration of the active form of MMP24 on the cell surface is probably short because of proteolytic degradation, although the identity of the proteolytic enzyme responsible for surface degradation of MMP24 is unknown. Together, these data indicate that under basal conditions, high levels of MMP9 and MMP24, predominantly in their inactive pro-forms, are distributed perisynaptically at excitatory synapses that are located on dendritic spines. The observation that pro-forms of MMPs are located perisynaptically has important implications for where they exert their proteolytic effects when activated and which proteins they target (see below). Of note, various proteins have been identified that are involved in the targeting of some MMPs to synapses (BOX 4).

Box 3

Gelatin-substrate zymography techniques

In gel gelatin-substrate zymography

An SDS-PAGE-based technique for the detection of enzyme activity based on the ability of the enzyme to cleave a substrate (gelatin) that is co-polymerized with the polyacrylamide gel. The advantages of this technique are that it is sensitive enough to detect picogram levels of enzyme and it can resolve pro-forms and active forms of gelatin-cleaving matrix metalloproteinases (MMPs) (for example, MMP2 and MMP9) on the basis of molecular mass. The disadvantages are that it lacks cellular resolution, is not applicable to non-gelatinolytic proteases, and does not accurately report on endogenous enzyme activity because of some auto-activation within the gel and the loss of any endogenous tissue inhibitor of metalloproteinase (TIMP) binding during preparation.

***In vivo* gelatin substrate zymography**

An extension of *in situ* gelatin-substrate zymography, but in this case, the dye-quenched (DQ)-gelatin is injected directly into the brain of an intact animal *in vivo*. The animal is subsequently perfused with aldehydes and the fluorescent gelatinolytic puncta can be visualized by fluorescent microscopy. The advantage of this approach, in addition to those of *in situ* zymography, is that brains are not sliced prior to the gelatinolytic reaction, thereby eliminating the potentially confounding effects of the processing steps on enzymatic activity.

***In situ* gelatin substrate zymography**

A technique for visualizing gelatinolytic enzyme activity within semi-intact tissues or cells. The approach is to expose acute tissue slices or cell cultures to dye-quenched (DQ)-gelatin, which is gelatin-coupled to a fluorescent molecule (fluorescein) that is masked until cleaved. Upon cleavage by endogenous gelatinases, the fluorescein becomes visible, and after aldehyde fixation, can be detected by fluorescent microscopy. The advantages of this approach are that it provides cellular and synaptic anatomical resolution of endogenous gelatinolytic enzymes, and can be combined with immunolocalization of other proteins of interest. The disadvantage is that it requires numerous controls to show

that enzyme activity is not altered as a result of tissue slicing or other processing steps prior to visualization.

Box 4

How are MMPs directed to or near synapses?

Recent studies show that ABP (AMPA-receptor (AMPA) binding protein) and GRIP1 (glutamate receptor interacting protein 1), two postsynaptic scaffolding proteins that bind to and direct AMPAR subunits to the synaptic membrane¹⁴⁶, also bind to the carboxy-terminal tail of matrix metalloproteinase 24 (MMP24), which is a transmembrane protein, and direct it to synaptic sites²³. In the case of secreted MMPs, the basis for such synaptic targeting is less well understood. Image analysis of intracellular trafficking of MMP2, MMP9 or the endogenous MMP inhibitor TIMP1 (tissue inhibitor of metalloproteinase 1) fused to green fluorescent protein expressed in cultured hippocampal neurons¹⁴⁷ and glia¹⁴¹ shows that these MMPs and TIMP1 are transported in distinct 160–200 nm-wide vesicles that, for neurons, are concentrated in the somatodendritic domain. Interestingly, although TIMP1 is colocalized in vesicles carrying either MMP2 or MMP9, these two MMPs are distributed into separate vesicle populations in both neurons and astrocytes, which suggests that there is differential control of their trafficking and release. Such vesicles associate with cytoskeletal components and the molecular motors dynein, kinesin and myosin Va, are found in dendritic spines, and can be secreted, as exosomes, from these sites¹⁴⁷. In non-neuronal cells, release of such MMP-containing vesicles depends on proteins of the SNARE (soluble NSF attachment protein receptor) family¹⁴⁸, although it is unclear at present whether regulated release in neurons occurs by a similar mechanism. Additionally, whether non-vesicular release of soluble MMPs or TIMPs occurs in neurons or glia also remains to be explored.

MMPs drive synaptic remodelling

Our emerging understanding of how MMPs contribute to normal synaptic and behavioural plasticity comes mostly from detailed studies of MMP9. The focus on MMP9 reflects the availability of a broad set of investigative reagents for this MMP and the fact that it has a well-recognized role in pathophysiological neural circuit remodelling^{19, 25, 28}. These new insights into the role of MMP9 in normal synaptic plasticity have been borne largely from experiments examining loss- and gain-of-function effects of MMP9 on persistent forms of synaptic structural and functional modifications such as LTP (BOX 5).

Box 5

Hippocampal long-term potentiation

Hippocampal long-term potentiation (LTP) is the archetypal cellular model of synaptic functional and structural plasticity that enables memory formation⁶³, information processing¹⁴⁹ and skill learning¹⁵⁰. Tetanic or theta-burst stimulation protocols applied to

the Schaffer collaterals (axons of CA3 pyramidal neurons) elicit NMDA receptor (NMDAR)-dependent LTP at CA3→CA1 glutamatergic synapses, which are located mostly on dendritic spines. At these synapses, LTP reflects functional changes in the magnitude of synaptic neurotransmission that are coordinated with structural changes in synaptic spine morphology, size and density¹⁵¹. Functionally, LTP has at least two mechanistically distinct phases, the occurrence of which depends on the strength of the induction protocol. Weak stimulation protocols elicit so-called early phase (E-LTP), which is transient and involves post-translational modifications of synaptic proteins but is protein synthesis-independent. By contrast, stronger, repetitive stimulation protocols elicit E-LTP followed by a late-phase (L-LTP), which is protein synthesis-dependent and persistent¹⁵². In L-LTP, spines undergo actin-dependent increases in spine-head volume, changes in density, and enlargement of synapses. These phenomena are all thought to be important structural correlates of stable changes in synaptic strength¹⁵³.

LTP and the accompanying dendritic spine plasticity, particularly those evoked by theta-burst stimulation, which mimics endogenous patterns of neural activity during learning¹⁵⁴, are initially labile and subject to reversal by a number of protocols before transitioning, via a consolidation-like process, to a stable form that is resistant to reversal protocols¹⁵⁵.

LTP-inducing stimuli rapidly activate MMP9

Levels of both the pro-form and the active form of MMP9 are notably elevated in CA1 60 min after induction of late-phase LTP (L-LTP) at CA1 synapses by a strong tetanic stimulation protocol, and 15 min following the end of a chemical induction protocol that selectively elicits L-LTP^{18,21}. The increase in MMP9 levels induced by these L-LTP induction protocols is transient, as MMP9 levels return to baseline values by 120 min post-induction. Weaker tetanic stimulation that induces only early phase LTP (E-LTP), or any one of several protocols that induce LTD at CA1 synapses, does not cause any detectable changes in the levels of the pro-form or active-form of MMP9²¹. Collectively, these observations suggest that upregulation of MMP9 is related mechanistically to L-LTP. Consistent with this idea, NMDAR antagonists and protein synthesis inhibitors abrogate the L-LTP-associated increase in protein levels²¹.

The elevation in MMP9 levels that results from L-LTP-inducing stimuli has been documented in both rats and mice and is consistent across *ex vivo* (acute hippocampal slices) and *in vivo* preparations (urethane-anaesthetized animals). Moreover, the increase in MMP9 levels with L-LTP is readily evident in preparations from animals of different ages (from 3 postnatal weeks to adulthood). These observations suggest that MMP9 plays a fundamental role in synaptic plasticity across species and the lifespan. Additionally, in terms of the gelatinases (that is, MMP2 and MMP9), L-LTP-inducing stimuli affect MMP9 levels selectively, as LTP induction has no effect on the levels of the pro-form or active form of MMP2²¹. Whether the levels of other MMPs are altered following the induction of LTP (or other forms of synaptic plasticity) is unknown.

Anatomical localization methods have shown that following induction of L-LTP, marked elevations in the numbers of MMP9 immunoreactive puncta and gelatinolytic puncta can be seen in the neuropil of CA1 — the location of the potentiated synapses (FIG. 1B–D)^{18, 21}. The majority of the LTP-associated gelatinolytic puncta colocalize with MMP9 (FIG. 1E–G) and these puncta are found mostly at or surrounding synapses, or along short stretches of dendrites. When LTP is blocked by NMDAR antagonists administered just before the tetanic stimulation used to induce LTP, the increase in the number of gelatinolytic puncta is also blocked¹⁸ (FIG. 1B–D). This result indicates that the appearance of gelatinolytic puncta is not a non-specific effect of high-frequency stimulation, but rather is related specifically to the LTP that results from it. Whether protein synthesis is also required to generate such L-LTP-associated hotspots of proteolytic activity has not been determined.

When these data are considered together, a striking picture emerges which indicates that MMP9 is upregulated and perisynaptically activated on a timescale (~15 min) that is roughly an order of magnitude faster than the timescales of induction and duration of MMP activity (several hours to weeks) following injury or onset of disease (BOX 2). What does a proteolytically active MMP9 contribute to synaptic form and function?

Blocking MMP9 impairs L-LTP and spine remodelling

The contribution of MMP9 to synaptic plasticity has been investigated through acute loss-of-function (exogenous reagents that inhibit MMP9) and chronic loss-of-function (genetic deletion) experimental approaches. Field recordings in CA1 show that L-LTP-inducing stimuli applied to the Schaffer collaterals in the presence of pharmacological MMP inhibitors or MMP9-specific neutralizing antibodies induce LTP at CA1 synapses that is indistinguishable from that induced in controls (by L-LTP-inducing stimuli applied without inhibitors) for 30–45 min, but which then declines rapidly to baseline values^{18, 21, 29, 30}. This suggests that MMP9 inhibitors have no effect on E-LTP, but block L-LTP selectively.

The contribution of MMP9 activity to the spine enlargement that accompanies persistent LTP has been examined by combining whole-cell recordings from CA1 pyramidal cells with simultaneous two-photon imaging of their dendritic spines²⁹. In a theta-burst stimulation paradigm applied in the presence of MMP inhibitors, spine enlargement and LTP were both induced normally but both subsequently collapsed to baseline, which is consistent with the timecourse of the effects of the inhibitors observed in the field recording experiments discussed above. The impaired stability of both LTP and spine structural plasticity was evident only when inhibitors were applied for a period of up to ~30 min post-induction; when they were applied after this point, L-LTP and spine enlargement were resistant to decay and therefore MMP9-independent effects. These observations suggest that MMP9 contributes to a consolidation-like process during the first 30 min of LTP in which coordinated functional and structural plasticity becomes stable and immutable. Similar time-dependent effects of pharmacological MMP inhibitors on LTP persistence have been described for synapses in other hippocampal circuits³¹ and in other brain regions, including the amygdala⁵ and prefrontal cortex³².

Interestingly, the acute MMP inhibitors that interfere with LTP persistence have no effect on the magnitude or timecourse of paired-pulse facilitation (PPF), a form of presynaptic

plasticity. These inhibitors also have no effect on E-LTP that is induced by weaker tetanic stimulation protocols, or on NMDAR- or metabotropic glutamate receptor (mGluR)-dependent forms of LTD. Thus, MMP9 seems to have a specific role in the persistence of L-LTP, which is consistent with the lack of upregulation or activation of MMP9 in response to LTD, PPF or E-LTP as discussed above. MMP inhibitors also have no effects on the baseline properties of synaptic neurotransmission, spine size or spine morphology, which suggests that MMPs become operative only under the demands of dynamic synaptic remodelling, and is consistent with the observation that much of the MMP9 present within the neuropil under basal conditions is in the inactive pro-form. This notion stands in contrast to a recent study showing that members of the ADAM (a disintegrin and metalloproteinase) family of metalloproteinases may have a constitutive role in regulating baseline levels of synaptic neurotransmission, at least in cultured neurons²⁶.

The lack of any direct involvement of MMP9 in hippocampal baseline neurotransmission, PPF or LTD is also supported by studies of MMP9-deficient mice (*Mmp9*^{-/-}), in which none of these processes is affected by the absence of this MMP²¹. However, results from other studies using broad-spectrum metalloproteinase inhibitors (FN439 and GM6001) suggest that other metalloproteinases (that is, other MMPs or ADAMs) may contribute to these forms of plasticity. In one study, hippocampal slices were exposed to FN439 and exhibited deficits in both PPF and LTD³⁰. Speculatively, deficits in PPF might arise through FN439-mediated inhibition of MMP7, as exogenous application of active MMP7 impairs synaptic vesicle release and uptake dynamics³³, possibly through intracellular cleavage of synaptic vesicle proteins³⁴. Similarly, diminished presynaptic vesicle release has also been reported following long-term (overnight) exposure of hippocampal cultures to GM6001²⁶. Deficits in LTD could arise if broad-spectrum pharmacological blockers affected the activity of ADAM17 (also known as TACE), as this enzyme is required for mGluR-dependent LTD³⁵.

Two studies have reported that non-specific MMP inhibitors impair LTP induction in CA1^{30, 36}, which is in contrast to the data discussed above that suggests that MMP9 does not contribute to LTP induction. As most MMP inhibitors lack selectivity, it is possible that such discrepant results reflect off-target effects of the inhibitors on synaptic physiology. For example, barring clear differences across studies in LTP induction protocols and other experimental conditions, other metalloproteinases (MMPs and/or ADAMs) that are affected by the broad-spectrum inhibitors may be contributing to the induction of LTP. It is notable, however, that acute slices prepared from *Mmp9*^{-/-} mice also exhibit impaired LTP induction, as well as maintenance, both of which are restored to wild-type levels in the presence of bath-applied proteolytically active MMP9²¹. One potential confounding factor in interpreting results from MMP9 null-mutant mice is the possibility of developmental compensation for the chronic loss of MMP9, which could include compensation by other MMPs or by other types of molecules. Indeed, recent studies suggest, for example, that ECM-dependent regulation of neuronal survival in developing hippocampus is modulated by MMP9 and is therefore altered in *Mmp9*^{-/-} mice³⁷. The observation that adding proteolytically active MMP9 to hippocampal slices from *Mmp9*^{-/-} mice restores normal levels of LTP could reflect an MMP9-dependent enhancement of synaptic neurotransmission (discussed below).

MMP9 activation proactively drives synaptic remodelling

Although loss-of-function approaches indicate that MMP9 is necessary for the persistence of functional and structural synaptic plasticity, acute gain-of-function experiments conducted in slices or *in vivo* show that MMP9 is also sufficient for driving functional and structural synaptic remodelling in the absence of conventional LTP-inducing stimuli^{21, 29}.

Brief, local application of proteolytically active MMP9 to unstimulated dendritic spines of CA1 pyramidal neurons induces an LTP-like increase in synaptic signal strength and concurrent spine enlargement, both of which reach a maximum in the 15–20 min period after the spines are first exposed to the enzyme. Such plasticity is not observed when spines are exposed to pro-MMP9. Moreover, spines and synapses are unresponsive to active MMP2. Both functional and structural plasticity induced by MMP9 depends on signalling through $\beta 1$ subunit-containing **integrins**, and are associated with integrin-dependent phosphorylation (and hence inactivation) of the actin-depolymerizing factor **cofilin** within dendritic spines. Additionally, such MMP9-induced synaptic and spine plasticity occludes subsequent potentiation and spine enlargement that are induced by theta-burst or tetanic stimulation^{18, 29}, which suggests that common mechanisms of synaptic potentiation and spine remodelling are shared by MMP9 and electrical stimulation protocols. Consistent with this suggestion, MMP9-induced potentiation and persistent spine enlargement are both sensitive to inhibitors of protein synthesis as well as reagents that interfere with postsynaptic actin polymerization and blockers of **SNARE**-dependent exocytosis in postsynaptic neurons²⁹. Such sensitivity to these reagents is identical to those of electrically evoked LTP and spine plasticity³⁸.

Together, these findings support a working model of the molecular cascade of MMP9 activity in driving synaptic remodelling: from MMP9 activation by LTP induction to its downstream target effects driving persistent potentiation. According to this model (Fig. 2), perisynaptic pro-MMP9 is rapidly activated by NMDAR-mediated signalling that occurs during LTP induction. Active MMP9 then signals, via proteolysis of a yet unidentified target (see section below for candidates), through $\beta 1$ -containing integrins on dendritic spines³⁹ to couple actin-dependent structural changes in spine shape with actin-dependent functional changes in synaptic neurotransmission.

Integrins are crucial for the consolidation of LTP^{40, 41}; they activate downstream signaling cascades that increase synaptic glutamate receptor-mediated currents^{42, 43}, regulate surface expression and composition of AMPARs⁴⁴, and promote spine actin polymerization^{45, 46}. Persistence of LTP requires the insertion of AMPARs into the synaptic membrane^{38, 47}, which in turn stabilizes LTP-associated spine enlargement^{38, 48}. Consequently, MMP9-mediated potentiation of synaptic transmission probably arises ultimately from integrin-directed trafficking of AMPARs into the synaptic membrane, which in turn stabilizes the spine enlargement, thereby consolidating both forms of MMP9-driven plasticity (Fig. 2).

In contrast to the potentiating and spine-enlarging effects of local, transient MMP9 exposure, widespread, chronic application of active MMP9 produces seemingly opposing effects, at least on spine morphology. In dissociated hippocampal cell or organotypical-slice cultures, active MMP9 that is bath-applied for up to 90 min induces the production of long,

thin dendritic spines with smaller spine-heads^{24, 49}, which represents an immature spine morphology. Additionally, although bath-applied MMP9 has no apparent effects on synaptic physiology for the first 90 min of exposure, after this point **mEPSCs** (miniature excitatory postsynaptic currents) display longer deactivation time-constants. Other studies involving cultured neurons have revealed that active MMP9 increases the lateral mobility⁵⁰ and desensitization kinetics⁵¹ of surface NMDARs, without having any effects on surface mobility of AMPARs. This observation is interesting in light of the fact that enzymatic degradation of the ECM that normally surrounds neurons and synapses increases the surface mobility of AMPARs, but not that of NMDARs⁵². These differences in glutamate receptor-subtype mobility following degradation of the ECM may indicate that the loss of the ECM is permissive for AMPAR mobility, while NMDAR mobility may be regulated selectively by an MMP9-mediated mechanism. Indeed, although the precise relationships between the altered kinetics of synaptic and NMDAR-mediated currents, the increased rates of NMDAR diffusion and the transition towards an immature spine morphology observed in cultures is presently uncertain, all these MMP9-linked effects in neuronal cultures are dependent on β 1-containing integrins.

Such stark differences in MMP9-driven, β 1-integrin-dependent effects on synaptic form and function between the different gain-of-function experimental approaches might be attributed to the nature and timecourse of MMP9 application. Global exposure of an entire network of cultured neurons to an active MMP could generate homeostatic adjustments in synaptic currents or spine morphology that differ substantially from local and delimited effects on a small subset of spines⁵³, as all neurons and their synapses would be affected simultaneously during bath-exposure to the active MMP. Indeed, bath-application of MMP7 to dissociated hippocampal cultures also results in a transition from mature to immature-looking spines, global increases in the phosphorylation of the NMDAR subunits NR2A and NR2B⁵⁴, and in the ectodomain cleavage of NR1 and NR2A⁵⁵. An increasingly more widespread exposure to active MMPs, coupled with long exposure times, may underlie how MMPs transition from adaptive, acute mediators of local synaptic remodelling to maladaptive, deleterious effectors of aberrant synaptic and neuronal networks (Fig. 3). Indeed, transgenic rats that constitutively overexpress an autocatalytically active form of MMP9 not only possess hippocampal neurons with immature-looking spines, but also exhibit a lower threshold for epileptogenesis²⁵, which suggests that global and/or chronic MMP activity contributes to a pathophysiological profile of cellular and synaptic architecture.

It should be noted that the different states of neuronal development represented by culture models versus intact circuitry of adult hippocampus *in vivo* or in acute *ex vivo* slices could also contribute to different outcomes of MMP9-driven integrin activation. Direct activation of integrins on young cultured neurons produces spine elongation and decreased filamentous actin content within dendritic protrusions⁵⁶, whereas integrin activation in mature intact circuitry drives actin polymerization in spine-heads and is associated with spine enlargement⁴⁵. In addition, important differences between model systems in the extracellular milieu may also be relevant to different outcomes, as some of the MMP7-mediated effects on neurons in culture vary depending on the substrate upon which the neurons are grown⁵⁴.

MMPs contribute to behavioural and experience-dependent plasticity

The mounting evidence indicating that MMPs are a necessary component of the molecular mechanisms that couple and consolidate synaptic functional and structural synaptic remodelling suggests that MMP activity would also influence cognitive tasks that depend on such synaptic plasticity. This is indeed the case, as several associative and non-associative learning and memory paradigms, conducted in conjunction with *in vivo* delivery of pharmacological blockers, or MMP antisense oligonucleotides, or MMP overexpression, have shown that MMPs, particularly MMP3 and MMP9, have important roles in cognitive performance^{20, 21, 57–61}.

Inhibitory avoidance (IA) learning, which is a single-trial fear-conditioning type of learning and memory task⁶² that elicits an LTP-like increase in the strength of synaptic transmission in CA1⁶³, leads to markedly elevated levels of both pro-forms and active forms of MMP3⁶⁰ and MMP9²⁰. In the case of MMP9, the increase in levels of the active form is matched by increased gelatinolytic activity: both are markedly higher 6 hours after IA training and remain elevated for ~48 hours. Importantly, such upregulation is not evident following exposure to either the chamber (the context) or the aversive stimulus (the shock) alone, but only when these two are coupled in time. Functionally, blockade of MMP activity with intrahippocampal administration of pharmacological blockers or MMP9-specific neutralizing antisera starting ~3 hours after IA training, just before the onset of training-induced MMP activity, completely abrogates memory for the IA response when tested days later. These data, when allied with the emerging understanding of how MMPs contribute at the cellular and molecular levels to synapse and spine remodelling, suggest that learning induces a period of focal MMP-mediated proteolysis that is vital for driving long-lasting synaptic modifications of the kind that underlie memory consolidation.

Considerable synaptic remodelling also occurs during rapid-eye movement (REM) sleep⁶⁴, and recent studies suggest that MMPs may be involved in this remodeling process. Sleep deprivation in rats is associated with diminished levels of *Mmp9* mRNA in cortical structures⁶⁵, and treatment of sleep-deprived rats with modafinil, a wake-promoting pharmacological reagent that is used to treat narcolepsy, increases *Mmp9* mRNA levels and restores cognitive function of sleep-deprived animals⁶⁶.

Finally, recent *in vivo* studies of visual⁶⁷ and somatosensory (barrel) cortex⁶⁸ suggest that MMPs facilitate functional and structural changes in connectivity that underlie experience-dependent alterations in representational maps.

Together, the data discussed above indicate that MMPs have an active role in remodelling synaptic structure and function during LTP, a role that is crucial for cognitive and sensory function. This raises important questions about how the onset and termination of MMP activity is coupled to LTP and the target molecules through which MMPs influence synaptic form and function.

Timecourse and targets of MMP activity

There are several distinct mechanisms that could activate, sustain and terminate perisynaptic MMP proteolysis following LTP induction. Although the precise onset of proteolysis relative to the onset of LTP induction remains to be definitively determined, in principle, the latent, perisynaptic pool of pro-MMPs could be activated quickly by molecules that are both capable of converting pro-MMPs to their active forms and rapidly released by LTP induction. One attractive candidate molecule is nitric oxide (NO), a gaseous, diffusible cellular messenger. NO can activate MMP9^{69, 70}, is released from postsynaptic neurons by theta-burst-stimulated LTP in an NMDAR-dependent manner, and contributes to L-LTP⁷¹. Additionally, the release of NO is presumably localized to potentiated synapses, where it could activate MMP proteolysis locally (Fig. 2). MMP9 can also be activated by the serine protease tissue plasminogen activator (tPA) in some cell types⁷². tPA is encoded by an immediate early gene that is rapidly upregulated by LTP induction⁷³; tPA is then released from dendritic spines⁷⁴. However, the gain-of-function effects of tPA on neuronal activity and morphology are markedly different to those induced by MMP9⁷⁵. When applied to hippocampal slices, active tPA converts a normally transient potentiation induced by a weak stimulus protocol into persistent LTP, but does not, by itself, induce synaptic potentiation. Moreover, in cultured neurons, tPA increases axon growth and the formation of presynaptic boutons. These different effects on synaptic plasticity and morphology in comparison with those described above for active MMP9 make it unlikely that tPA is immediately upstream of MMP9 in the context of LTP-triggered proteolytic remodelling. Instead, such differences suggest that tPA, MMPs and other LTP-activated extracellular proteases^{76, 77} operate along largely independent pathways, but act synergistically to remodel the synaptic microenvironment.

Once pro-MMPs are activated at the synapse following LTP induction, new MMP synthesis and secretion probably replenishes the pro-MMP pool, since the LTP-triggered increase in MMP9 levels is sensitive to protein synthesis inhibitors (discussed above). Studies suggested that *Mmp9* mRNA is localized to dendrites and dendritic spines of granule cells in rat dentate gyrus. Moreover, 12–24 hours following kainate-induced epileptogenesis in rats, levels of *Mmp9* mRNA increased within the molecular (dendritic) layer of the dentate gyrus, attributed to both dendritic and glial expression^{19, 78}. Similarly, increases in *Mmp9* mRNA levels have been detected in hippocampus 2 hours post-seizure onset following administration of the GABA_A receptor antagonist pentylentetrazole⁷⁹. Together, these findings suggest that *Mmp9* mRNA could be translocated into dendrites and locally translated at synaptic sites under activity-dependent conditions. However, in CA1, there is no evidence yet for increased levels of *Mmp9* mRNA, either in somata or dendrites, at 75 min following LTP induction by strong tetanic stimuli delivered *in vivo*¹⁸. Thus, LTP-related transcriptional regulation probably occurs at later time points when it might serve to sustain MMP activity levels. More studies are required to characterize potential transcriptional regulation of MMPs in the context of LTP.

Behaviourally, the upregulation in MMP9 levels following IA training²⁰ probably results from increases in translation and transcription. The *Mmp9* promoter contains binding sites for the transcription factor nuclear factor-kappa B (NF-κB)⁸⁰, and IA training increases the

transcriptional activity of NF- κ B during the period over which MMP9 levels rise⁸¹. Other studies⁷⁹ suggest that *Mmp9* mRNA synthesis in neurons is subject to epigenetic regulation of the transcriptional repressor YY1 (Yin Yang 1), while under conditions of epileptogenesis, transcriptional regulation of mRNAs encoding MMP9 or tissue inhibitor of metalloproteinases 1 (TIMP1) may be controlled by the transcription factor activator protein 1 (AP1)^{82, 83}. At the translational level, recent studies implicate microRNA-dependent control of MMP9 levels⁸⁴. An important goal is to determine how these various transcriptional and translational mechanisms contribute to MMP-driven modulation of synaptic or behavioural plasticity.

It also remains to be determined precisely how focal MMP-mediated proteolysis is terminated following synaptic remodelling. TIMPs are likely to be key regulators of this termination step (Fig. 2), as *Timp1* mRNAs are rapidly upregulated following LTP induction or epileptogenesis in the hippocampus^{85, 86}. Importantly, viral-mediated TIMP1 overexpression in rat prefrontal cortex (PFC) abolishes the persistence but not the induction of LTP at subiculum→PFC synapses³². Behaviourally, the results of several studies, using a variety of approaches to interfere with or augment TIMP activity, are consistent with the idea that TIMPs are critical modulators of MMP activity that is necessary for performance of a variety of behaviours, including olfactory learning^{87, 88}, pre-pulse inhibition of the startle reflex⁸⁹, spatial navigation in a water maze and habituation in an open field⁹⁰.

Targets of MMP proteolysis underlying synaptic remodeling

Several molecules have been identified or suggested to be MMP targets through which MMP-mediated proteolysis contributes to synaptic remodelling.

The involvement of integrins as mediators of almost all aspects of MMP9-driven forms of synapse and spine plasticity discovered to date casts the spotlight on endogenous integrin ligands as MMP proteolytic targets that drive synaptic remodelling. In this context, recent studies show that intercellular adhesion molecule 5 (ICAM5; also known as telencephalin) may be an important MMP target. ICAM5 is a transmembrane protein that is a member of the immunoglobulin superfamily and binds to and activates integrins in the immune system⁹¹. In brain, ICAM5 localizes to dendrites and filopodia⁹², where it negatively regulates spine number and maturation during development⁹³. Recent studies show that LTP-inducing tetanic stimuli of CA1 synapses promotes MMP-dependent shedding of an amino-terminal ectodomain fragment of ICAM5 that is detectable at 15 min after LTP induction³⁶ (Fig. 2). Such cleavage can be mimicked by exposing cultured neurons to NMDA and can be blocked by the application of MMP inhibitors^{36, 94}, which is consistent with the idea that NMDARs are upstream of MMP activation as discussed above. Similarly, active forms of MMP3, MMP7 and MMP9 can promote ICAM5 ectodomain shedding in cultured neurons³⁶. Biochemical studies demonstrate that the cleaved ICAM5 fragment associates with β 1-integrins, and when applied to cultured neurons, stimulates β 1-integrin-dependent phosphorylation of cofilin⁹⁵.

Members of the cadherin family of cell adhesion molecules are also targets of MMP-mediated perisynaptic proteolysis. MMP-dependent ectodomain cleavage of neuronal- (N-) and epithelial- (E-) cadherin has been demonstrated in several cell types, including cultured

neurons⁹⁶, and may reflect the activity of MMP24²³. Nevertheless, it is not clear at present how MMP-mediated processing of synaptic cadherins would contribute to LTP and associated spine enlargement, since L-LTP-inducing stimuli promote N-cadherin synthesis and recruitment to synapses⁹⁷, where it has a vital function in stabilizing both LTP and spine enlargement in CA1 neurons⁹⁷⁻⁹⁹. One possibility is that such MMP-mediated proteolysis may affect only a subset of cadherin molecules, perhaps ones that are highly localized to a particular synaptic or perisynaptic domain.

Another activity-dependent target of MMPs that has been identified in neuronal culture assays is β -dystroglycan¹⁰⁰. This molecule is a transmembrane protein that, together with α -dystroglycan, couples the ECM to the actin cytoskeleton¹⁰¹. The onset of seizure activity in hippocampus increases the levels of a proteolytic fragment of β -dystroglycan, but this fragment is absent in *Mmp9*^{-/-} mice¹⁰⁰. These data suggest that such cleavage is MMP9-dependent, and occurs in an activity-dependent manner under pathophysiological conditions. Whether such MMP-directed proteolysis of β -dystroglycan contributes to synaptic remodelling in non-pathophysiological or other contexts has not been studied.

Several studies indicate that myelin-associated, axon growth-inhibitory molecules (that is, MAG (myelin-associated glycoprotein), Nogo66, and OMGP (oligodendrocyte myelin glycoprotein)) and one of their associated receptors, NGR1 (Nogo66 receptor 1), are important in gating the expression of ocular dominance plasticity in the visual cortex¹⁰², and LTP and spine plasticity in the hippocampus¹⁰³. Cell culture assays demonstrate that MAG can be cleaved by MMP2, MMP7 or MMP9, releasing bioactive fragments that inhibit axonal outgrowth from dorsal root ganglion neurons¹⁰⁴. Similarly, NGR1 can be cleaved by MMP14, MMP15, MMP16 or MMP24, which diminishes growth-cone collapse in response to myelin-associated inhibitors¹⁰⁵. Interestingly, the cleaved ectodomain fragment of NGR1 retains the ability to bind Nogo66, suggesting it can act as a dominant-negative entity under physiological conditions¹⁰⁵. These data suggest that MMP-mediated proteolytic regulation of this inhibitory signalling system directly influences the molecular cascade through which myelin-derived inhibitors and their receptors regulate experience-dependent synaptic plasticity.

Members of the family of insulin-like growth factors and their related receptors and binding proteins have recently been implicated in the molecular mechanisms underlying memory consolidation¹⁰⁶, and some of these are targets of MMP-mediated proteolysis. Levels of insulin-like growth factor 2 (IGF2) mRNA and protein are upregulated in rat hippocampus 20 hours following IA training¹⁰⁷. Antisense-mediated knockdown of IGF2 or its interacting IGF2 receptor (IGF2R) markedly impairs IA memory retention, whereas administration of exogenous IGF2 promotes persistent hippocampal LTP. In other systems, the bioavailability of IGF2 is regulated by sequestration through binding to insulin-like growth factor binding protein 2 (IGFBP2)¹⁰⁸. Studies show that the IGF2-IGFBP2 complex is proteolytically processed by MMP9¹⁰⁹, thereby releasing IGF2 for full bioactivity. Notably, MMP9 upregulation by IA training coincides with the period over which IGF2 is operative in memory consolidation, which raises the possibility that MMP9 critically regulates bioavailability of IGF2 as part of an orchestrated sequence of molecular mechanisms that consolidate and enhance memory.

Finally, in addition to candidate MMP targets described above, there is a growing list of additional molecules with documented roles in synapse development, plasticity and function that are also known targets of MMPs¹¹⁰. These include: tenascins; synaptogenic proteins such as the thrombospondins, hevin and SPARC; neurotrophins; tumor necrosis factor- α (TNF α); and ephrins and their eph receptors. Whether MMP-mediated processing of these molecules contributes to synaptic remodelling during LTP or in any other context remains to be rigorously tested.

MMP proteolysis in brain disorders

It is increasingly recognized that several neurological and psychiatric brain disorders have deficient or maladaptive synaptic functional and structural plasticity at their core^{111–115}. The growing recognition that fast, focal MMP-mediated perisynaptic proteolysis is a crucial driver of ongoing, adaptive synaptic remodelling, with direct consequences on behavioural plasticity, has established a new conceptual framework for linking MMP-mediated proteolysis to pathophysiological, maladaptive synaptic remodelling. Some recent studies that examine the relationship between MMP activity and potentially maladaptive synaptic remodelling capture these emerging links, and are briefly highlighted here.

Repeated exposure to the illicit psychostimulant methamphetamine is associated, in rodent models, with persistent abnormalities in the density and morphology of dendrites and spines¹¹⁶, and alterations in LTP¹¹⁷ in a number of forebrain structures. Protein and mRNA levels of MMP2, MMP9 and TIMP2 are elevated in rat prefrontal cortex, nucleus accumbens and hippocampus following repeated methamphetamine exposure^{95, 118}, whereas abrogating MMP expression or function blocks behavioural sensitization to methamphetamine¹¹⁹. Moreover, the reinstatement of cocaine-induced **conditioned place preference** (CPP) by cocaine-priming is associated with increased expression and activity of MMP9 in rat medial prefrontal cortex¹²⁰ and blocking such MMP9 activity impairs acquisition of cocaine-induced CPP or subsequent reinstatement of CPP upon cocaine-priming¹²¹. Mechanistically, acute methamphetamine-treatment *in vivo* stimulates cleavage of ICAM5 in striatum and hippocampus by ~6 hours post-treatment; such methamphetamine-induced cleavage is blocked by MMP inhibitors when it is recapitulated *in vitro*⁹⁵. Additionally, the expression and activity levels of β 1- and β 3-containing integrins — critical signalling mediators of MMP9 remodelling as well as of ICAM5 activity — are bidirectionally regulated by cocaine treatment, withdrawal and relapse in the nucleus accumbens¹²². Additionally, exposure to morphine drives aberrant MMP9 activity in neurons and glia in the spinal cord dorsal horn, whereas inhibiting MMP9 blocks morphine dependence⁷⁰. As persistent drug craving resembles dysfunctional synaptic plasticity processes that are involved in memory consolidation¹¹¹, these studies together suggest that drugs of abuse co-opt normal, ongoing and adaptive MMP-mediated synaptic plasticity by driving maladaptive MMP expression, circuit remodeling and addictive behaviour.

Neuropathic pain is also associated with aberrant synaptic circuit rewiring in the spinal cord¹¹³. Following ligation of the fifth lumbar spinal nerve — a standard rodent model for neuropathic pain — levels of MMP9 are rapidly but transiently elevated in injured dorsal root ganglion neurons, whereas upregulation of MMP2 occurs at later time points, which

suggests a role for these different MMPs in different phases of neuropathic pain¹²³. Consistent with this, suppression of MMP9 inhibits the initial phase of aberrant pain sensation, but suppression of MMP2 inhibits persistence. Intrathecal delivery of active MMP2 or MMP9 delivery causes behavioral changes that mimic behavioural manifestations of neuropathic pain. The MMP-driven effects may be mediated through proteolytic processing of the proinflammatory cytokine interleukin-1 β ¹²³.

Fragile X syndrome (FXS) is the most common inherited form of intellectual disability and autism¹²⁴, and is caused by a trinucleotide expansion that inactivates the fragile X mental retardation 1 gene (*FMR1*), leading to the loss of fragile X mental retardation protein (FMRP). FMRP is a postsynaptically localized RNA-binding protein that regulates local translation of a select subset of mRNAs at synapses¹²⁵. One abnormal structural hallmark of humans and mice lacking FMRP is an overabundance of long, thin, immature-appearing dendritic spines with corresponding defects in synaptic and experience-dependent plasticity¹²⁶. The spine structural abnormalities associated with FXS are very similar to those described above following global and prolonged MMP9 exposure in hippocampal cultures, which suggests that aberrant expression, localization and/or activity of MMP9 could contribute to spine and plasticity deficits in FXS. Indeed, recent studies show that levels of MMP9 as well as gelatinolytic activity are both elevated in hippocampal lysates from *Fmr1*^{-/-} mice in comparison with those from wild-type mice²⁴. Importantly, treatment of *Fmr1*^{-/-} mice with minocycline, a tetracycline derivative that — among many of its targets — blocks MMP9 activity¹²⁷, reduced levels of endogenous MMP9 activity, reversed the abnormal behaviours that these mice display and restored dendritic spines to normal morphologies²⁴. These findings have recently been largely validated in a *Drosophila melanogaster* model of FXS¹²⁸. Together, these data indicate that a strong correlation exists between aberrant MMP9 function and abnormal spine and circuit-level architecture in FXS, and potentially offers a realistic therapeutic approach to reversing dendritic spine, circuit and behavioural abnormalities that are driven by excessive MMP proteolysis.

Conclusions and perspective

MMPs can no longer be regarded solely as injury-related molecules in the mature nervous system. Multiple and compelling lines of evidence now show that MMPs are vital molecular drivers of synaptic structural and functional remodelling of the kind that supports ongoing cognitive functions.

One important area for future investigation is to determine precisely how MMP activity transitions from its normal, adaptive roles in synaptic circuit remodelling to its aberrant and deleterious roles that have important pathophysiological cellular and synaptic consequences. At its core, the transition in MMP-mediated cellular responses is probably dictated by the relative scale in amount, duration and spatial extent of MMP activity. In the case of disease or injury, this scale is potentially quite large (Fig. 3). Neurons, glia and a variety of invading inflammatory cell types all contribute to a massive elevation in MMP activity that spans days to weeks or longer, affects many cells and cell types over large areas, and permits broad access of MMPs to potential proteolytic targets that they may not normally encounter under non-pathophysiological conditions^{5, 129}. It will therefore be crucial in the future to define

precisely the regulatory mechanisms that govern focal release, localization, activation and termination of perisynaptic MMP activity, as these become overridden and dysregulated under conditions of injury or disease, leading to aberrant proteolytic processing of synaptic molecules such as NR1^{130, 131}. A thorough characterization of the regulatory mechanisms that govern MMP activity at the synapse will also serve the design of therapeutic strategies aiming to harness the adaptive roles of MMPs as molecular drivers of synaptic circuit remodelling to promote restoration and repair of damaged brain circuits, while at the same time diminishing the deleterious effects of these molecules that could impede recovery of synaptic function following injury¹³².

Another area of future research will be to see whether other metalloproteinases have functional roles in synaptic structural and behavioural plasticity. Pursuing this question is potentially daunting because of the sheer number of MMPs and other metalloproteinases and the relative lack of reliable reagents to detect them. However, one potential strategy to identify families of metalloproteinases whose proteolytic activity is regulated by plasticity-inducing stimuli is to use MMP active site-directed chemical probes¹³³ in a high-throughput proteomic screen for plasticity-regulated metalloproteinases, akin to how such probes have been used to identify and distinguish metalloproteinases that are active in certain cancers¹³⁴.

Relatedly, another limitation — both experimentally and therapeutically — is the lack of MMP-specific inhibitors. It is already known that different MMPs have distinct cell-type expression patterns, react differently to synaptic stimulation, affect synaptic structural and functional plasticity differently, and exert different pathophysiological effects, and so the need for advancing the development of MMP type-specific inhibitors is self-evident. The formidable obstacle to developing MMP-specific small-molecule antagonists of the catalytic site is the high structural homology across MMPs and other metzincins in this region. However, recent advances in next-generation small-molecule inhibitors have been described that target **exosites** or other allosteric regions¹³⁵, and these strategies may prove effective in overcoming the barriers to producing MMP-specific antagonists.

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Glossary

Metzincin

a clan of metalloproteases comprising the matrix metalloproteinases (MMPs), the ADAMs (a disintegrin and metalloproteinase) and ADAMs-TSs (ADAMs with thrombospondin repeats). It is so-named by an amalgam of two structural hallmarks of the active-site region: a conserved methionine-containing turn (Met-turn) downstream of—and positioned underneath to stabilize—a conserved zinc-binding motif (HExxHxxGxxH), in

	which the three histidine residues are zinc-binding ligands within the catalytic site.
Dendritic spines	small actin-rich dendritic protrusions that harbour most of the excitatory glutamatergic synapses.
Gelatinases	any proteolytic enzyme capable of cleaving gelatin (denatured collagen). In the MMP lexicon, the gelatinases are matrix metalloproteinase 2 (MMP2) (gelatinase A) and MMP9 (gelatinase B), as gelatin is a canonical substrate for these MMPs.
Integrins	heterodimers composed of an α - and a β -subunit. In mammals, there are eighteen α - and eight β -subunits. They are canonical receptors of extracellular matrix and other proteins. In hippocampus, most integrin heterodimers contain the β -subunit.
Cofilin	an actin binding protein that is enriched in dendritic spines. Cofilin is a member of the ADF (actin-depolymerizing factor)/cofilin family, and regulates the disassembly of filamentous actin. Cofilin is negatively regulated by phosphorylation at a single site (Ser3).
SNARE	soluble NSF (<i>N</i> -ethylmaleimide-sensitive factor) attachment protein (SNAP) receptors. A family of proteins comprising vesicular (v)-snares and target (t)-snares that mediate intracellular membrane fusion.
mEPSCs	miniature excitatory postsynaptic currents. These are the postsynaptic responses to a quantum of excitatory neurotransmitter (usually glutamate).
Conditioned place preference	a behavioural task used to evaluate an animal's preference for stimuli or an environment associated with positive or negative reward.
Exosites	a secondary substrate-binding site on an enzyme that is distinct from the catalytic active site. Exosites are often important for positioning substrates for full proteolytic cleavage.

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Biography

George W. Huntley is Associate Professor of Neuroscience and a member of the Friedman Brain Institute at the Mount Sinai School of Medicine, New York, USA. His research focuses on development and plasticity of cortical connectivity, the role of adhesion proteins in synaptic function, and extracellular proteolytic remodelling of synaptic circuits.

Online summary

- Matrix metalloproteinases (MMPs) are a large family of mostly secreted, extracellularly acting proteolytic enzymes. In brain, they have well described roles in slowly emerging, but long-lasting pathophysiological processes of cell loss and synaptic dysfunction associated with acute injury, ischaemia, neurodegeneration, and demyelination.
- Remodelling of synapse structure and function also underlies normal cognitive processes, such as learning and memory. This Review focuses on recent studies that indicate that MMPs have important roles in driving such synapse plasticity under non-pathological conditions that is distinct from their roles in neuropathophysiology.
- MMPs are secreted as inactive pro-enzymes (zymogens). Under basal conditions, a large pool of mostly pro-MMPs are situated perisynaptically, poised for activation by plasticity-inducing stimuli such as long-term potentiation (LTP).
- Upon induction of LTP, but not other forms of short- or long-lasting plasticity, pro-MMPs are rapidly (within ~15 min) converted to proteolytically active MMPs through an NMDAR-dependent mechanism. Such proteolytically active MMPs then signal through β 1-containing integrins to promote dendritic spine enlargement and synaptic potentiation concurrently.
- Intercellular adhesion molecule 5 (ICAM5), which binds to and activates integrins, may be a direct target of perisynaptic MMP proteolysis during LTP. LTP-associated MMP proteolysis is probably then terminated by an increase in the activity of endogenous inhibitors called tissue inhibitors of metalloproteinases (TIMPs).
- When MMP activity is blocked pharmacologically or genetically, LTP, spine enlargement and behavioral measures of cognitive function are all impaired.
- Several psychiatric and neurological disorders, including drug addiction, neuropathic pain syndromes, and Fragile X mental retardation syndrome, are associated with abnormal or deficient synaptic plasticity. Recent studies indicate that aberrant MMP expression, localization and function may contribute to synaptic plasticity deficits associated with such disorders.
- A key area for future research is to elucidate how MMP activity transitions from normal, adaptive roles in local synaptic remodelling to deleterious roles that have important pathophysiological cellular and synaptic consequences. This transition likely involves abnormal regulatory mechanisms leading to excessive, prolonged, and widespread MMP activity.

FIGURE 1. MMP9 localization and activity in hippocampus before and after LTP

a Under basal conditions in adult hippocampal area CA1, numerous matrix metalloproteinase 9 (MMP9) immunofluorescent puncta are present within the synaptic neuropil of stratum radiatum (**a**). Many such puncta codistribute with labelling for other synaptic molecular markers, for example, vGluTs (vesicular glutamate transporters), which is a marker of excitatory presynaptic terminals (insets), indicating perisynaptic localization. Much of this perisynaptic immunoreactivity is probably the inactive pro-form, as there are few gelatinolytic puncta (hotspots of proteolytic activity) present under control conditions (**b**). Following induction of long-term potentiation (LTP) (**c**, image taken 75 minutes after LTP induction), there is a significant increase in numbers of gelatinolytic puncta. In the absence of LTP because of prior administration of the NMDA receptor (NMDAR) antagonist 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), there is no increase in proteolysis (**d**), thus, MMP activation is related specifically to LTP and is not a non-specific effect of trains of electrical stimuli. At higher-power, many of the gelatinolytic puncta (**e**, gel'lysis) codistribute with MMP9 immunoreactivity (**f,g**, arrows), which consistent with the activation of MMP9 with LTP induction. Images B–G are adapted from REF ¹⁸. Bars represent 50 μ m (A); 10 μ m (B–D); 3 μ m (E–G).

FIGURE 2. A model of MMP9 activity in driving synaptic structural and functional remodelling Under basal conditions, mostly pro-forms of matrix metalloproteinase 9 (MMP9) are localized perisynaptically in CA1. Upon LTP induction, postsynaptic NMDA-type glutamate receptor (NMDAR) activity triggers rapid and local nitric oxide (NO) release into the perisynaptic microenvironment, which in turn converts pro-MMP9 to proteolytically active MMP9. Once proteolytically active, MMP9 cleaves perisynaptic intracellular adhesion molecule 5 (ICAM5). The ICAM5 ectodomain then binds to and activates postsynaptic β 1-containing integrins, which in turn triggers actin polymerization in part through phosphorylation of cofilin. Actin polymerization is the basis for an integrin-mediated consolidation process of spine structural enlargement as well as AMPA receptor (AMPA) trafficking into the synaptic membrane. New pro-MMP9 synthesis and release by pre- and postsynaptic neurons and/or perisynaptic astrocytes replenishes the extracellular pool of pro-MMP9. In time, tissue inhibitors of metalloproteinases (TIMPs) are released to terminate MMP9 activity.

FIGURE 3. Transition of MMP activity to pathophysiological processes

a | Both experimentally induced¹⁵⁵ and behaviorally induced⁶³ forms of synaptic plasticity affect a minority of synapses that are widely distributed. Thus, during long-term potentiation LTP, matrix metalloproteinases (MMPs) are activated locally at a minority of synapses distributed widely throughout the neuropil. Within ~15 min, active MMP9 coordinately drives dendritic spine enlargement (**arrow**) and potentiation as part of an integrin-dependent consolidation process. After 1–2 hours, MMP activity is terminated, most likely by increased tissue inhibitor of metalloproteinase (TIMP) activity.

b | By contrast, under pathophysiological conditions, ischaemic or inflammatory disruption of the blood–brain barrier (BBB) results initially from aberrant expression of a number of MMPs within the neurovascular unit. Such MMP-mediated activity leads to subsequent invasion into the brain parenchyma of inflammatory and other cell types, which in turn triggers further aberrant MMP expression and secretion by astrocytes, neurons and microglia. Under these conditions, MMPs are activated by each other and by free radicals such as nitric oxide. Such excessive and active MMPs then infiltrate networks of neurons and their synapses. Broad and sustained MMP exposure at all or most synapses of a network lead to conversion of dendritic spines to immature-like filopodia (**inset**), followed by synapse breakdown and, eventually, neuronal apoptosis.