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Time dependent integration of matrix metalloproteinases and their targeted substrates directs axonal sprouting and synaptogenesis following central nervous system injury

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Abstract

Over the past two decades, many investigators have reported how extracellular matrix molecules act to regulate neuroplasticity. The majority of these studies involve proteins which are targets of matrix metalloproteinases. Importantly, these enzyme/substrate interactions can regulate degenerative and regenerative phases of synaptic plasticity, directing axonal and dendritic reorganization after brain insult. The present review first summarizes literature support for the prominent role of matrix metalloproteinases during neuroregeneration, followed by a discussion of data contrasting adaptive and maladaptive neuroplasticity that reveals time-dependent metalloproteinase/substrate regulation of postinjury synaptic recovery. The potential for these enzymes to serve as therapeutic targets for enhanced neuroplasticity after brain injury is illustrated with experiments demonstrating that metalloproteinase inhibitors can alter adaptive and maladaptive outcome. Finally, the complexity of metalloproteinase role in reactive synaptogenesis is revealed in new studies showing how these enzymes interact with immune molecules to mediate cellular response in the local regenerative environment, and are regulated by novel binding partners in the brain extracellular matrix. Together, these different examples show the complexity with which metalloproteinases are integrated into the process of neuroregeneration, and point to a promising new angle for future studies exploring how to facilitate brain plasticity.

Key Words: neuroregeneration; reactive synaptogenesis; matrix metalloproteinases; brain injury; adaptive and maladaptive neuroplasticity; metalloproteinase inhibition; osteopontin; lipocalin 2

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Introduction

While major central nervous system (CNS) and peripheral nervous system (PNS) fiber tracts have been principal models for defining the role of extracellular matrix (ECM) proteins in axonal growth and regeneration (Busch and Silver, 2007; Pizzi and Crowe, 2007; Gonzalez-Perez et al., 2013), these matrix molecules also mediate synapse formation (Ethell and Ethell, 2007). The success of axonal regeneration depends, ultimately, upon formation of functional connections between axon terminals and their postsynaptic sites. Such connections occur in an environment which includes neuroglia and vascular elements, all surrounded by a host of matrix molecules. Among the more important matrix proteins are those that provide structural scaffolding, such as collagens, fibronectins and proteoglycans (Hubert et al., 2009; Andrews et al., 2012; Howell and Gottschall, 2012). Networks of these proteins give shape to the synaptic environment, reorganize in response to CNS insult, and affect the distribution of immune signaling molecules like cytokines, chemokines, and secreted growth factors, each of which can direct different cellular phases of regeneration. The successful distribution of these matrix proteins during synaptogenesis depends upon activation of secreted and membrane bound matrix metalloproteinases (MMPs). These Zn^{2+}/Ca^{2+} activated enzymes are members of a highly homologous protease family, tightly regulated under normal conditions (Handsley et al., 2005). Upon stimulus of CNS injury or disease, their proteolytic activity is turned on, often to very high levels. They can then target principal ECM proteins associated with the synapse, affecting structural integrity of the surrounding extracellular space, distribution of ECM bound proteins, and cleaving membrane associated proteins which stabilize intact synapses to permit the reshaping required for plasticity (Ethell and Ethell, 2007). As the process of synapse regeneration develops, the same enzymes may selectively breakdown ECM proteins in order to facilitate stabilization of favored new synaptic connections (Reiss et al., 2005; Warren et al., 2012).

Metalloproteinases mediate plasticity in both development and CNS disease

The role of MMPs in CNS synaptic development and remodeling is complex, involving a variety of enzyme family members and targeted substrates (Ethell and Ethell, 2007; Van Hove et al., 2012; Verslegers et al., 2013). A number of published studies support elevated MMP activity during synapse organization in a variety of CNS models (Van Hove et al., 2012; Verslegers et al., 2013). The time dependent profiling of MMP response has been demonstrated in each of the established phases of synaptogenesis. These include the acute ECM reorganization around affected synapses, subsequent axonal sprouting and new synapse formation, and the period of nascent synapse stabilization. In normal CNS development, secreted MMPs-2, -3 and -9, along with membrane type MT5 and MT1-MMPs, act to mediate axonal growth, dendritogenesis and synaptogenesis. For example, MMPs-2, -3 and -9 appear to regulate the extension of granule neuron processes as they migrate through the cerebellar ECM (Vaillant et al., 2003; Luo, 2005). Other studies provide evidence that Purkinje cell MT5-MMP expression directs axonal growth in nascent synapses and prescribe the layout of dendritic cytoarchitecture for the complex synaptic morphology of these principal cerebellar neurons (Hayashita-Kinoh et al., 2001; Sekine-Aizawa et al., 2001). Notably, animals in which MMPs-2 and -9 expression is deficient or knocked out show abnormal cerebellar synaptic formation and functional motor deficits (Van Hove et al., 2012).

The interaction between ECM proteins and their regulatory MMPs is similarly correlated with successful axonal growth and synaptic organization in the developing neocortex, where MMP-2 and MMP-3 are each localized at growing dendrite tips and associated with semaphorin growth promotion activity (Gonthier et al., 2007; Gonthier et al., 2009). Both MMPs-2 and -9 are linked to cleavage of neuroadhesion molecule ICAM-5 (Tian et al., 2007), as well as to ephrins, hippocampal ECM axon guidance proteins, which can be regulated through MMP-9 cleavage of their Eph-2B receptor (Pizzi and Crowe, 2007). As in the cerebellum, hippocampal MT5-MMP is also localized to growing axon and dendritic filipodia during synapse development (Monea et al., 2006). MMP role in mediating synaptic organization extends to developing sensory systems as well. For example, in the olfactory pathway, a disintegrin and metalloproteinase 21 (ADAM-21) MMP is found in growing axonal tracts, often co-localized with olfactory marker protein, indicating its role in the maturation of olfactory sensory neuron projections (Yang et al., 2005). Likewise, a rise in MMP-2 protein level was correlated with increased growth associated protein 43 (GAP43) expression during olfactory epithelial process maturation (Tsukatani et al., 2003). As olfactory bulb glomeruli develop, many other MMP substrates (*e.g.*, laminin-1, perlecan, CSPG, tenascin) act to restrict and guide afferent axons during synaptogenesis (Treolar et al., 2009).

In another context, MMP response is documented to accompany many types of CNS neurodegenerative disease, each condition offering potential for restorative repair. With Alzheimer's disease (AD), elevated MMP-9 expression occurs at sites of neurofibrillary tangle and senile plaque accumulation (Asahina et al., 2001). Differential MMP response may be present in the aging brain as well, where MMP-2 activity is actually reduced by one third in AD and Huntington's disease (Safciuc et al., 2007). Elevating amyloid precursor protein (APP) proteolysis by increased ADAM-10 alpha secretase expression/activity offers potential for stabilizing neuronal connections in both diseases (Endres and Fahrenholz, 2010).

Plasticity following stroke and epilepsy is also linked with altered MMP expression. Conditions of stroke and ischemia were some of the first to be associated with elevated MMP response (Rosenberg and Navratil, 1997; Asahi et al., 2000). After stroke, compromise of the blood brain barrier has been attributed, in part, to the elevated activity of MMPs-2, -3 and -9, reducing the integrity of the vascular interface and exacerbating hemorrhage (Montaner et al., 2001). Clinical interventions have been built upon targeting this MMP activity, damping down its effect on the weakened neurovascular unit to attenuate further damage from bleeding (Lo et al., 2004). However, MMP association with the pathophysiology of stroke is complex and, more recently, a positive role for MMP-2 in cortical recovery ipsilateral to the stroke locus was posited (Liu et al., 2011). Using DWI mapping, these researchers provide proof that restorative amphetamine treatment is correlated with ipsilateral axonal integrity, as well as the elevation of synaptic proteins, brain derived neurotrophic factor (BDNF) and MMP-2 activity. This MMP function during stroke induced synaptogenesis expands our understanding of how important matrix enzymes are in the overall context of circuit reorganization after injury.

A hallmark of epilepsy is its generation of hippocampal seizure activity, mediated, in part, by abnormal recurrent mossy fiber sprouting and synaptogenesis in the dentate gyrus (Ziemianska et al., 2012). Interestingly, protein level and enzyme activity for MMP-9 are elevated in both the kainic acid (KA) and pentylenetetrazole induced rodent models of seizure (Wilczynski et al., 2008), with MMP-9 knockout resulting in the attenuation of evoked seizures and aberrant mossy fiber sprouting. One mechanism for MMP-9 mediation of such aberrant synaptogenesis is the enzyme's capacity to convert pro-BDNF to its mature growth factor form, facilitating recurrent axonal sprouting into the dentate (Mizoguchi et al., 2011). At least three other MMPs have been linked to neuronal survival and synapse stabilization after seizure induction. MMP-3 induced by KA seizure can be attenuated under conditions of metallothionein over expression, a model which rescues neurons from decay after epileptic neuroexcitation (Penkowa et al., 2005). By contrast, elevated matrilysin, MMP-7, is postulated to offer neuroprotection through its cleavage of pro-NGF at sites of KA induced seizure, preventing pro-growth factor p75 receptor signaling of apoptosis (Le and Friedman, 2012). Further, membrane type MMP ADAM-10 exhibits a complex molecular interaction affecting neural survival and synaptic function after seizures (Clement et al., 2008). Using conditional ADAM-10 expressing mice, Clement and colleagues demonstrated pronounced differences in outcome depending on enzyme level, indicat-

ing that benefits of APP secretase cleavage may be offset by the action of ADAM-10 derived cleavage products, which exacerbate seizure activity. Interestingly, more recent studies support the potential for genetic screening of ADAM-22 mutations as a tool for predicting vulnerability to seizures or demyelinating disease (Sagane et al., 2010).

Finally, it is important to point out that MMPs are often produced by the various resident glia within the nervous system. In the context of immune response, microglia can synthesize and release a variety of MMPs capable of lysing ECM proteins to signal cytokine recruitment (Peng et al., 2012), and can drive phagocytic activity which precedes synaptic regeneration (Shin et al., 2005). Astrocytes often produce MMPs-2, -3 at local sites of synapse reorganization, presumably facilitating their hypertrophy and migration to sites of synaptogenesis (Falo et al., 2006; Ogier et al., 2006). MMP-9 is also produced by oligodendrocytes, and released at the tips of their growing processes in order to control their migration through the local ECM to reach specific targets (Oh et al., 1999), as well as identify axonal surfaces for myelination (Uhm et al., 1998). It is this relationship between MMP-9 and myelin formation which has lead to the hypothesis that MMP dysfunction plays a role in the etiology of multiple sclerosis (Yong et al., 2007) and other white matter CNS pathologies (Belien et al, 1999; Asahi et al., 2000; Noble et al., 2002). Overall, a wide range of published evidence highly supports MMP family members as critical regulators of neuroplasticity in CNS development, maturation and identified diseased states.

Metalloproteinase response after traumatic injury

Assigning a regulatory function for MMPs during neuroplasticity induced by CNS injury is equally justified. Both spinal cord injury (SCI) and traumatic brain injury (TBI) induce profound changes in the transcription and translation of several MMPs (Rosenberg, 1995; Zhang et al., 2010). Many of the substrate targets of these MMPs are the same as those affecting axonal integrity in AD and epilepsy. Moreover, common MMPs mediating hemorrhagic stroke may also contribute to the vascular pathology of SCI and TBI. This suggests that MMP role in the context of postinjury plasticity is likely to be similar to that of neurodegenerative disease. For several years, we have investigated MMP role in the synaptogenic response to TBI. Our results show that at least five MMP family members (MMPs-2, -3,-9; MT-5 MMP; ADAM-10) and four of their targeted substrates (agrin, phosphacan, N-cadherin, osteopontin) contribute to successful reactive synaptogenesis in the injured hippocampus (Phillips and Reeves, 2001; Kim et al., 2005; Falo et al., 2006; Warren et al., 2012; Chan et al., in review). We have also identified specific matrix enzyme/substrate pairs which we hypothesize affect different phases of the recovery process. Published studies of MMP response in a wide range of CNS lesion models support this hypothesis.

In both cortical stab wound (Kyrkanides et al., 2001) and hippocampal neuroexcitatory insult (Szklarczyk et al., 2002), MMP-9 was found to be elevated during periods of dendritic remodeling and plasticity. Different MMP/substrate pairs have been identified by other investigators as players in hippocampal synaptic reorganization after entorhinal deafferentation, including MMP-3 and several proteoglycan family members (Deller et al., 2001), as well as ADAM-TS and brevican (Mayer et al., 2005). This enzyme/substrate interaction is considered critical for promoting neurite outgrowth and creating an open, accessible environment for reconnection (Muir et al., 2002). When the cerebellum is challenged to repair after either excitotoxic kainic acid lesion (Zhang et al., 1998) or collagenase induced hemorrhage (Lekic et al., 2011), gelatinases MMPs-2 and -9 are elevated within the first 24 hours postinjury. Similarly, sensory synaptic remodeling evokes matrix molecules for control of axonal and dendritic growth. For example, when unilateral cochlear ablation drives synaptogenesis within the cochlear nucleus, expression of MMP-2 coincides with the emergence of GAP-43 containing presynaptic terminals (Fredrich and Illing, 2010). Sensory deprivation in the rodent barrel cortex also causes collateral sprouting of spared fibers, a process which is attenuated in MMP-9 deficient mice (Kaliszewska et al., 2012). Further, the transection of either visual or olfactory tracts can induce gelatinase expression over the time course of reactive synaptogenesis (Costanzo et al., 2006; Costanzo and Perrino, 2008; Penedo et al., 2009), a response which can also be induced by the more focal methyl bromine gas lesion of olfactory neuroepithelium, selectively targeting sensory receptor neurons (Bakos et al., 2010).

Lastly, MMP activation following traumatic injury to the CNS illustrates a major role for these ECM enzymes in both neural pathophysiology and synaptic recovery (Rosenberg, 1995; Zhang et al., 2010). In humans, injured spinal cord and brain each induce a rapid rise in MMPs (Buss et al., 2007; Vilalta et al., 2008; Grossetete et al., 2009; Copin et al., 2012), which is coupled with acute immune cell activation (Ziebell and Morganti-Kossmann, 2010). This pattern of MMP response is reproducible in rodent models of SCI (de Castro et al., 2000; Goussev et al., 2003), where increased enzyme activity is associated with neutrophil infiltration and neovascularization. Outcome may be either positive or negative, depending upon MMP type and postinjury time frame. For example, acute pharmacological inhibition of MMP-9 reduces SCI pathology and astrocytic glial scarring (Hsu et al., 2008; Zhang et al., 2011), while blockade of MMP-2 interferes with subsequent wound healing by increasing the size of the glial scar and extent of white matter damage (Hsu et al., 2006). Such results suggest that the two gelatinases have different, perhaps opposing, effects on neurite outgrowth through the extracellular space after injury.

In the case of TBI, a more direct link between MMPs and different aspects of synaptic plasticity has been reported. MMPs-2, -3 and -9 are also elevated acutely (Kim et al., 2005; Falo et al., 2006; Ding et al., 2009; Li et al., 2009) and, as for SCI, beneficial effects of MMP inhibition have been reported, with both reduced presynaptic terminal loss (Ding et al., 2009), and edema (Higashida et al., 2011). MMP-9 knockout conditions were also shown to be neuroprotective after TBI (Wang et al., 2000). Interestingly, neuroprotective

hypothermia differentially affects gelatinase response in TBI, attenuating MMP-9, but having no effect on MMP-2 (Truettner et al., 2005). Our studies with targeted hippocampal deafferentation have provided further evidence of MMP role in TBI neuroplasticity, showing that time dependent inhibition of MMP activity can shift the synaptogenic outcome between adaptive and maladaptive patterns (Reeves et al., 2003; Falo et al., 2006; Warren et al., 2012). This attenuation of enzyme activity appears to be associated with either the disruption or restoration of MMP substrates which can support axonal sprouting and stabilize synapse cytoarchitecture. For several decades it has been known that MMP substrates, most prominently proteoglycans, can attenuate the success of axonal regrowth and synaptic recovery (Snow et al., 1991; McKeon et al., 1995). However, it is becoming clear that optimal regenerative output depends upon a critical balance between 'good' and 'bad' levels of protein expression (Grimpe and Silver, 2002; Jones et al., 2003). More recently several of these ECM proteins have been examined after TBI, showing both region and time dependent expression, and suggesting that, while they can inhibit recovery, they may also act to support neuroplasticity (Falo et al., 2008; Harris et al., 2009; Harris et al., 2011; Warren et al., 2012; Yi et al., 2012). MMP family members not only target ECM proteoglycans after injury (Pizzi and Crowe, 2007), affecting axonal pathfinding and perineuronal net segregation of synaptic contacts, but MMPs-3 and -7 are reported to cleave membrane bound NMDA receptor complexes (Pauly et al., 2008; Szklarczyk et al., 2008), altering synaptic structure (Szepesi et al., 2013) and signaling properties (Szklarczyk et al., 2008). Other studies show that NMDA receptor induced cleavage of adhesion protein ICAM-5 to promote dendrite remodeling is mediated through MMP activity (Tian et al., 2007). Interestingly, we have reported a link between NMDA receptor activity and MMP-3/substrate interaction during hippocampal reactive synaptogenesis, where the NMDA antagonist MK-801 attenuates upregulation of MMP-3 transcript under conditions of maladaptive plasticity (Falo et al., 2008). Thus, the field of neural regeneration research has generated considerable evidence suggesting that the interaction between MMPs and their extracellular substrates is critical to the neural reorganization of synaptic structure lost after brain injury.

Importance of ECM and axonal regeneration at the synapse

Collectively, our findings and those of others, point to principal roles for stromelysins (MMPs-3, -7), gelatinases (MMPs-2, -9), membrane type metalloproteinases (MT-1, MT-5 MMP, ADAM family members), and their targeted substrates in synaptic reconstruction. These matrix enzymes are integral to the evolution of synaptic plasticity after a wide range of CNS injuries. It is our thesis that their time dependent response during TBI induced synaptic reconstruction will determine the success or failure of the process. In the following sections, we will present our results from experiments which probed paired MMP and identified ECM substrates active during different phases of synaptic reorganization, and provided evidence that regeneration can be positively and negatively affected by MMP activity. We have taken the approach of contrasting rodent TBI models which generate either adaptive or maladaptive synaptic plasticity, with the goal of dissecting differences in MMP response as possible targets for altering structural and functional outcome (Phillips and Reeves, 2001). The first set of data summarizes our published findings which link certain MMPs and ECM substrates to the different phases of postinjury plasticity (Kim et al., 2005; Falo et al., 2006; Falo et al., 2008; Harris et al., 2011; Warren et al., 2012). To test the feasibility of targeting these molecules for improved recovery, we have inhibited MMP activity pharmacologically at different postinjury intervals and examined the effect on outcome measures which reflect synaptic integrity (Reeves et al., 2003; Kim et al., 2005; Falo et al., 2006; Warren et al., 2012). In the second data summary, we will provide key examples of how such MMP inhibition alters the profile of synaptogenesis. Future studies will no doubt identify new molecular mechanisms affecting MMP response during synaptic remodeling which will add to these observations. We will conclude with two examples from our current research, described here for the first time, linking MMPs to novel immune signaling mechanisms activated with TBI induced synaptogenesis, and second, showing how a unique MMP interaction with ECM carrier proteins has the potential to influence nascent synapse stabilization. Together, this evidence supports the importance of understanding the role of MMPs in neural regeneration and, along with the other reviews in this journal issue, will illustrate how ECM molecules integrate with cellular response to determine outcome after CNS injury.

Benefit of contrasting TBI models of adaptive and maladaptive synaptic regeneration

Given that human TBI generates profound, persistent cognitive deficits, and that circuitry disruption in the hippocampus may contribute to these deficits (Povlishock and Katz, 2005), we have modeled reactive synaptogenesis in the rodent using two deafferentation paradigms, one which induces adaptive synaptic plasticity, the other producing a maladaptive version of the response. The entorhinal cortical lesion delivered unilaterally (UEC) generates targeted deafferentation of the hippocampal dentate gyrus, inducing reactive synaptogenesis with structural and functional recovery over a period of two weeks postinjury (Steward, 1989). In the acute postinjury period (0–4 days), the deafferented dendritic zone (outer 2/3 of the dentate molecular layer) undergoes terminal degeneration, glial activation, dendritic retraction and spine atrophy. At 6–7 days, the remaining intact axon terminals sprout collateral processes which grow through the ECM to identify appropriate postsynaptic targets on reemerging dendritic spines. This exponential growth generates nascent synapses in the deafferented zone, which are further refined through pruning and stabilization at the 15 day period and beyond. By contrast, when acute neuroexcitatory insult (in our case produced by fluid percussion TBI) is paired with bilateral entorhinal lesion (TBI + BEC), these time dependent phases of the synaptogenic

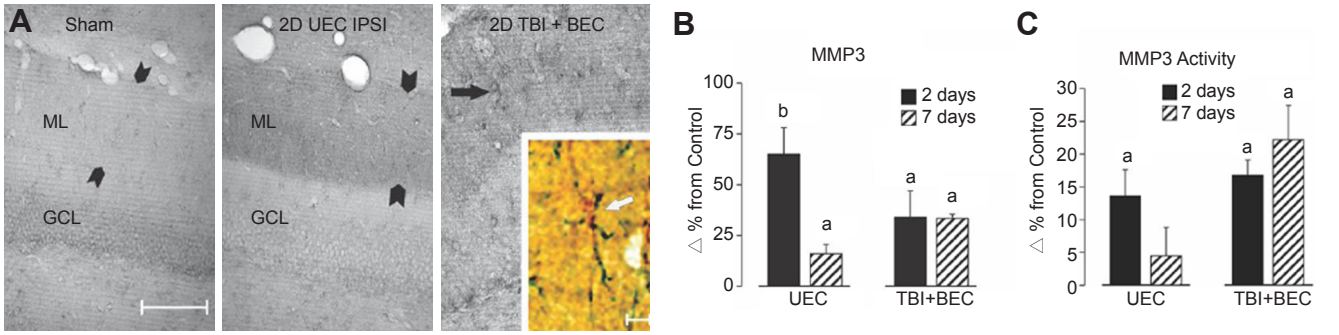

Figure 1 Hippocampal MMP-3 expression increases during the acute degenerative phase of reactive synaptogenesis.
Comparison of adaptive (UEC) and maladaptive (TBI+BEC) models showed differences in MMP-3 expression and distribution (A). With poor recovery, high MMP-3 expression was seen in reactive astrocytes of the deafferented zone (inset). Tissue assays revealed a persistent postinjury elevation of both MMP-3 protein and enzyme activity within the maladaptive condition (B, C). [a]$P < 0.05$, [b]$P < 0.001$, *vs.* paired controls; bar = 100 μm in A, 10 μm in inset. Panel (A) from Falo et al. (2006), with permission. MMP: Matrix metalloproteinase; MT-5: membrane type; TBI: traumatic brain injury; UEC: unilateral entorhinal lesion; BEC: bilateral entorhinal lesion.

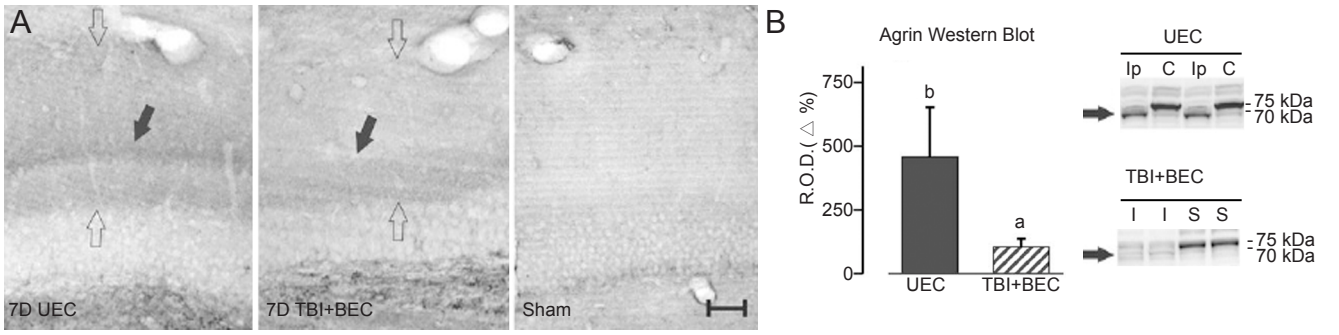

Figure 2 Assessment of hippocampal synaptic MMP-3 substrate agrin.
Results revealed that the intensity and positioning of this matrix boundary protein was altered in the maladaptive model (A). Analysis of agrin protein expression revealed reduction in agrin fragment peptides with TBI + BEC (B). [a]$P < 0.05$, [b]$P < 0.001$, *vs.* paired controls; bar = 100 μm. From Falo et al. (2008), with permission. MMP: Matrix metalloproteinase; TBI: traumatic brain injury; UEC: unilateral entorhinal lesion; BEC: bilateral entorhinal lesion.

process fail to proceed normally. Acute clearance of degenerating presynaptic terminals is attenuated, sprouting is delayed and the newly generated synapses show an underdeveloped profile. This model results in maladaptive plasticity and poor outcome, mimicking the deficits observed with human brain injury (Phillips et al., 1994; Reeves et al., 1997; Phillips and Reeves, 2001), and providing a paradigm to test how the combined pathologies of TBI affect structural synaptic reorganization. Our approach has been to first generate a temporal profile of MMP expression and activity in these two models, along with the expression map of selected substrate targets relevant to synaptic organization. A direct comparison of the two models allowed the identification of differences which correlate with the shift from adaptive to maladaptive synaptic reorganization. These studies revealed specific MMPs and ECM substrates most closely associated with each of the three different phases of reactive synaptogenesis, as well as distinct changes in expression profile for the enzyme/substrate pairs between adaptive and maladaptive plasticity. Further, when separate cohorts of each model were subjected to injury and time dependent MMP inhibition, a comparison of outcome between the two insults revealed an MMP mediation of successful recovery. In the following sections we describe how the study of two TBI models with different synaptogenic outcome can be used to identify time dependent roles for different MMPs during reactive neuroplasticity. Importantly, we demonstrate that MMP manipulation, depending upon model pathology, can have either positive or negative effects on outcome.

Time dependent MMP and ECM substrate interaction with synaptogenesis

While several studies do show differences in MMP expression during adult synaptogenesis (Van Hove et al., 2012; Verslegers et al., 2013), few have directly assessed these expression differences relative to the specific phases of remodeling synaptic structure after injury (Szklarczyk et al., 2002; Mayer et al., 2005; Falo et al., 2006; Warren et al., 2012). The principal approach has been to map individual MMP response after CNS insult, most prominently under conditions of stroke (Asahi et al., 2000), spinal cord injury (Hsu et al., 2006; Hsu et al., 2008) and TBI (Grossetete et al., 2009; Li et al., 2009; Jia et al., 2010). Overall, postinjury increases in MMP expression and activity were hypothesized to cause poor outcome, and thus targeted with pharmacological inhibitors to attenuate enzyme proteolysis and improve recovery (Asahi et al., 2000; Hsu et al., 2008; Zhang et al., 2011).

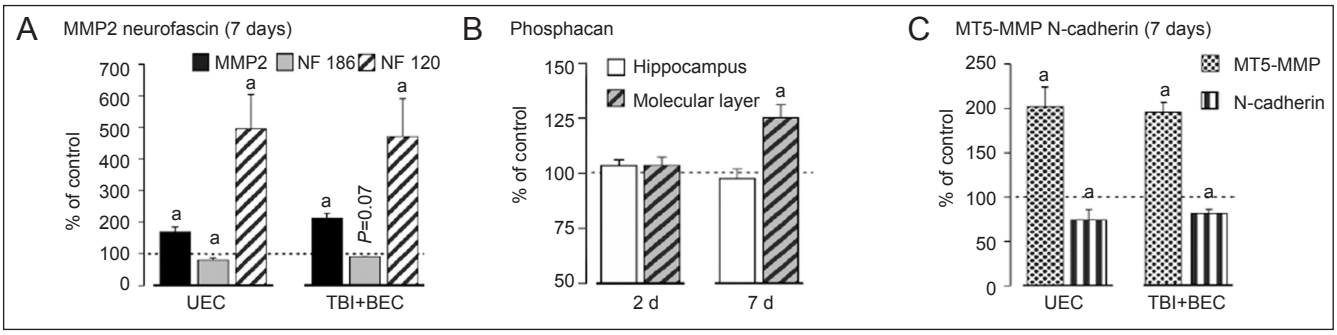

Figure 3 Hippocampal MMP level correlates with expression of proteins directing the early regenerative phase of synaptic reorganization.
Sustained increase in MMP-2 7 days postinjury paralleled production of neurofascin fragment (NF120), supportive of axonal growth (A). By contrast, 7 day MMP-3 normalization was linked to elevation of its substrate phosphacan within the zone of collateral axonal sprouting (molecular layer in B). MT5-MMP, whose membrane localization apposes its target N-cadherin, remained elevated at 7 days, likely cleaving N-cadherin to permit flexible synaptic junctions during reinnervation (C). Unlike MMP-3, both MMP-2 and MT5-MMP showed similar temporal expression in adaptive (UEC) and maladaptive (TBI+BEC) models. [a]$P < 0.05$, *vs.* paired controls. Panel (B) from Harris et al. (2011), with permission. MMP: Matrix metalloproteinase; MT-5: membrane type-5; TBI: traumatic brain injury; UEC: unilateral entorhinal lesion; BEC: bilateral entorhinal lesion.

Other studies have employed specific MMP knockout mice to reduce postinjury MMP activity and reverse pathophysiology (Asahi et al., 2000; Wang et al., 2000; Hsu et al., 2008). To our knowledge, adaptive and maladaptive plasticity conditions had not been compared in this context, nor had specific enzyme/substrate interaction been profiled for each phase of structural synaptic repair.

We began by investigating the role of individual MMPs in synaptogenesis, contrasting our two models with different repair efficacy (UEC *vs.* TBI + BEC). Our results have identified specific MMP/substrate pairs which influence different phases of this process. In the acute 2 day postinjury phase (Figure 1A), we showed that elevated glial MMP-3 expression and activity was correlated with the period of degenerating axon terminal removal (Falo et al., 2006). Notably, when this MMP-3 response persisted into the initial regenerative phase at 7 days, the resulting synaptic plasticity was maladaptive. These persistently higher levels of MMP-3 were associated with the loss of agrin boundary formation at the sites of new synapse formation within the deafferented zone of the TBI + BEC insult (Falo et al., 2008). Assay of MMP-3 protein expression and enzyme activity (Figure 1 B, C), as well as attenuated agrin protein level within the maladaptive model (Figure 2), support this interpretation. Further, parallel qRT-PCR studies confirmed a persistent 7 day elevation of MMP-3 mRNA with maladaptive insult (Falo et al., 2006), as well as an increase in agrin gene transcription at 7 days postinjury when molecular layer agrin protein level was reemerging (Falo et al., 2008). Collectively, we interpreted these findings as evidence that acute elevation of MMP-3 is critical for adequate matrix morphing, the preliminary removal of degenerating terminals, and the establishment of dendritic boundaries for the successful onset of synaptic regeneration. Consistent with its role in the patterning of peripheral neuromuscular junction synapse structure (Van-Saun et al., 2003), the MMP-3 substrate agrin is one of the required matrix mediators when this regeneration is induced by TBI. Indeed, several prior studies report a correlation between MMP-3 activity and expression of ECM peptides that direct synaptogenesis, ranging from transcriptional signaling, to modifications of postsynaptic membrane and degradation of inhibitory CSPGs prior to axonal growth (Deller et al., 2001; Muir et al., 2002; Dityatev and Schachner, 2003; Pizzi and Crowe, 2007).

In our study of MMP response to TBI, we also observed that injury induced elevation in gelatinase (MMPs-2, -9) activity (Phillips and Reeves, 2001). Further analysis showed that MMP-2 activity was elevated above controls at both 2 and 7 days, but significantly reduced by 15 days, suggesting that it may act as a switch for 7 day onset of presynaptic terminal sprouting and the reemergence of postsynaptic spines. Interestingly, our results pointed to a similar MMP-2 activity in both adaptive and maladaptive models at this time point (Figure 3A). In probing for potential matrix substrates which might influence this initial phase of axonal sprouting and dendritic reformation, we tested whether lysis of the neural adhesion protein neurofascin was correlated with the 7 day changes in MMP-2 activity (Figure 3A). Neurofascin is a highly conserved transmembrane protein linked to sub-plasmalemmal ankyrin-spectrin networks, as such influencing mobility of axon terminals and growth cones. Anchored neurofascin contains a RGD integrin binding sequence, giving it the potential to affect adhesion molecules like NCAM, ultimately stabilizing neurite extensions and fixing axon growth (Volkmer et al., 1996). By contrast, soluble forms of neurofascin do not appear to facilitate such fixed growth, where complex interaction with matrix proteins can affect the molecule's glycosylation, slowing down process extension. Interestingly, such altered posttranslational modification as a consequence of MMP lysis may act to free axons during early collateral sprouting phases and postsynaptic targeting (Maier et al., 2006). In our TBI models, we found a 120 kDa form of neurofascin elevated, a fragment similar in size to that seen by Maier and colleagues, which they suggest can be shed, bind NCAM or F11 and, once fixed in the local synaptic environment, induce axonal sprouting. Notably, this shedding can be blocked by the MMP inhibitor GM6001.

When the UEC model was examined in more detail (Harris et al., 2011), we also observed that the proteoglycan phosphacan, a potential mediator of axonal growth (Garwood

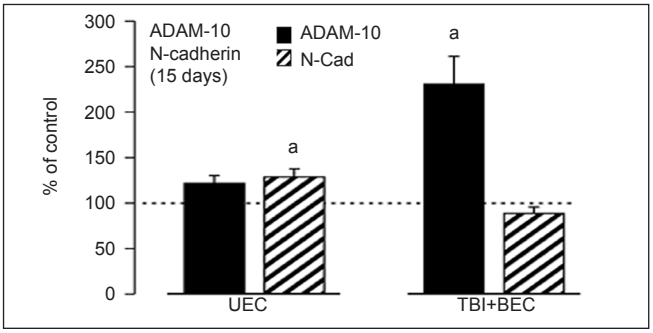

Figure 4 Hippocampal ADAM-10, also capable of N-cadherin lysis, is persistently elevated with maladaptive traumatic brain injury (TBI) + bilateral entorhinal lesion (BEC) plasticity.
This membrane type MMP can cleave matrix substrates well into the 15 day synaptic stabilization period, and was correlated with a failed reemergence of N-cadherin, in direct contrast to the response seen during unilateral entorhinal lesion (UEC) adaptive synaptogenesis. [a]$P < 0.05$, *vs.* paired controls. From Warren et al. (2012), with permission. ADAM-10: A disintegrin and metalloproteinase-10; MMP: matrix metalloproteinase; TBI: traumatic brain injury; UEC: unilateral entorhinal lesion; BEC: bilateral entorhinal lesion.

et al., 1999), and synaptic substrate of MMP-3 (Muir et al., 2002), exhibited a shift in distribution within the deaffwerented zone as MMP-3 activity was attenuated. Specifically, at 7 days when hippocampal MMP-3 lysis was no longer different from controls, phosphacan expression was significantly elevated in protein extracts enriched for deafferented dentate molecular layer (Figure 3B). This response is consistent with a matrix modulatory function for phosphacan during the onset of synapse regrowth. It was also clear that reactive glia within the deafferented dendritic regions of the UEC were a principal source of MMP-2 production. These findings are consistent with published studies profiling gelatinase activation (both MMPs-2 and -9) during recovery phases induced by spinal cord injury (Hsu et al., 2006; Hsu et al., 2008), olfactory deafferentation (Costanzo et al., 2006; Costanzo and Perrino, 2008) and cortical impact brain injury (Wang et al., 2000). Interestingly, it has been documented that MMP-3 cleaves upstream peptide sequences from inactive proenzyme forms of the gelatinases, converting them to active enzymes (Ogata et al., 1992). Early activation of MMP-3 and MMP-2 following deafferentation is consistent with such an interaction, possibly regulating acute degenerative and initial regenerative phases of reactive synaptogenesis.

In another study, we examined the injury-induced change in membrane type 5 metalloproteinase (MT5-MMP) and its synapse stabilizing substrate N-cadherin (Warren et al., 2012). Here we found an interesting relationship between the 7 day postinjury MT5-MMP and N-cadherin protein levels. N-cadherin is a transmembrane molecule whose homodimeric binding between pre and postsynaptic terminals can act as an adhesion stabilizer to maintain structural synaptic integrity (Shapiro and Coleman, 1999; Fercakova, 2001). This MMP substrate was significantly reduced during the phase of regenerative sprouting and spine reformation at 7 days after UEC, matching the time period when MT5-MMP was elevated (Figure 3C). Interestingly, when MT5-MMP expression was no longer elevated over control, N-cadherin protein levels show a reversal, increasing over baseline expression.

In summary, we have identified MMP-2 and MT5-MMP as two MMPs that exhibit rapid injury-induced activation and subsequent interaction with substrates targeted to the early regenerative phase of reactive synaptogenesis. These substrates, which are critical to both the fluidity of the synaptic junctional complex (N-cadherin), and the modulation of terminal sprouting (neurofascin), are both spatially and temporally correlated with elevated MMP response during the onset of synapse regeneration. Our data, taken with prior published reports, suggest that MMP-2 and MT5-MMP target transmembrane stabilizing proteins like neurofascin and N-cadherin, resulting in the shedding of their extracellular domains into the local regenerative environment. These shed forms can induce greater flexibility of synaptic elements for reorganization and morphing, as well as fix local growth promoting proteins at the sites of this activity.

During injury-induced synaptogenesis, the brain produces an excess of nascent synapses, overshooting the necessary number of replacement connections. At the end of the regenerative phase, a pruning and stabilization period begins (15 days postinjury), when selected new synaptic junctions are made more permanent (Steward, 1989). We have explored MMP role in this phase of synaptogenesis as well, gathering evidence suggesting that a second membrane type MMP, a disintegrin and metalloproteinase-10 (ADAM-10), may serve as a switch to support successful synapse stabilization (Warren et al., 2012). As with MT5-MMP, we have investigated N-cadherin as a possible synaptic substrate for ADAM-10. Because the adhesive properties of N-cadherin contribute to the control of synapse destabilization/stabilization cycling, its modulation by MMPs, such as ADAM-10, would be predicted in this later phase of synaptogenesis. We found that, like MT5-MMP, ADAM-10 protein expression was elevated acutely after TBI and, when recovery is adaptive (UEC model), the enzyme returned to normal levels by 15 days (Figure 4). As with MT5-MMP, this normalization of ADAM-10 was correlated with increased expression of hippocampal N-cadherin. Interestingly, N-cadherin was localized to reactive neuroglia (Figure 5), cells that may provide a local source of the protein for regenerating synapses along deafferented dendrites. Perhaps more importantly, the examination of 15 day ADAM-10 response in the TBI + BEC model revealed a persistent elevation of enzyme expression under maladaptive conditions, an effect accompanied by failed N-cadherin recovery (Figure 4). These results point to ADAM-10 as another time dependent MMP regulator of reactive synaptogenesis, one whose persistent expression appears to negatively affect recovery. Unlike MMPs-2, -3 and MT5-MMP, whose 15 day postinjury expression was not different between the adaptive and maladaptive plasticity conditions, 15 day ADAM-10 level in the deafferented hippocampus remained high when recovery was poor. Parallel qRT-PCR experiments also showed that ADAM-10 transcript was elevated over controls as late as 7 days after TBI + BEC, and thus, may contribute to the persistent increase of the enzyme observed at 15 days (Warren et al., 2012). Together, these results suggest that appropriate ADAM-

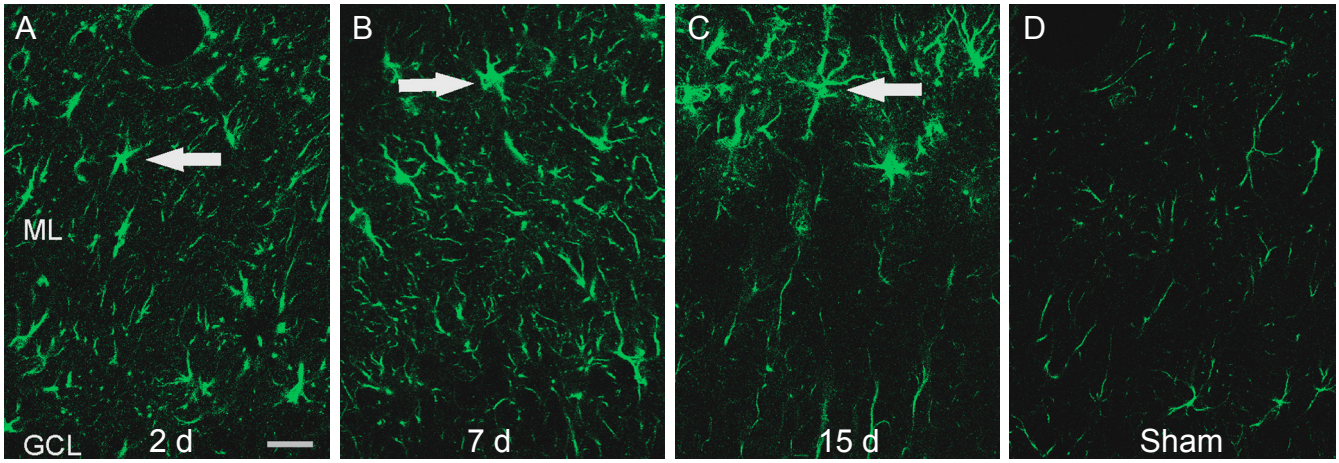

Figure 5 ADAM-10 protein shows time dependent differences in distribution within the deafferented molecular layer after traumatic brain injury (TBI) + bilateral entorhinal lesion (BEC) insult.
In the maladaptive model, hippocampal ADAM-10 was localized within reactive glia at the sites of synaptic reorganization (arrows in A–C), a signal which was strong during the 15 day postinjury interval (C) when its tissue levels were persistently elevated in molecular layer (ML; compared with Figure 4). Bar = 20 μm. From Warren et al. (2012), with permission. ADAM-10: A disintegrin and metalloproteinase-10; ML: molecular layer; GCL: granule cell layer; d: days.

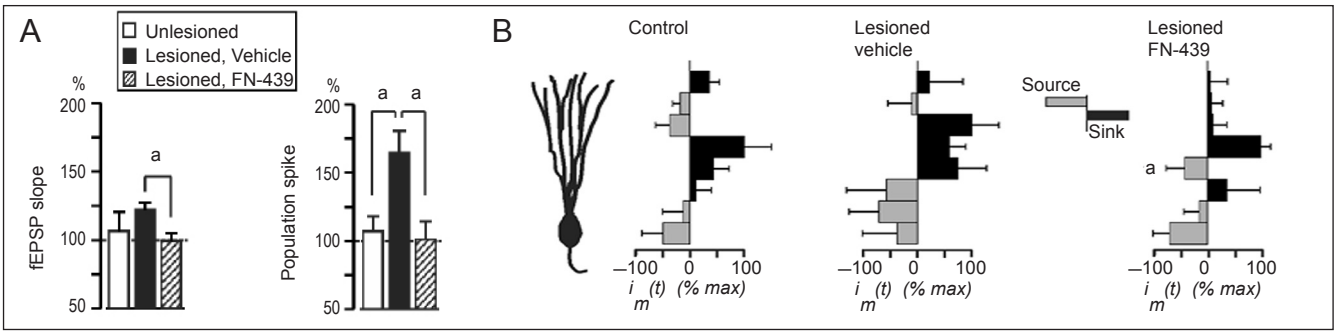

Figure 6 Acute post-injury MMP inhibition attenuates LTP induction and alters dendritic reinnervation at 7 days after UEC.
In (A), tetanus-induced increases in fEPSP initial slope and in population spike amplitude are plotted as percent of pretetanus baseline. LTP was blocked by a single acute (30 minutes postinjury) dosing of MMP inhibitor FN-439. Average current source density across UEC deafferented dendrites at 7 days showed that synaptic input (current sink) was abnormally distributed in animals subjected to MMP inhibition (B). $^{a}P < 0.01$, *vs.* control and vehicle treated. From Reeves et al. (2003), with permission. MMP: Matrix metalloproteinase; LTP: long-term potentiation; UEC: unilateral entorhinal lesion; fEPSP: field excitatory post-synaptic potentials.

10 expression is critical for successful stabilization of new synaptic junctions at the 15 day phase of recovery. Although the 15 day postinjury interval was not examined, Mayer and Gotschall (2005) did report that another ADAM family member, ADAMTS, degraded dentate brevican at 7 days after UEC. Their finding is consistent with our ADAM-10 data and support the hypothesis that ADAMs play a more prominent role during the period of synaptic reinnervation.

MMP inhibition produces positive and negative effects on synaptogenesis

Several studies have provided evidence that inhibition of MMP activity alters synaptic activity and functional efficacy of neuronal circuits in both the healthy and injured brain (Szklarczyk et al., 2002; Bozdagi et al., 2007; Wang et al., 2008; Wilczynski et al., 2008; Penedo et al., 2009). The majority of these reports have focused on hippocampal LTP as a model of synaptic plasticity. They showed that by blocking MMP proteolysis, its intracellular substrates critical to synaptic function are altered, ranging from cytoskeleton and adhesion proteins that shape postsynaptic spines, to molecules like collapsin response mediator protein (CRMP-2), which regulates Ca^{2+} uptake, controlling presynaptic vesicle release of transmitter (Brittain et al., 2009). Notably, this effect on synaptic function appears to be a general phenomenon, demonstrated throughout the brain, including prefrontal cortex and cerebellum (Okulski et al., 2007; Piccolini et al., 2012).

Despite the clear evidence that MMP inhibition can alter synaptic efficacy, it remained unknown how the manipulation of time dependent differences in MMP expression during reactive synaptogenesis could alter the extent of synaptic recovery. It was unclear whether targeting these MMP responses would be beneficial or harmful to synaptic recovery. To address this question, we have conducted several *in vivo* studies using common pharmacological inhibitors of MMP activity, with the caveat that these drugs

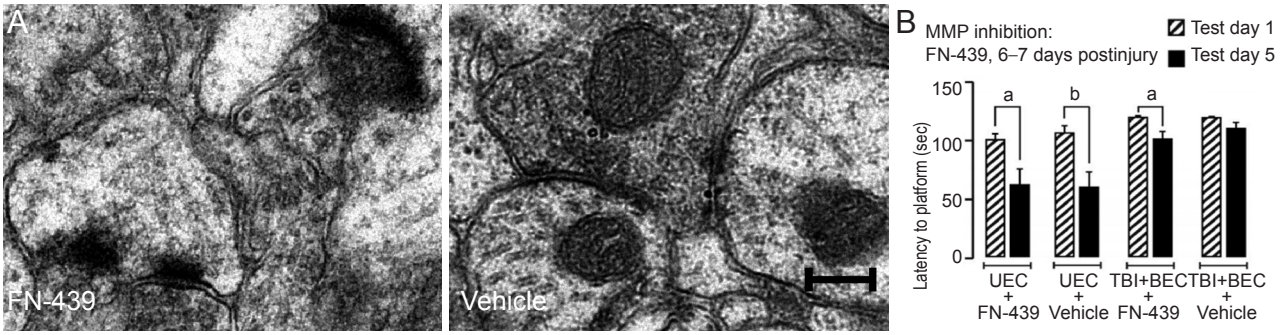

Figure 7 MMP inhibition in the maladaptive plasticity model improves synaptic regeneration and attenuates long term cognitive deficits.
Ultrastructural analysis showed a mature synaptic cytoarchitecture in TBI + BEC after FN-439 treatment relative to vehicle controls (A). Dosing of FN-439 during 6–7 day elevation of MMP-3 in TBI + BEC significantly reduced latency to platform in the Morris Water Maze, while vehicle cases continued to show behavioral deficit (B). Similar dosing had no effect on UEC performance. $^{a}P < 0.05$, $^{b}P < 0.001$ (days 1 *vs.* 5); bar = 0.2 μm in A. From Falo et al. (2006), with permission. MMP: Matrix metalloproteinase; TBI: traumatic brain injury; UEC: unilateral entorhinal lesion; BEC: bilateral entorhinal lesion.

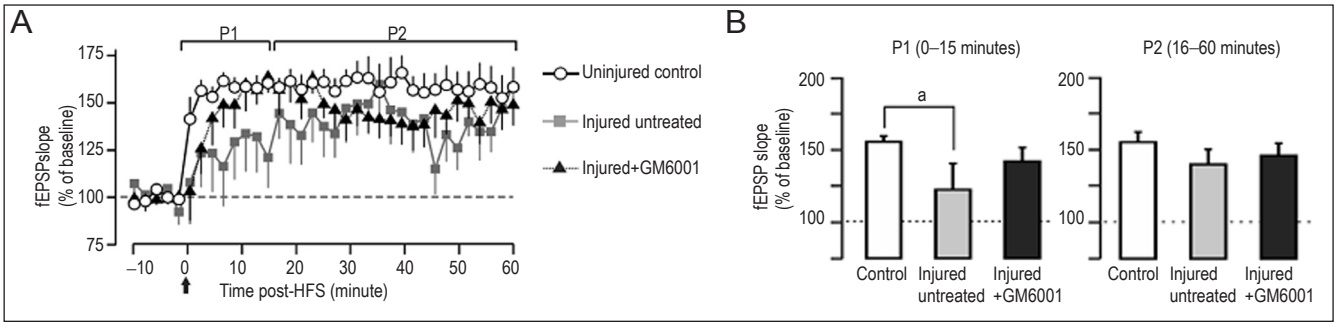

Figure 8 ADAM-10 inhibition attenuates synaptic functional deficits after traumatic brain injury (TBI) + bilateral entorhinal lesion (BEC).
Time course of mean fEPSP slopes spanning 10 minutes (min) pre-HFS to 60 min post-HFS (A, arrow = HFS theta burst) showed that, relative to sham controls, LTP induction was attenuated. Notably, prior treatment at 6–7 days with MMP inhibitor GM6001 appeared to alleviate this deficit in the initial 15 min interval (P1). Group analysis of fEPSP slope (B) revealed that mean LTP during the initial period of induction was significantly suppressed for injured untreated cases, but after GM6001, the signal was not different from controls. These results suggest that deficits in a cell model of synaptic efficacy generated under conditions of maladaptive plasticity can be reversed by time dependent MMP inhibition. $^{a}P < 0.05$, *vs.* sham controls. From Warren et al. (2012), with permission. ADAM-10: A disintegrin and metalloproteinase-10; fEPSP: field excitatory post-synaptic potentials; HFS: high frequency stimulation; LTP: long-term potentiation; TBI: traumatic brain injury; MMP: matrix metalloproteinase.

are not specific to any one MMP family member. Each drug has established MMP inhibitory function, usually for several enzymes, but can be demonstrated to significantly block function of selected MMPs of interest. The first study tested whether inhibition of MMP-3 at 30 minutes after UEC would affect synaptic reorganization and efficacy under conditions of adaptive recovery. To do this, we used the hydroxyamate MMP inhibitor FN-439 (MMP inhibitor 1), which significantly attenuated MMP-3 activity in our TBI models (Kim et al., 2005). We hypothesized that acute MMP-3 inhibition would alter the environmental reorganization preceding synapse regeneration, resulting in poor reinnervation and altered synaptic function. Essentially, we proposed that blocking MMP activity in the early phases of synaptogenesis would shift an adaptive recovery process into a maladaptive form. This was what we observed. Two outcome measures illustrated this finding. In Figure 6A, we found that a dose of FN-439 sufficient to generate approximately a 40% reduction in MMP-3 activity blocked the reemergence of hippocampal LTP at 7 days postinjury, a functional correlate marking synaptic reorganization in this model (Reeves et al., 2003). The effect was demonstrated with both fEPSP slope and population spike measures. Further, when the synaptic input to deafferented dentate granule cell dendrites was mapped using current source density analysis, we found additional evidence that acute postinjury inhibition of MMP attenuated the successful redistribution of new presynaptic terminals during the period of sprouting and synapse reformation (Figure 6B). Together, these findings show that blocking MMP activity during the acute degenerative phase of reactive synaptogenesis can attenuate the extent of recovery attainable in an adaptive model. This result points to the positive role for these matrix enzymes during the early phase of injury response, where synaptic morphing is required to optimize the local environment for successful synaptic regeneration.

Another important finding in our studies comparing adaptive and maladaptive synaptic plasticity was the observation that some MMPs activated after injury remained elevated under conditions of poor recovery (Figures 1 and 4). In the case of MMP-3, we documented a persistent elevation of MMP expression and activity in the TBI + BEC model when compared with the adaptive UEC condition, resulting in severe attenuation of both structural and functional re-

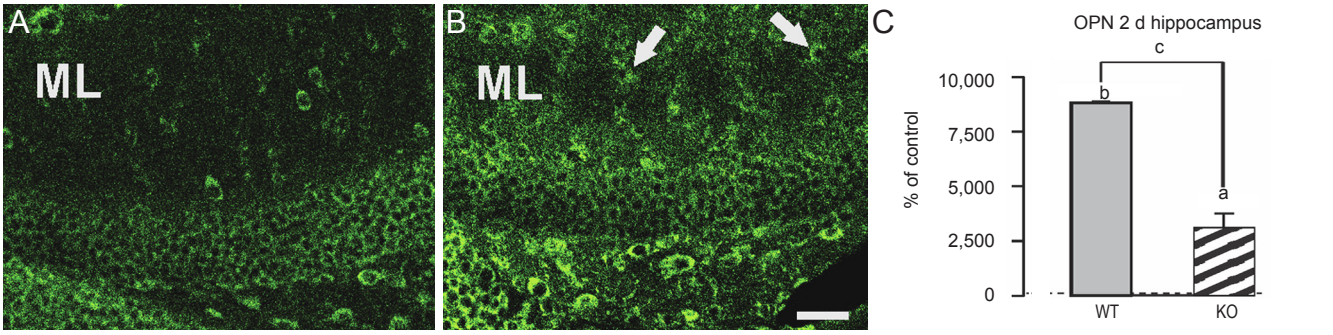

Figure 9 UEC acutely elevates OPN protein within the deafferented zone, a response partially dependent upon MMP-9 expression.
Control mouse hippocampus (A) showed principal OPN signal within granule cells of the dentate gyrus and secondary, low labeling within the dendritic zone. At 2 days after UEC (B), OPN was notably increased in the deafferented ML, expressed as both punctuate and cellular labeling (arrows). OPN increase was confirmed with western blot analysis, and MMP-9 knockout mice showed a significant reduction of injury-induced OPN (C). $^{a}P < 0.05$, $^{b}P < 0.001$, *vs.* paired controls; $^{c}P < 0.001$. Bar = 30 μm in A, B. OPN: Osteopontin; MMP-9: matrix metalloproteinase-9; UEC: unilateral entorhinal lesion; ML: molecular layer.

covery (Falo et al., 2006). We reasoned that if MMP activity could be inhibited at the postinjury interval when enzyme showed aberrant increase, the maladaptive consequences might be shifted to a recovery pattern more like the adaptive model. It was hypothesized that targeting FN-439 treatment at 6 and 7 days after TBI + BEC, the time when MMP-3 was persistently elevated, would dampen enzyme activity at the onset of reinnervation and stabilize the local synaptic environment for more successful repair. We examined the effect of FN-439 treatment on both hippocampal synaptic structure and hippocampal dependent cognitive function (Figure 7). Our results showed that, even with a partial MMP-3 inhibition generated by FN-439, we could elicit an improved structural recovery of dentate synapses (Figure 7A), and significant reduction of postinjury behavioral deficits in the maladaptive TBI + BEC model (Figure 7B).

In a second approach, we explored the finding that membrane MMP ADAM-10 was persistently elevated between 7–15 days postinjury, the period of synapse regeneration and stabilization in the maladaptive TBI + BEC model. Our hypothesis was that this increase in ADAM-10 expression contributed to abnormal lysis of synapse stabilizing proteins like N-cadherin, attenuating the maturation of nascent synapses required for functional efficacy. To test this hypothesis, we employed a second broad based MMP inhibitor, GM6001, in order to include target ADAM-10 enzyme activity. TBI + BEC animals were treated with GM6001 during the active phase of fiber remodeling, then functional neuroplasticity was evaluated in the hippocampus using the LTP paradigm (Figure 8). We first confirmed the efficacy of GM6001 in altering ADAM-10 and N-cadherin expression. Our analyses showed that enzyme expression was reduced; a commonly reported effect of MMP inhibitory drugs and, more importantly, N-cadherin protein was elevated above control levels, similar to that seen at 15 days after UEC (Warren et al., 2012). Our electrophysiological results suggested that the GM6001 treatment at 6–7 days altered injury-induced fiber remodeling and synaptogenesis which were detectable in LTP induction trials conducted at 15 days postinjury. When the capacity for synaptic potentiation was examined, we found that GM6001 treatment attenuated TBI + BEC deficits in the early phases of LTP induction, shifting the fEPSP slope toward uninjured control levels (Figure 8A). This effect was quantified with group analysis, showing that fEPSP slope measures were significantly reduced in the maladaptive TBI + BEC model during this early P1 phase of synaptic potentiation. With GM6001 dosing that both attenuated ADAM-10 and increased N-cadherin levels, this deficit in LTP generation was no longer present (Figure 8B). We conclude that ADAM-10 over expression and activity is an aberrant condition during the process of synaptogenesis, marking poor recovery and serving as a reasonable target for manipulation to improve outcome.

In summary, by contrasting adaptive UEC and maladaptive TBI + BEC injury models, we have provided evidence that persistent MMP activation during different postinjury phases of reactive synaptogenesis can influence extent of recovery. Using these differences to identify targets for MMP manipulation, we have improved outcome under conditions of maladaptive plasticity, shifting recovery to be more in line with that produced by adaptive synaptogenesis. This approach has potential for translational application and appears to be feasible with different types of MMPs over a range of regenerative phases. Overall, our studies show that inhibition of MMP enzyme activity can be both good and bad with respect to synaptic recovery. Blocking MMPs at times when their elevation is critical to matrix reshaping is detrimental to regeneration, but damping down their activity when pathology drives an excess response can facilitate better outcome. Timing may be quite important, with an understanding of MMP role in each phase of the synaptogenic process critical to designing interventions for improved recovery.

Novel MMP interaction with immune and carrier proteins affects synaptogenesis

Given that many lines of research point to MMP mediation of synaptic plasticity, and that these time dependent effects influence different phases of synapse reorganization, our current studies have focused on how MMPs mediate cellular response to CNS insult, and how their lytic activity might be

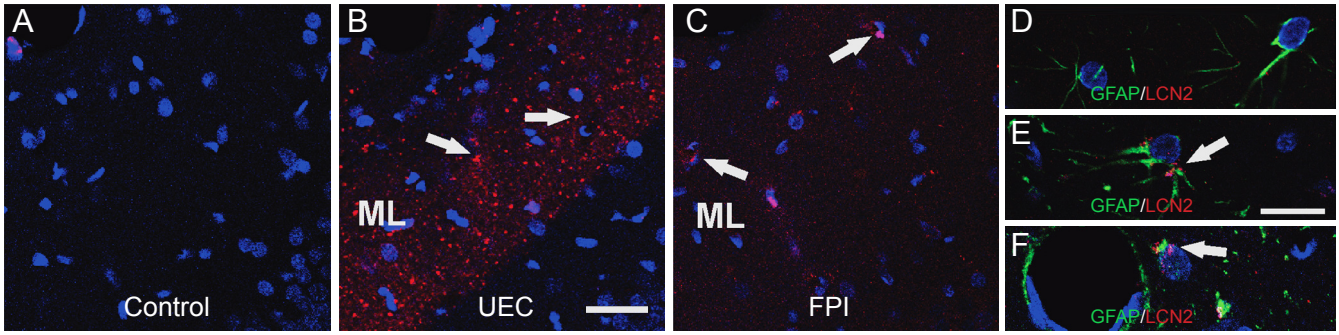

Figure 10 Siderophore and MMP activator lipocalin 2 (LCN2) is increased within deafferented hippocampus during acute postinjury phases. Control dentate gyrus showed no detectable LCN2 signal (A). At 2 days after UEC (B), LCN2 protein was elevated over the deafferented dentate molecular layer (ML), often appearing in punctuate aggregates (arrows). When fluid percussion injury was probed (C), LCN2 was also increased over the dentate gyrus, but overall LCN2 distribution was reduced and ML staining most prominent in cells adjacent to vessels (arrows). LCN2 co-labeled experiments showed siderophore present in ML reactive astrocytes (arrow, E), and in astrocytes adjacent to ML vessels (arrow, F), with lack of LCN2 signal in astrocytes of uninjured controls (D). Bar = 40 μm in A–C, 20 μm in D–F.

regulated. To do this we have utilized Affymetrix microarray data to identify MMP targets and regulators which are upregulated during synaptogenesis. These analyses revealed two new promising candidates, osteopontin (OPN), a secreted cytokine and MMP substrate, and lipocalin 2 (LCN2), a matrix siderophore with the capacity to bind and persistently activate MMP-9. Multiple studies have shown that immune molecules induced by cell stress (*i.e.*, cytokines, chemokines and growth associated peptides) can upregulate MMP activity (Clark et al., 2008). Since we knew that both MMP and OPN transcripts are acutely elevated after UEC, and that MMP generated OPN fragments can mediate integrin receptor signaling during the acute immune response (Wang et al., 2008), we hypothesized that UEC deafferentation would increase OPN expression 1–2 days postinjury, and that OPN levels would be dependent upon MMP interaction. We first profiled acute OPN protein and mRNA expression following UEC, and found not only elevations in the hippocampus at 1–2 days postinjury, but a concomitant increase in expression of OPN fragments containing exposed integrin binding sites (Chan et al., in review). In parallel studies of the mouse UEC model, OPN protein was increased within non neuronal cells of the deafferented dendritic zone when compared with control samples (Figure 9A, B). When hippocampal tissue extracts were evaluated by Western blot, this elevation was found to be over 75 fold (Figure 9C). Interestingly, if MMP-9 knockout mice were subjected to UEC, their 2 day hippocampal extracts showed significant reduction in OPN protein expression (Figure 9C). This result supports an enzyme/substrate interaction between MMP-9 and OPN during the acute UEC postinjury phase, however, MMP-9 knockout did not fully normalize OPN elevation, indicating that the regulation of this MMP substrate is complex, involving multiple mechanisms. Additional studies of synaptogenic time course will be required to sort out other ECM contributors to OPN regulation during this process. It is encouraging that change in OPN expression has been reported with a variety of TBI animal models (Israelsson et al., 2006; Cernak et al., 2011; Risling et al., 2011), but these studies are broad mechanism analyses and do not provide evidence for OPN mediation of synaptic recovery. Nevertheless, OPN has been documented to direct cellular reactivity after cortical cryolesion (Shin et al., 2005), stab wounds (Plantman, 2012), hypoxia-ischemia (Chen et al., 2011) and SCI (Hashimoto et al., 2007). Our new data suggest that MMPs can target OPN, potentially mediating intercellular signals to direct cell activation and mobility during the acute degenerative phase of reactive synaptogenesis.

In a second set of experiments, we have examined the response of inflammation induced matrix carrier protein LCN2 in hippocampus after both UEC and fluid percussion TBI. While it is understood that MMP activity is tightly regulated by endogenous tissue inhibitors of metalloproteinases (TIMPs), control of MMP activation can be achieved in other ways after CNS insult (Ra and Parks, 2007). For example, vascular pathology increases local production of cytokines, which can then activate MMPs secreted by reactive glia. Some MMP family members can also cleave pro forms of other MMPs to facilitate further enzyme lysis. More recently, a novel mechanism for control of MMP-9 activity has been identified through its binding to LCN2 (Tschesche et al., 2001). Interestingly, *in vitro* studies show that LCN2 regulates both microglial and astrocyte activation in response to inflammatory stimuli (Lee et al., 2009; Jang et al., 2013), providing a common cellular base for LCN2/MMP-9 interaction after TBI. Given that LCN2 transcript was significantly elevated following both UEC and concussive TBI, we hypothesized that the siderophore increases during the acute postinjury interval, serving to regulate MMP-9 activity in the early phases of reactive synaptogenesis. Our initial immunohistochemical studies confirm LCN2 elevation over the deafferented dentate molecular layer at 2 days after UEC when compared with control cases (Figure 10A, B). Diffuse hippocampal deafferentation produced by fluid percussion TBI also increases LCN2, but the response is reduced, and broadly distributed throughout the dentate, with the strongest signal around blood vessels (Figure 10C). When dual label confocal imaging was performed, LCN2 signal was co-localized within reactive GFAP positive astrocytes of the deafferented zone (Figure 10E) and in astrocytes adjacent to neuropil vasculature (Figure 10F). Studies mapping LCN2 expression and MMP-9 activity over time postinjury are

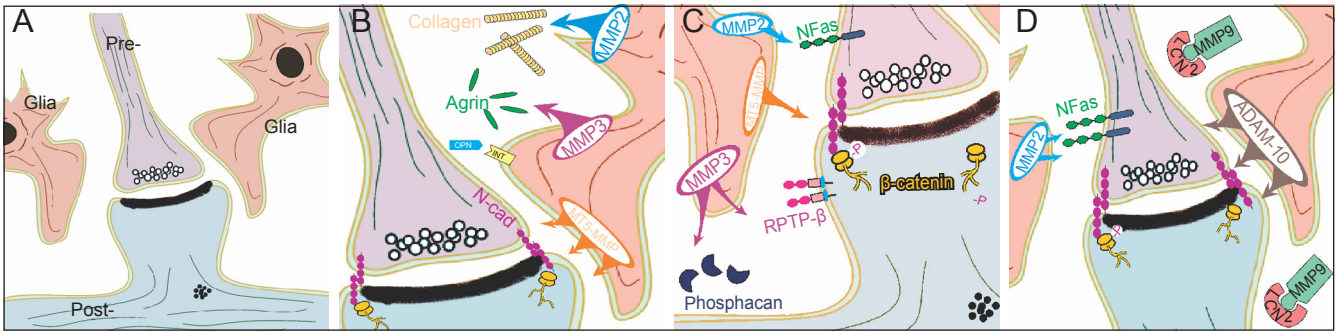

Figure 11 A model predicting how MMP family members and their substrates act in concert to mediate different phases of adaptive synaptogenesis.
Acute postinjury elevation and activation is common among both secreted and membrane type MMPs, however, the time course of these changes and the substrates targeted may differ. MMP-3 function appears focused on the acute degenerative phase, while MMP-2, MMP-9, MT5-MMP and ADAM-10 likely influence both degenerative and regenerative cell interactions. Cleavage of substrates like neurofascin and N-cadherin would be increased when synaptic structure is being broken down and reshaped, while the lysis of agrin and phosphacan would be reduced at later phases, allowing their elevation to form network boundaries for axon terminal growth. Once new synaptic junctions are formed, their stability will depend upon a reduction of MMP activity and the reemergence of molecules like neurofascin and N-cadherin with important adhesion properties. When synaptogenesis is maladaptive, certain MMPs (*e.g.*, MMP-3, ADAM-10) become persistently activated, abnormally degrading substrates critical to successful synaptic regeneration. Interesting new data suggest that MMPs target the cytokine OPN to provide autocrine and paracrine cell activation in the early stages of synaptic reorganization. Further, novel enzyme activation through binding to secreted carrier proteins like LCN2 support the complexity with which certain MMPs (*i.e.*, MMP-9) are integrated into the different processes of synapse regeneration. MMP: Matrix metalloproteinase; ADAM-10: a disintegrin and metalloproteinase-10; MT-5: membrane type.

underway to better dissect the extent of LCN2/MMP-9 interaction during the course of injury-induced synaptogenesis. Together, these new data with OPN and LCN2 reinforce the fact that MMPs are highly integrated mediators of CNS plasticity. They illustrate the complexity of different MMP roles, interfacing with a wide range of cellular components and signaling mechanisms to direct synaptic reorganization after injury. We believe that these new MMP interactions can serve as prototypes for future research on MMP role in synaptic plasticity.

Conclusions and matrix role in synaptic remodeling

Here we have reviewed historical and novel data supporting MMP regulation of axonal and synaptic reorganization. From these results it is clear that changes in matrix enzyme/substrate expression are correlated with the time dependent phases of axonal sprouting and reactive synaptogenesis, and specific combinations of these matrix proteins are associated with different phases of the process (Figure 11). Both secreted and membrane bound matrix proteins exhibit cellular profiles supporting structural and functional plasticity. Importantly, this expression profile is altered under maladaptive conditions and targeted MMP manipulation can influence the efficacy of both adaptive and maladaptive neuroplasticity. This targeting can be optimized by understanding the time dependent profile of matrix proteins affecting each phase of plasticity. New studies show that immune MMP substrates not typically associated with the CNS may be up-regulated after injury and have the potential to influence both axonal and synaptic regeneration. Osteopontin is one such molecule, which current evidence suggests can regulate cell mediated synaptic turnover during the early phases of reactive synaptogenesis. Secreted carrier proteins also have potential for novel regulation of matrix enzyme function during synaptogenesis. One of these carriers, lipocalin 2, binds MMP-9 in a persistently activated state, and the two molecules are concurrently elevated during certain phases of reactive synaptogenesis. Future prospects appear promising for identifying how direct MMP-mediated neuroplasticity after brain injury, however, this goal will require careful examination of each postulated mechanism in the context of time-dependent synaptic recovery.

Author contributions: *Phillips LL and Reeves TM were responsible for paper writing and figure preparation. Chan JL provided osteopontin data and edited text. Doperalski AE provided lipocalin 2 data and edited text. All authors approved the final version of this paper.*
Conflicts of interest: *None declared.*

References

Andrews EM, Richards RJ, Yin FQ, Viapiano MS, Jakeman LB (2012) Alterations in chondroitin sulfate proteoglycan expression occur both at and far from the site of spinal contusion injury. Exp Neurol 235:174-187.

Asahi M, Asahi K, Jung JC, del Zoppo GJ, Fini ME, Lo EH (2000) Role for matrix metalloproteinase 9 after focal cerebral ischemia: effects of gene knockout and enzyme inhibition with BB-94. J Cereb Blood Flow Metab 20:1681-1689.

Asahina M, Yoshiyama Y, Hattori T (2001) Expression of matrix metalloproteinase-9 and urinary-type plasminogen activator in Alzheimer's disease brain. Clin Neuropathol 20:60-63.

Bakos SR, Schwob JE, Costanzo RM (2010) Matrix metalloproteinase-9 and -2 expression in the olfactory bulb following methyl bromide gas exposure. Chem Senses 35:655-661.

Beliën AT, Paganetti PA, Schwab ME (1999) Membrane-type 1 matrix metalloprotease (MT1-MMP) enables invasive migration of glioma cells in central nervous system white matter. J Cell Biol 144:373-384.

Bozdagi O, Nagy V, Kwei KT, Huntley GW (2007) In vivo roles for matrix metalloproteinase-9 in mature hippocampal synaptic physiology and plasticity. J Neurophysiol 98:334-344.

Brittain JM, Piekarz AD, Wang Y, Kondo T, Cummins TR, Khanna R (2009) An atypical role for collapsin response mediator protein 2 (CRMP-2) in neurotransmitter release via interaction with presynaptic voltage-gated calcium channels. J Biol Chem 284:31375-31390.

Busch SA, Silver J (2007) The role of extracellular matrix in CNS regeneration. Curr Opin Neurobiol 17:120-127.

Buss A, Pech K, Kakulas BA, Martin D, Schoenen J, Noth J, Brook GA (2007) Matrix metalloproteinases and their inhibitors in human traumatic spinal cord injury. BMC Neurol 7:17.

Cernak I, Merkle AC, Koliatsos VE, Bilik JM, Luong QT, Mahota TM, et al (2011)The pathobiology of blast injuries and blast-induced neurotrauma as identified using a new experimental model of injury in mice. Neurobiol Dis 41:538-551.

Chan JL, Reeves TM and Phillips LL (2013) Osteopontin expression during acute immune response mediates reactive synaptogenesis and adaptive outcome. In Review.

Chen W, Ma Q, Suzuki H, Hartman R, Tang J, Zhang JH (2011) Osteopontin reduced hypoxia-ischemia neonatal brain injury by suppression of apoptosis in a rat pup model. Stroke 42:764-769.

Clark IM, Swingler TE, Sampieri CL, Edwards DR (2008) The regulation of matrix metalloproteinases and their inhibitors. Int J Biochem Cell Biol 40:1362-1378.

Clement AB, Hanstein R, Schröder A, Nagel H, Endres K, Fahrenholz F, Behl C (2008) Effects of neuron-specific ADAM10 modulation in an in vivo model of acute excitotoxic stress. Neuroscience 152:459-468.

Copin JC, Rebetez MM, Turck N, Robin X, Sanchez JC, Schaller K, Gasche Y, Walder B (2012) Matrix metalloproteinase 9 and cellular fibronectin plasma concentrations are predictors of the composite endpoint of length of stay and death in the intensive care unit after severe traumatic brain injury. Scand J Trauma Resusc Emerg Med 20:83.

Costanzo RM, Perrino LA, Kobayashi M (2006) Response of matrix metalloproteinase-9 to olfactory nerve injury. Neuroreport 17:1787-1791.

Costanzo RM, Perrino LA (2008) Peak in matrix metaloproteinases-2 levels observed during recovery from olfactory nerve injury. Neuroreport 19:327-331.

de Castro RC Jr, Burns CL, McAdoo DJ, Romanic AM (2000) Metalloproteinase increases in the injured rat spinal cord. Neuroreport 11:3551-3554.

Deller T, Haas CA, Frotscher M (2001) Sprouting in the hippocampus after entorhinal cortex lesion is layer-specific but not translaminar: which molecules may be involved? Restor Neurol Neurosci 19:159-167.

Ding JY, Kreipke CW, Schafer P, Schafer S, Speirs SL, Rafols JA (2009) Synapse loss regulated by matrix metalloproteinases in traumatic brain injury is associated with hypoxia inducible factor-1alpha expression. Brain Res 1268:125-134.

Dityatev A, Schachner M (2003) Extracellular matrix molecules and synaptic plasticity. Nat Rev Neurosci 4:456-468.

Endres K, Fahrenholz F (2010) Upregulation of the alpha-secretase ADAM10--risk or reason for hope? FEBS J 277:1585-1596.

Ethell IM, Ethell DW (2007) Matrix metalloproteinases in brain development and remodeling: synaptic functions and targets. J Neurosci Res 85:2813-2823.

Falo MC, Fillmore HL, Reeves TM, Phillips LL (2006) Matrix metalloproteinase-3 expression profile differentiates adaptive and maladaptive synaptic plasticity induced by traumatic brain injury. J Neurosci Res 84:768-781.

Falo MC, Reeves TM, Phillips LL (2008) Agrin expression during synaptogenesis induced by traumatic brain injury. J Neurotrauma 25: 769-783.

Fercakova A (2001) Cell adhesion molecules in the neural development and plasticity. Bratisl Lek Listy 102:552-555.

Fredrich M, Illing RB (2010) MMP-2 is involved in synaptic remodeling after cochlear lesion. Neuroreport 21:324-327.

Garwood J, Schnädelbach O, Clement A, Schütte K, Bach A, Faissner A (1999) DSD-1-proteoglycan is the mouse homolog of phosphacan and displays opposing effects on neurite outgrowth dependent on neuronal lineage. J Neurosci 19:3888-3889.

Gonthier B, Koncina E, Satkauskas S, Perraut M, Roussel G, Aunis D, Kapfhammer JP, Bagnard D (2009) A PKC-dependent recruitment of MMP-2 controls semaphorin-3A growth-promoting effect in cortical dendrites. PLoS One 4:e5099.

Gonthier B, Nasarre C, Roth L, Perraut M, Thomasset N, Roussel G, Aunis D,Bagnard D (2007) Functional interaction between matrix metalloproteinase-3 and, semaphorin-3C during cortical axonal growth and guidance. Cereb Cortex 17:1712-1721.

Gonzalez-Perez F, Udina E, Navarro X (2013) Extracellular matrix components in peripheral nerve regeneration. Int Rev Neurobiol 108:257-275.

Goussev S, Hsu JY, Lin Y, Tjoa T, Maida N, Werb Z, Noble-Haeusslein LJ (2003) Differential temporal expression of matrix metalloproteinases after spinal cord injury: relationship to revascularization and wound healing. J Neurosurg 9:188-197.

Grimpe B, Silver J (2002) The extracellular matrix in axon regeneration. Prog Brain Res 137:333-349.

Grossetete M, Phelps J, Arko L, Yonas H, Rosenberg GA (2009) Elevation of matrix metalloproteinases 3 and 9 in cerebrospinal fluid and bloodin patients with severe traumatic brain injury. Neurosurgery 65:702-708.

Handsley MM, Cross J, Gavrilovic J, Edwards DR (2005) The matrix metalloproteinases and their inhibitors. In: Matrix metalloproteinases in the central nervous system (Conant K, Gottschall PE, eds), pp3-16. London: Imperial College Press.

Harris JL, Reeves TM, Phillips LL (2011) Phosphacan and receptor protein tyrosine phosphatase β phosphacan and receptor protein tyrosine phosphatase β expression mediates deafferentation-induced synaptogenesis. Hippocampus 21:81-92.

Harris NG, Carmichael ST, Hovda DA, Sutton RL (2009) Traumatic brain injury results in disparate regions of chondroitin sulfate proteoglycan expression that are temporally limited. J Neurosci Res 87:2937-2950.

Hashimoto M, Sun D, Rittling SR, Denhardt DT, Young W (2007) Osteopontindeficient mice exhibit less inflammation, greater tissue damage, and impaired locomotor recovery from spinal cord injury compared with wild-type controls. J Neurosci 27:3603-3611.

Hayashita-Kinoh H, Kinoh H, Okada A, Komori K, Itoh Y, Chiba T, Kajita M, Yana I, Seiki M (2001) Membrane-type 5 matrix metalloproteinase is expressed in differentiated neurons and regulates axonal growth. Cell Growth Differ 12:573-580.

Higashida T, Kreipke CW, Rafols JA, Peng C, Schafer S, Schafer P, Ding JY,Dornbos D 3rd, Li X, Guthikonda M, Rossi NF, Ding Y (2011) The role of hypoxia-inducible factor-1α, aquaporin-4, and matrix metalloproteinase-9 in blood-brain barrier disruption and brain edema after traumatic brain injury. J Neurosurg 114:92-101.

Howell MD, Gottschall PE (2012) Lectican proteoglycans, their cleaving metalloproteinases, and plasticity in the central nervous system extracellular microenvironment. Neuroscience 217:6-18.

Hsu JY, Bourguignon LY, Adams CM, Peyrollier K, Zhang H, Fandel T, Cun CL, Werb Z, Noble-Haeusslein LJ (2008) Matrix metalloproteinase-9 facilitates glial scar formation in the injured spinal cord. J Neurosci 28:13467-1377.

Hsu JY, McKeon R, Goussev S, Werb Z, Lee JU, Trivedi A, Noble-Haeusslein LJ (2006) Matrix metalloproteinase-2 facilitates wound healing events that promote functional recovery after spinal cord injury. J Neurosci 26:9841-9850.

Hubert T, Grimal S, Carroll P, Fichard-Carroll A (2009) Collagens in the developing and diseased nervous system. Cell Mol Life Sci 66:1223-1238.

Israelsson C, Lewén A, Kylberg A, Usoskin D, Althini S, Lindeberg J, Deng CX, Fukuda T, Wang Y, Kaartinen V, Mishina Y, Hillered L, Ebendal T (2006) Genetically modified bone morphogenetic protein signaling alters traumatic brain injury-induced gene expression responses in the adult mouse. J Neurosci Res 84:47-57.

Jang E, Lee S, Kim JH, Kim JH, Seo JW, Lee WH, Mori K, Nakao K, Suk K (2013) Secreted protein lipocalin-2 promotes microglial M1 polarization. FASEB J 27:1176-1190.

Jia F, Pan YH, Mao Q, Liang YM, Jiang JY (2010) Matrix metalloproteinase-9 expression and protein levels after fluid percussion injury in rats: the effect of injury severity and brain temperature. J Neurotrauma 27:1059-1068.

Jones LL, Sajed D, Tuszynski MH (2003) Axonal regeneration through regions of chondroitin sulfate proteoglycan deposition after spinal cord injury: a balance of permissiveness and inhibition. J Neurosci 23:9276-9288.

Kaliszewska A, Bijata M, Kaczmarek L, Kossut M (2012) Experience-dependent plasticity of the barrel cortex in mice observed with 2-DG brain mapping and c-Fos: effects of MMP-9 KO. Cereb Cortex 22:2160-2170.

Kim HJ, Fillmore HL, Reeves TM, Phillips LL (2005) Elevation of hippocampal MMP-3 expression and activity during trauma-induced synaptogenesis. Exp Neurol 192:60-72.

Kyrkanides S, O'Banion MK, Whiteley PE, Daeschner JC, Olschowka JA (2001) Enhanced glial activation and expression of specific CNS inflammation-related molecules in aged versus young rats following cortical stab injury. J Neuroimmunol 119:269-277.

Le AP, Friedman WJ (2012) Matrix metalloproteinase-7 regulates cleavage of pro-nerve growth factor and is neuroprotective following kainic acid-induced seizures. J Neurosci 32:703-712.

Lee S, Park JY, Lee WH, Kim H, Park HC, Mori K, Suk K (2009) Lipocalin-2 is an autocrine mediator of reactive astrocytosis. J Neurosci 29:234-249.

Lekic T, Rolland W, Hartman R, Kamper J, Suzuki H, Tang J, Zhang JH (2011) Characterization of the brain injury, neurobehavioral profiles, and histopathology in a rat model of cerebellar hemorrhage. Exp Neurol 227(1):96-103.

Li RB, Guo XC, Liang HX, Wang FY, Zhu BL (2009) Study on changes of MMP-3 expression after brain contusion in rats. Leg Med (Tokyo) 11 Suppl 1:S176-179.

Lin KT, Sloniowski S, Ethell DW, Ethell IM (2008) Ephrin-B2-induced cleavage of EphB2 receptor is mediated by matrix metalloproteinases to trigger cell repulsion. J Biol Chem 283:28969-28979.

Liu HS, Shen H, Harvey BK, Castillo P, Lu H, Yang Y, Wang Y (2011) Post-treatment with amphetamine enhances reinnervation of the ipsilateral side cortex in stroke rats. Neuroimage 56:280-289.

Lo EH, Broderick JP, Moskowitz MA (2004) tPA and proteolysis in the neurovascular unit. Stroke 35:354-356.

Luo J (2005) The role of matrix metalloproteinases in the morphogenesis of the cerebellar cortex. Cerebellum 4:239-245.

Maier O, van der Heide T, Johnson R, de Vries H, Baron W, Hoekstra D (2006) The function of neurofascin155 in oligodendrocytes is regulated by metalloprotease-mediated cleavage and ectodomain shedding. Exp Cell Res 312:500-511.

Mayer J, Hamel MG, Gottschall PE (2005) Evidence for proteolytic cleavage of brevican by the ADAMTSs in the dentate gyrus after excitotoxic lesion of the mouse entorhinal cortex. BMC Neurosci 6:52.

McKeon RJ, Höke A, Silver (1995) Injury-induced proteoglycans inhibit the potential for laminin-mediated axon growth on astrocytic scars. J Exp Neurol 136:32-43.

Mizoguchi H, Yamada K, Nabeshima T (2011) Matrix metalloproteinases contribute toneuronal dysfunction in animal models of drug dependence, Alzheimer's disease, and epilepsy. Biochem Res Int 2011:681385.

Monea S, Jordan BA, Srivastava S, DeSouza S, Ziff EB (2006) Membrane localization of

membrane type 5 matrix metalloproteinase by AMPA receptor binding protein and cleavage of cadherins. J Neurosci 26:2300-2312.

Montaner J, Alvarez-Sabín J, Molina C, Anglés A, Abilleira S, Arenillas J, González MA,

Monasterio J (2001) Matrix metalloproteinase expression after human cardioembolic stroke: temporal profile and relation to neurological impairment. Stroke 32:1759-1766.

Muir EM, Adcock KH, Morgenstern DA, Clayton R, von Stillfried N, Rhodes K, Ellis C, Fawcett JW, Rogers JH (2002) Matrix metalloproteases and their inhibitors are produced by overlapping populations of activated astrocytes. Brain Res Mol Brain Res 100:103-117.

Noble LJ, Donovan F, Igarashi T, Goussev S, Werb Z (2002) Matrix metalloproteinases limit functional recovery after spinal cord injury by modulation of early vascular events. J Neurosci 22:7526-7535.

Ogata Y, Enghild JJ, Nagase H (1992) Matrix metalloproteinase 3 (stromelysin) activates the precursor for the human matrix metalloproteinase 9. J Biol Chem 267:3581-3584.

Ogier C, Bernard A, Chollet AM, LE Diguardher T, Hanessian S, Charton G, Khrestchatisky M, Rivera S (2006) Matrix metalloproteinase-2 (MMP-2) regulates astrocyte motility in connection with the actin cytoskeleton and integrins. Glia 54:272-284.

Oh LY, Larsen PH, Krekoski CA, Edwards DR, Donovan F, Werb Z, Yong VW (1999) Matrix metalloproteinase-9/gelatinase B is required for process outgrowth by oligodendrocytes. J Neurosci 19:8464-8475.

Okulski P, Jay TM, Jaworski J, Duniec K, Dzwonek J, Konopacki FA, Wilczynski GM, Sanchez-Capelo A, Mallet J, Kaczmarek L (2007) TIMP-1 abolishes MMP-9-dependent long-lasting long-term potentiation in the prefrontal cortex. Biol Psychiatry 62:359-362.

Pauly T, Ratliff M, Pietrowski E, Neugebauer R, Schlicksupp A, Kirsch J, Kuhse J (2008) Activity-dependent shedding of the NMDA receptor glycine binding site by matrix metalloproteinase 3: a putative mechanism of postsynaptic plasticity. PLoS One 3:e2681.

Penedo LA, Oliveira-Silva P, Gonzalez EM, Maciel R, Jurgilas PB, Melibeu Ada C, Campello-Costa P, Serfaty CA (2009) Nutritional tryptophan restriction impairs plasticity of retinotectal axons during the critical period. Exp Neurol 217:108-115.

Peng H, Wu Y, Duan Z, Ciborowski P, Zheng JC (2012) Proteolytic processing of SDF-1α by matrix metalloproteinase-2 impairs CXCR4 signaling and reduces neural progenitor cell migration. Protein Cell 3:875-882.

Penkowa M, Florit S, Giralt M, Quintana A, Molinero A, Carrasco J, Hidalgo J (2005) Metallothionein reduces central nervous system inflammation, neurodegeneration, and cell death following kainic acid-induced epileptic seizures. J Neurosci Res 79:522-534.

Phillips LL, Lyeth BG, Hamm RJ, Povlishock JT (1994) Combined fluid percussion brain injury and entorhinal cortical lesion: A model for assessing the interaction between neuroexcitation and deafferentation. J Neurotrauma 11:641-656.

Phillips LL, Reeves TM (2001) Interactive Pathologies Modify Hippocampal Plasticity. Restor Neurol Neurosci 19:213-235.

Piccolini VM, Avella D, Bottone MG, Bottiroli G, Bernocchi G (2012) Cisplatin induces changes in the matrix metalloproteinases and their inhibitors in the developing rat cerebellum. Brain Res 1484:15-28.

Pizzi MA, Crowe MJ (2007) Matrix metalloproteinases and proteoglycans in axonal regeneration. Exp Neurol 204:496-511.

Plantman S (2012) Osteopontin is upregulated after mechanical brain injury and stimulates neurite growth from hippocampal neurons through β1 integrin and CD44. Neuroreport 23:647-652.

Povlishock JT, Katz DI (2005) Update of neuropathology and neurological recovery after traumatic brain injury. J Head Trauma Rehabil 20:76-94.

Ra HJ, Parks WC (2007) Control of matrix metalloproteinase catalytic activity. Matrix Biol 26:587-596.

Reeves TM, Prins ML, Zhu J, Povlishock JT, Phillips, LL (2003) Matrix metalloproteinase inhibition alters functional and structural correlates of deafferentation-induced sprouting in the dentate gyrus. J Neurosci 23:10182-10189.

Reeves TM, Zhu J, Povlishock JT, Phillips LL (1997) The effect of combined fluid percussion and entorhinal cortical lesions on long-term potentiation. Neuroscience 77:431-444.

Reiss K, Maretzky T, Ludwig A, Tousseyn T, de Strooper B, Hartmann D, Saftig P (2005) ADAM10 cleavage of N-cadherin and regulation of cell-cell adhesion and beta-catenin nuclear signaling. EMBO J 24:742-752.

Risling M, Plantman S, Angeria M, Rostami E, Bellander BM, Kirkegaard M, et al (2011) Mechanisms of blast induced brain injuries, experimental studies in rats. Neuroimage 54:S89-97.

Rosenberg GA, Navratil M (1997) Metalloproteinase inhibition blocks edema in intracerebral hemorrhage in the rat. Neurology 48:921-926.

Rosenberg GA (1995) Matrix metalloproteinases in brain injury. J Neurotrauma 12:833-842.
Safciuc F, Constantin A, Manea A, Nicolae M, Popov D, Raicu M, Alexandru D, Constantinescu E (2007) Advanced glycation end products, oxidative stress and metalloproteinases are altered in the cerebral microvasculature during aging. Curr Neurovasc Res 4:228-234.
Sagane K, Sugimoto H, Akaike A. Biological characterization of ADAM22 variants reveals the importance of a disintegrin domain sequence in cell surface expression. J Recept Signal Transduct Res. 2010;30:72-77.
Sekine-Aizawa Y, Hama E, Watanabe K, Tsubuki S, Kanai-Azuma M, Kanai Y, Arai H, Aizawa H, Iwata N, Saido TC (2001) Matrix metalloproteinase (MMP) system in brain: identification and characterization of brain-specific MMP highly expressed in cerebellum. Eur J Neurosci 13:935-948.
Shapiro L, Colman DR (1999) The diversity of cadherins and implications for a synaptic adhesive code in the CNS. Neuron 23:427-430.
Shin T, Ahn M, Kim H, Moon C, Kang TY, Lee JM, Sim KB, Hyun JW (2005) Temporal expression of osteopontin and CD44 in rat brains with experimental cryolesions. Brain Res 1041:95-101.
Snow DM, Watanabe M, Letourneau PC, Silver J (1991) A chondroitin sulfate proteoglycan may influence the direction of retinal ganglion cell outgrowth. Development 113:1473-1485.
Steward O (1989) Reorganization of neuronal connections following CNS trauma: principles and experimental paradigms. J Neurotrauma 6:99-152.
Szepesi Z, Bijata M, Ruszczycki B, Kaczmarek L, Wlodarczyk J (2013) Matrix metalloproteinases regulate the formation of dendritic spine head protrusions during chemically induced long-term potentiation. PLoS One 8:e63314.
Szklarczyk A, Ewaleifoh O, Beique JC, Wang Y, Knorr D, Haughey N, Malpica T, Mattson MP, Huganir R, Conant K (2008) MMP-7 cleaves the NR1 NMDA receptor subunit and modifies NMDA receptor function. FASEB J 22:3757-3767.
Szklarczyk A, Lapinska J, Rylski M, McKay RD, Kaczmarek L (2002) Matrix metalloproteinase-9 undergoes expression and activation during dendritic remodeling in adult hippocampus. J Neurosci 22:920-930.
Tian L, Stefanidakis M, Ning L, Van Lint P, Nyman-Huttunen H, Libert C, Itohara S, Mishina M, Rauvala H, Gahmberg CG (2007) Activation of NMDA receptors promotes dendritic spine development through MMP-mediated ICAM-5 cleavage. J Cell Biol 178:687-700.
Treloar HB, Ray A, Dinglasan LA, Schachner M, Greer CA (2009) Tenascin-C is an inhibitory boundary molecule in the developing olfactory bulb. J Neurosci 29:9405-9416.
Truettner JS, Alonso OF, Dalton Dietrich W (2005) Influence of therapeutic hypothermia on matrix metalloproteinase activity after traumatic brain injury in rats. J Cereb Blood Flow Metab 25:1505-1516.
Tschesche H, Zolzer V, Triebel S, Bartsch S (2001) The human neutrophil lipocalin supports the allosteric activation of matrix metalloproteinases. Eur J Biochem 268:1918-1928.
Tsukatani T, Fillmore HL, Hamilton HR, Holbrook EH, Costanzo RM (2003) Matrix metalloproteinase expression in the olfactory epithelium. Neuroreport 14:1135-1140.
Uhm JH, Dooley NP, Oh LY, Yong VW (1998) Oligodendrocytes utilize a matrix metalloproteinase, MMP-9, to extend processes along an astrocyte extracellular matrix. Glia 22:53-63.
Vaillant C, Meissirel C, Mutin M, Belin MF, Lund LR, Thomasset N (2003) MMP-9 deficiency affects axonal outgrowth, migration, and apoptosis in the developing cerebellum. Mol Cell Neurosci 24:395-408.
Van Hove I, Lemmens K, Van de Velde S, Verslegers M, Moons L (2012) Matrix metalloproteinase-3 in the central nervous system: a look on the bright side. J Neurochem 123:203-216.
VanSaun M, Herrera AA, Werle MJ (2003) Structural alterations at the neuromuscular junctions of matrix metalloproteinase 3 null mutant mice. J Neurocytol 32:1129-1142.
Verslegers M, Lemmens K, Van Hove I, Moons L (2013) Matrix metalloproteinase-2 and -9 as promising benefactors in development, plasticity and repair of the nervous system. Prog Neurobiol 105:60-78.
Vilalta A, Sahuquillo J, Rosell A, Poca MA, Riveiro M, Montaner (2008) Moderate and severe traumatic brain injury induce early over expression of systemic and brain gelatinases. J Intensive Care Med 34:1384-1392.
Volkmer H, Leuschner R, Zacharias U, Rathjen FG (1996) Neurofascin induces neurites by heterophilic interactions with axonal NrCAM while NrCAM requires F11 on the axonal surface to extend neurites. J Cell Biol 135:1059-1069.
Wang KX, Denhardt DT (2008) Osteopontin: role in immune regulation and stress responses. Cytokine Growth Factor Rev 19:333-345.
Wang XB, Bozdagi O, Nikitczuk JS, Zhai ZW, Zhou Q, Huntley GW (2008) Extracellular proteolysis by matrix metalloproteinase-9 drives dendritic spine enlargement and long-term potentitation coordinately. Proc Nat Acad Sci USA 105:19520-19525.
Wang X, Jung J, Asahi M, Chwang W, Russo L, Moskowitz MA, Dixon CE, Fini ME, Lo EH (2000) Effects of matrix metalloproteinase-9 gene knock-out on morphological and motor outcomes after traumatic brain injury. J Neurosci 20:7037-7042.
Warren KM, Reeves TM, Phillips LL (2012) MT5-MMP, ADAM-10, and N-cadherin act in concert to facilitate synapse reorganization after traumatic brain injury. J Neurotrauma 29:1922-1940.
Wilczynski GM, Konopacki FA, Wilczek E, Lasiecka Z, Gorlewicz A, Michaluk P, Wawrzyniak M, Malinowska M, Okulski P, Kolodziej LR, Konopka W, Duniec K, Mioduszewska B, Nikolaev E, Walczak A, Owczarek D, Gorecki DC, Zuschratter W, Ottersen OP, Kaczmarek L (2008) Important role of matrix metalloproteinase 9 in epileptogenesis. J Cell Biol 180:1021-1035.
Yang P, Baker KA, Hagg T (2005) A disintegrin and metalloprotease 21 (ADAM21) is associated with neurogenesis and axonal growth in developing and adult rodent CNS. J Comp Neurol 490:163-179.
Yi JH, Katagiri Y, Susarla B, Figge D, Symes AJ, Geller HM (2012) Alterations in sulfated chondroitin glycosaminoglycans following controlled cortical impact injury in mice. J Comp Neurol 520:3295-3313.
Yong VW, Zabad RK, Agrawal S, Goncalves Dasilva A, Metz LM (2007) Elevation of matrix metalloproteinases (MMPs) in multiple sclerosis and impact of immunomodulators. J Neurol Sci 259:79-84.
Zhang H, Adwanikar H, Werb Z, Noble-Haeusslein LJ (2010) Matrix metalloproteinases and neurotrauma: evolving roles in injury and reparative processes. Neuroscientist 16:156-170.
Zhang H, Chang M, Hansen CN, Basso DM, Noble-Haeusslein LJ (2011) Role of matrix metalloproteinases and therapeutic benefits of their inhibition in spinal cord injury. Neurotherapeutics 8:206-220.
Zhang JW, Deb S, Gottschall PE (1998) Regional and differential expression of gelatinases in rat brain after systemic kainic acid or bicuculline administration. Eur J Neurosci 10:3358-3368.
Ziebell JM, Morganti-Kossmann MC (2010) Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of traumatic brain injury. Neurotherapeutics 7:22-30.
Ziemiańska K, Konopka A, Wilczyński GM (2012) The role of extracellular proteolysis in synaptic plasticity of the central nervous system. Postepy Hig Med Dosw (Online) 66:959-975.

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