Long-term depression-inducing stimuli promote cleavage of the synaptic adhesion molecule NGL-3 through NMDA receptors, matrix metalloproteinases and presenilin/γ-secretase

Hyejin Lee¹,², Eun-Jae Lee¹,², Yoo Sung Song¹,² and Eunjoon Kim¹,²

¹Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 305-701, Korea
²Center for Synaptic Brain Dysfunctions, Institute for Basic Science (IBS), Daejeon 305-701, Korea

Long-term depression (LTD) reduces the functional strength of excitatory synapses through mechanisms that include the removal of AMPA glutamate receptors from the postsynaptic membrane. LTD induction is also known to result in structural changes at excitatory synapses, including the shrinkage of dendritic spines. Synaptic adhesion molecules are thought to contribute to the development, function and plasticity of neuronal synapses largely through their trans-synaptic adhesions. However, little is known about how synaptic adhesion molecules are altered during LTD. We report here that NGL-3 (netrin-G ligand-3), a postsynaptic adhesion molecule that trans-synaptically interacts with the LAR family of receptor tyrosine phosphatases and intracellularly with the postsynaptic scaffolding protein PSD-95, undergoes a proteolytic cleavage process. NGL-3 cleavage is induced by NMDA treatment in cultured neurons and low-frequency stimulation in brain slices and requires the activities of NMDA glutamate receptors, matrix metalloproteinases (MMPs) and presenilin/γ-secretase. These results suggest that NGL-3 is a novel substrate of MMPs and γ-secretase and that NGL-3 cleavage may regulate synaptic adhesion during LTD.

1. Introduction

Synaptic adhesion molecules play important roles in the regulation of synaptic development, function and plasticity [1–14]. A large number of synaptic adhesion molecules have recently been identified. These include neuroligins, neurexins, SynCAMs (synaptic cell adhesion molecules), LRRTMs (leucine-rich repeat transmembrane neuronal proteins, NGLs (netrin-G ligands), SALMs (synaptic adhesion-like molecules), netrin-Gs, LAR-RPTPs (leucocyte common antigen-related protein-receptor-type protein tyrosine phosphatases), EphB receptors (EphB class ephrin receptors), GluR2 (82 glutamate receptor), Cbln1 (cerebellin 1 precursor protein), TrkB (tropomyosin receptor kinase C), Slitrks (SLIT and NTRK-like proteins), MDGAs (MAM domain-containing glycosylphosphatidylinositol anchor proteins), ILIRAP1 (interleukin-1 receptor accessory protein-like 1) [15–28] and IL1RAcP (interleukin-1 receptor accessory protein) [29].

Synaptic adhesion molecules are thought to contribute to synaptic development largely through their trans-synaptic adhesions, but relatively little is known about how synaptic adhesions are structurally weakened and how this contributes to functional weakening of synapses. It has recently been shown that neurexin-1 is cleaved in an activity-dependent manner through mechanisms requiring the activation of metalloproteinases MMP-9 (matrix metalloproteinase 9) and ADAM-10.
(a disintegrin and metalloproteinase 10); this cleavage leads to both structural and functional weakening of synapses [30,31]. However, it remains unclear whether other synaptic adhesion molecules are similarly regulated. It is also not certain whether long-term depression (LTD), which is accompanied by the shrinkage of dendritic spines, loss of F-actin in spines and separation of pre- and postsynaptic structures [32–34], leads to proteolytic cleavage of synaptic adhesion molecules.

NGLS (netrin-G ligands) are a family of postsynaptic adhesion molecules with three known members: NGL-1, NGL-2 and NGL-3 [9]. The C-terminal tails of NGls interact with the postsynaptic scaffolding protein PSD-95 [19], suggesting that these interactions promote the recruitment of PSD-95-associated receptors and signalling molecules to the sites of presynaptic release [35]. Extracellular domains of NGL-1 and NGL-2 interact with netrin-G1 and netrin-G2, respectively [18,19]; these latter molecules are axonally enriched glycosylphosphatidylinositol (GPI)-anchored adhesion proteins [36–39]. NGL-3 interacts with members of the LAR family of receptor tyrosine phosphatases (LAR, PTPα and PTPμr) [20,21,40], which are critically involved in regulating presynaptic development and function [41]. More recently, LAR family proteins have been shown to interact with diverse postsynaptic adhesion molecules, including TrkC, Slitrks, IL1RAPL1 and IL1RacP [20–22,25–29] and have emerged as novel organizers of presynaptic development, although a postsynaptic role in synapse development and maintenance has also been suggested [42].

Matrix metalloproteinases (MMPs) are a group of zinc-dependent endopeptidases known to process extracellular matrix components and cell surface proteins. In the nervous system, MMPs are involved in various brain functions and dysfunctions, including brain development, synaptogenesis, synaptic plasticity, learning and memory, neuron–glia interactions, neuronal injury and neurological and neuropsychiatric disorders [43–54]. Of the numerous MMPs expressed in the nervous system, MMP-9 has been extensively characterized and shown to regulate late-phase long-term potentiation (LTP), dendritic spine morphology and learning and memory, through mechanisms including integrin signalling and NMDA (N-methyl-D-aspartate) receptor trafficking and function [55–65]. MMP-9, however, does not regulate early-phase LTP, presynaptic release, NMDA receptor-dependent LTD or metabotropic glutamate receptor (mGluR)-dependent LTD. Although a study using general MMP inhibitors has suggested a role for MMPs in the regulation of LTD [66], the regulation of LTD by MMPs, unlike MMP-dependent regulation of LTP, is not well understood. In addition, it remains unclear which substrate proteins mediate MMP-dependent regulation of LTP and LTD, although a recent study has identified subunits of NMDA receptors, which directly regulate synaptic plasticity [67], as novel substrates of MMP-7 [68]. Notably, many substrates of MMPs, including cadherins, ephrins, Eph receptors, β-dystroglycan and neurologin-1, are SynCAMs [30,31,69–76]. Given the increasing recognition that synaptic adhesion molecules are involved in the regulation of synaptic plasticity [77–81], it is possible that MMP-dependent cleavage of synaptic adhesion molecules could contribute to regulation of synaptic plasticity.

The presenilin/γ-secretase complex is a group of membrane-embedded proteases that is composed of four proteins, including presenilin, nicastrin, Aph-1 and Pen-2 [82]. Well-known substrates of γ-secretase include the amyloid β-protein precursor (APP) and Notch, which have been implicated in Alzheimer’s disease and brain development, respectively. A large number of γ-secretase substrates have recently been identified and the number is now approaching approximately 90 [83–85]. Notably, many γ-secretase substrates are synaptic surface proteins [83–85], including N-cadherin [86], ErbB4 [87,88], nectin-1α [89], syndecan-1/2/3 [85,90], GluR3 (GluA3) [91], ephrinB1/2 [92–94], EphA4/B2 [95,96], LAR [97], neurexin-1/3/8 [98,99] and neurologin-1 [30,31]. This suggests that γ-secretase could act on these proteins to regulate synapse structure and function.

In this study, we tested whether NGL-3, used as a model adhesion molecule, undergoes a series of proteolytic cleavages in a neuronal activity-dependent manner. We found that LTD-inducing chemical and electrical stimuli caused proteolytic cleavage of NGL-3 in a manner requiring the activation of NMDA receptors, MMPs and γ-secretase. These results suggest that NGL-3 cleavage may regulate synaptic structure and function during LTD.

2. Results

(a) NMDA treatment of cultured neurons induces NGL-3 cleavage

To test whether LTD induction leads to the cleavage of NGL-3, we first treated cultured hippocampal neurons with NMDA (20 μM, 3 min), which is known to induce chemical LTD in brain slices [100] and a long-lasting decrease in surface levels of AMPA receptors in cultured neurons [101,102]. Immunoblot analyses showed that NMDA treatment of cultured neurons increased the levels of an NGL-3 fragment (approx. 22 kDa) recognized by an NGL-3 antibody raised against the cytoplasmic region of NGL-3 (amino acids 622–690; #1948) (figure 1a). Three other antibodies raised against the cytoplasmic region of NGL-3 recognized the same 22 kDa band (see electronic supplementary material, figure S1), suggesting the authenticity of the band as NGL-3 C-terminal fragments (hereafter termed NGL-3-CTFs). The small levels of NGL-3-CTFs before NMDA treatment suggest that NGL-3 cleavage occurs under basal conditions. A faint band beneath the NGL-3-CTF band is not likely to be an alternative or subsequent cleavage product, because it is not recognized by other NGL-3 antibodies (see electronic supplementary material, figure S1).

1. Glutamate treatment (50 μM, 1 min) of cultured hippocampal neurons, which has been used to induce internalization of AMPA receptors in cultured neurons [103], induced the same NGL-3 cleavage (see electronic supplementary material, figure S2a), similar to the results of the NMDA treatment. In addition, KCl treatment (50 mM, 5 min) induced NGL-3 cleavage (figure 1b), suggesting that general depolarization of neurons can induce NGL-3 cleavage.

(b) NGL-3 cleavage requires NMDA receptor activity but not mGluR activity or chronic changes in neuronal activity

NMDA-induced cleavage of NGL-3 may involve the activation of NMDA receptors. Indeed, incubation of cultured neurons with APV (2-amino-5-phosphovalerate, 50 μM), an antagonist of NMDA receptors, 30 min before and during NMDA treatment (20 μM, 3 min) blocked NGL-3 cleavage (figure 1c). In addition, APV also inhibited
glutamate- and KCl-induced NGL-3 cleavage (see electronic supplementary material, figure S2a,b).

By contrast, incubation of cultured neurons with DHPG ((RS)-3,5-dihydroxyphenylglycine, 50 μM, 30 min), an agonist of group I mGluRs (mGluR1/5) known to induce mGluR-dependent LTD (mGluR-LTD) in brain slices [104–107] and AMPA receptor internalization in cultured neurons [108], had no effect on NGL-3 cleavage, suggesting that mGluR activation does not induce NGL-3 cleavage (figure 1d). In addition, blocking neuronal network activity with tetrodotoxin (1 μM, 48 h) or enhancing network activity with bicuculline (10 μM, 36 h) had no effect on NGL-3 cleavage (figure 1e). These results suggest that neither mGluR activation nor chronic modulation of neuronal activity induces NGL-3 cleavage.

(c) Long-term depression-inducing low-frequency stimulation causes NGL-3 cleavage in brain slices

We next tested whether LTD-inducing electrical stimulation in brain slices causes NGL-3 cleavage. To accomplish this, we stimulated the Schaffer collateral pathway in mouse hippocampal slices (three weeks) by low-frequency stimulation (LFS, 1 Hz, 900 pulses), a stimulation paradigm known to cause NMDA receptor-dependent LTD in rat and mouse hippocampal slices [109,110], followed by confirmation of LTD induction by electrophysiological measurements, and immunoblotting analysis of hippocampal lysates. We found that LFS-LTD induced a significant increase in NGL-3 cleavage (figure 2a), similar to the results of chemical LTD induction in cultured neurons (figure 1). The presence of NGL-3-CTFs in hippocampal slices before LTD induction could also be observed in rat slices stimulated by LFS (data not shown).

Figure 1. NGL-3 cleavage requires NMDAR activation, but not mGluR activation or chronic changes in neuronal activity. (a) NMDA treatment of cultured neurons induces NGL-3 cleavage. Rat hippocampal neurons at days in vitro (DIV) 18–21 were stimulated with NMDA (20 μM, 3 min), followed by direct lysis of the neurons in SDS-PAGE sample buffer and immunoblotting with NGL-3 antibodies (#1948). For quantitative analyses, the intensity of the 22 kDa band (indicated by an arrow) was normalized to that of α-tubulin and compared with other normalized intensities. The bar graphs represent mean ± s.e.m.; n = 3, *p < 0.05, Student's t-test. (b) KCl-dependent depolarization induces NGL-3 cleavage. Rat hippocampal neurons at DIV 18–21 were stimulated with KCl (50 mM, 5 min) followed by immunoblotting; n = 4, **p < 0.01, Student's t-test. (c) NMDA receptor activation is required for NMDA-induced NGL-3 cleavage. Rat hippocampal neurons pretreated with APV (NMDA receptor antagonist; 50 μM, 30 min) were stimulated with NMDA (20 μM, 3 min) and analysed by immunoblotting; n = 5, ***p < 0.001, one-way ANOVA. (d) Activation of NMDA receptors, but not mGluRs, leads to NGL-3 cleavage. Rat hippocampal neurons at DIV 18–21 were stimulated with NMDA (20 μM, 3 min) or DHPG (group I mGluR agonist; 50 μM, 30 min); n = 3, ***p < 0.001, one-way ANOVA. (e) Chronic inhibition or activation of cultured neurons does not induce NGL-3 cleavage. Rat hippocampal neurons at DIV 18–21 were stimulated with tetrodotoxin (TTX; 1 μM for 48 h) or bicuculline (Bic; 10 μM for 36 h); n = 3, n.s., not significant, one-way ANOVA. arb. units, arbitrary units; Ctrl, control.

Figure 2. NGL-3 cleavage occurs under basal conditions in hippocampal slices. (a) NGL-3 cleavage is detectable in CA1 hippocampal slices from 3-week-old mice. Representative immunoblot analysis of NGL-3 cleavage. Glutamate (NMDA) and bicuculline (Bic) stimulate NGL-3 cleavage. When NMDA receptor antagonist (APV) is added, stimulation-induced NGL-3 cleavage is blocked. (b) NGL-3 cleavage occurs in CA1 hippocampal slices under basal conditions. Representative immunoblot analysis of NGL-3 cleavage with and without treatment of NMDA receptor antagonist (APV) in CA1 hippocampal slices from 3-week-old mice. NGL-3 cleavage occurs under basal conditions in hippocampal slices from 3-week-old mice.

(c) Long-term depression-inducing low-frequency stimulation causes NGL-3 cleavage in brain slices

We next tested whether LTD-inducing electrical stimulation in brain slices causes NGL-3 cleavage. To accomplish this, we stimulated the Schaffer collateral pathway in mouse hippocampal slices (three weeks) by low-frequency stimulation (LFS, 1 Hz, 900 pulses), a stimulation paradigm known to cause NMDA receptor-dependent LTD in rat and mouse hippocampal slices [109,110], followed by confirmation of LTD induction by electrophysiological measurements, and immunoblotting analysis of hippocampal lysates. We found that LFS-LTD induced a significant increase in NGL-3 cleavage (figure 2a), similar to the results of chemical LTD induction in cultured neurons (figure 1). The presence of NGL-3-CTFs in hippocampal slices before LTD induction could also be observed in rat slices stimulated by LFS (data not shown).
(d) NGL-3 cleavage requires matrix metalloproteinase activity

NMDA receptor activation often leads to the activation of metalloproteinases [30,31,111]. We thus tested whether NMDA-induced NGL-3 cleavage requires MMP activity. Inhibition of MMPs with two different concentrations (2.5 and 25 μM) of GM6001, a broad-spectrum MMP inhibitor [112,113], for 30 min before and during NMDA treatment blocked NGL-3 cleavage in cultured neurons (figure 3a), suggesting that NGL-3 cleavage induced by NMDA receptor activation requires activation of MMPs.

(e) NGL-3 cleavage requires γ-secretase activity

MMP cleavage of surface membrane proteins is usually followed by subsequent cleavage of their CTFs by γ-secretase [82]. To test whether this also occurs for NGL-3, we treated...
Figure 3. NGL-3 cleavage is blocked by inhibition of MMPs and presenilin/γ-secretase. (a) MMP inhibition blocks NMDA-induced NGL-3 cleavage. Rat hippocampal neurons at DIV 18–21 were incubated with GM6001 (2.5 and 25 µM, 30 min) before and during NMDA stimulation (20 µM, 3 min), followed by immunoblotting. The bar graphs represent mean ± s.e.m; n = 4; **p < 0.01, ***p < 0.001, n.s., not significant, one-way ANOVA. (b) γ-Secretase inhibition by DAPT blocks NGL-3 cleavage, leading to an increase in the levels of NGL-3-CTFs. Hippocampal neurons pretreated with the γ-secretase inhibitor DAPT (250 nM, 2 h; 2 µM, 3 h) were stimulated with NMDA (20 µM, 3 min); n = 5, *p < 0.05, **p < 0.01, ***p < 0.001, n.s., not significant, one-way ANOVA. (c) γ-Secretase inhibition by L-685,458 blocks NGL-3 cleavage, leading to an increase in the levels of NGL-3-CTFs. Hippocampal neurons pretreated with the γ-secretase inhibitor L-685,458 (1 µM, 30 min) were stimulated with NMDA (20 µM, 3 min); n = 3, **p < 0.01, ***p < 0.001, one-way ANOVA.
cultured hippocampal neurons with DAPT (N-[N-(3,5-difluorophenacetyl)-l-alanyl]-S-phenylglycine t-butyl ester), a γ-secretase inhibitor, at two different concentrations (250 nM and 2 μM) for 2–3 h before and during NMDA treatment. The levels of NGL-3-CTFs in cultured neurons induced by NMDA treatment combined with DAPT inhibition (2 μM) were higher than those induced by NMDA alone (figure 3b). We obtained a similar result by using L-685,458 (1 μM), another γ-secretase inhibitor (figure 3c). These results suggest that NGL-3-CTFs can be further cleaved by γ-secretase.

3. Discussion

Our data indicate that LTD-inducing stimuli promote proteolytic cleavage of NGL-3 in a manner that requires the activation of NMDA receptors, MMPs and γ-secretase. These results suggest that (i) NGL-3 is a novel substrate of MMPs and γ-secretase, (ii) LTD induction promotes NGL-3 processing and (iii) NGL-3 may regulate excitatory synapse structure and function during LTD.

What might be the consequences of NGL-3 cleavage by MMPs and γ-secretase? One straightforward possibility would be the weakening of NGL-3-mediated trans-synaptic adhesion at excitatory synapses that undergo LTD (figure 4). In addition, γ-secretase-mediated cleavage of NGL-3-CTFs, the step following MMP cleavage, would further remove the C-terminal tail from NGL-3, destabilizing the interaction between NGL-3 and PSD-95 (figure 4).

NGL-3 trans-synaptically interacts with LAR family receptor protein tyrosine phosphatases (LAR, PTPβ and PTPγ) [20,21]. Therefore, NGL-3 cleavage may cause the removal of LARs from synapses, similar to the removal of presynaptic neurexins observed at the site of neuroligin-1 cleavage [30]. Functionally, LAR is a well-known regulator of presynaptic development and function [41,114–116]. Synaptic removal of LAR induced by NGL-3 cleavage is thus likely to exert a significant influence on presynaptic structure and function. Notably, LAR can be processed by ADAM-17/TACE (an α-secretase) and γ-secretase [97,117,118]. It is conceivable that NGL-3 and LAR may undergo simultaneous proteolytic cleavages by specific MMPs/ADAMs during LTD, although we could not test LTD-induced LAR cleavage in this study owing to a lack of suitable LAR antibodies.

Our results are reminiscent of the recently reported activity-dependent cleavage of neuroligin-1 mediated by MMP-9/ADAM-10 and γ-secretase [30,31]. Neurexin-1β and neurexin-3β, which interact with neuroligins, have also been shown to be substrates of γ-secretase [98,99]. The complex of neuroligins and neurexins has been clearly demonstrated to regulate diverse aspects of synapse development and function [2–4,13]. Therefore, these previous observations taken together with the results of this study support the notion that the cleavage of synaptic adhesion molecules by MMPs/ADAMs and γ-secretase regulates diverse aspects of synapse structure and function. One rather unique finding of the current study is that LTD-inducing low-frequency stimulation in slices leads to NGL-3 cleavage. It thus may be worth testing whether neuroligin-1 undergoes a similar LTD-induced cleavage and whether neuroligin-1 and NGL-3 act in a synergistic manner during LTD.

NGL-3 cleavage induced by LTD-inducing stimuli requires MMP activation. Because MMPs have been implicated in the...
regulation of synaptic plasticity including LTP and LTD [55–62,66], our data suggest that NGL-3 may be one of the downstream effectors that mediate MPP-dependent regulation of LTD. With regard to specific MMPs that may mediate NGL-3 cleavage, MMP-9 is unlikely to be involved because LTD is unaffected in MMP-9-deficient mice [58] and in slices with specific inhibition of MMP-9 [66]. Other possible MMPs include MMP-3 and MMP-7, which have been shown to regulate NMDA receptor function, synaptic plasticity, dendritic spine morphology, and learning and memory [62,68,119–126]. GM6001 used in this study is a broad-spectrum inhibitor that acts on both MMPs and ADAMs [112,113]. Therefore, ADAMs may also act on NGL-3, similar to the cleavage of neuroligin-1 by both MMP-9 and ADAM-10 [30,31]. However, ADAM-17 (also known as TACE) is unlikely to process NGL-3 because it is known to mainly regulate mGluR1/5-dependent LTD [127], and DHPG stimulation in cultured neurons or mGlur-LTD in brain slices does not induce NGL-3 cleavage (figures 1d and 2c).

NGL-3 cleavage requires γ-secretase activity. γ-Secretase has been shown to enhance LTD through the production of amyloid-β and endocytosis of NMDA receptors [128–130]. It is thus possible that NGL-3 also contributes to γ-secretase-dependent regulation of LTD. In addition, γ-secretase acts on synaptic adhesion molecules, including neurexins, neuroligins, cadherins, nectins, syndecans, LARs, ephrins and Eph receptors [83–85], and many of these molecules regulate synaptic plasticity [77,78,131–136]. Thus, the results of our study, taken together with these previous observations, corroborate the notion that γ-secretase regulates synaptic adhesion and plasticity through activity-dependent cleavage of synaptic adhesion molecules.

γ-Secretase action is usually preceded by the MMP-mediated cleavage of membrane proteins into N- and C-terminal fragments (NTFs and CTFs) [82–85]. CTFs are further processed by γ-secretase to generate intracellular domains (ICDs), which are known to regulate intracellular signalling in the cytoplasm or nucleus by, for instance, interacting with transcriptional regulators. Alternatively, ICDs are degraded by the proteasome for protein turnover. In this study, we could not observe detectable levels of NGL-3-ICDs. This suggests that NGL-3-ICDs are labile and degraded by the proteasome, similar to the case of ICDs from neuroligin-1 [31]. However, this does not exclude the possibility that NGL-3-ICDs have some cytoplasmic functions. Notably, ICDs derived from NGL-3-interacting LAR translocate to the nucleus and regulate β-catenin-dependent gene expression [97].

A member of the LAR family, PTPD (encoded by the PTPRD gene), has been associated with attention deficit/hyperactivity disorder (ADHD) [137] and restless leg syndrome [138], a disorder often comorbid with ADHD [139], autism spectrum disorder [140] and bipolar disorder [141]. This suggests the possibility that abnormalities in the trans-synaptic interactions between LAR family proteins and their postsynaptic partners including NGL-3 may contribute to the development of these disorders.

In summary, our data suggest that induction of LTD in neurons leads to proteolytic cleavage of NGL-3 in a manner requiring the activation of NMDA receptors, MMPs and γ-secretase. This cleavage may lead to the weakening of NGL-3-dependent trans-synaptic adhesion at excitatory synapses and contribute to structural and functional weakening of excitatory synapses during LTD.

4. Material and methods

(a) Antibodies and animals

Guinea pig polyclonal NGL-3 antibodies (#2020 and #2021) were raised in this study using synthetic peptides mimicking the last 30 amino acid residues of NGL-3 (CGAKGPGLNSHEPLFKSCGS KENVQVETQI). Rabbit polyclonal pan-NGL (#1583; against last 15 amino acid residues of NGL-2; CIIQTHTKDKVQETQI) [17] and rabbit polyclonal NGL-3 antibodies (#1948; against GST-NGL-3 amino acids 622–690) [21] have been described. α-Tubulin antibody was purchased from Sigma. Experiments on animals were performed in accordance with the guidelines of the Animal Welfare Committee of KAIST, Korea.

(b) Quantification of immunoblot results

NGL-3-CTF bands were quantified by normalizing the integrated intensities of the 22 kDa bands to those of tubulin bands, and comparing these normalized values from treated cultured neurons or stimulated brain slices with those from untreated control neurons or slices.

(c) Primary rat hippocampal neuron culture and drug treatment

Cultured hippocampal neurons were prepared from embryonic day 18 Sprague-Dawley rat brains. Dissociated neurons on poly-L-lysine-coated (1 mg ml⁻¹) coverslips were placed in neumobasal medium supplemented with R27 (Invitrogen), 0.5 mM L-glutamate and penicillin–streptomycin. For activation, cultured neurons at days in vitro (DIV) 18–21 were treated with NMDA (Sigma; 20 μM, 3 min), KCl (Sigma; 50 mM, 5 min) or L-glutamate (Sigma; 50 μM, 30 min). For induction of NMDA receptor-dependent LTD or mGlur-LTD, cultured neurons were treated with NMDA (20 μM, 3 min) and DHPG (Tocris; 50 μM, 30 min), respectively, and returned to normal conditioning medium. For the blockade of NMDA receptors, cultured neurons were pre-treated with APV (Tocris; 50 μM, 30 min), followed by stimulation with NMDA (20 μM, 3 min) in the presence of APV or with L-glutamate (50 μM, 1 min) or KCl (30 mM, 1 min) in the absence of APV. For chronic neuronal inhibition or activation, neurons were treated with tetrodotoxin (Tocris; TTX; 1 μM, 48 h) or bicuculline (Tocris; Bic; 10 μM, 36 h). For blockade of metalloprotease and γ-secretase activity, neurons were pretreated with GM6001 (Enzo Life Sciences; 2.5 and 25 μM, 30 min), DAPT (Sigma; 250 nM, 2 h; 2 μM, 3 h) and L-685,458 (Calbiochem; 1 μM, 30 min) before and during NMDA stimulation (20 μM, 3 min).

(d) Electrophysiology

C57BL/6 background wild-type mice of both sexes at the age of postnatal day 21–28 were used for electrophysiological experiments. Sagittal hippocampal slices (400 μm thick) were prepared using a vibratome (Leica VT1200) in ice-cold dissection buffer containing (in mM) 212 sucrose, 25 NaHCO3, 5 KCl, 1.25 NaH2PO4, 0.5 CaCl2, 3.5 MgSO4, 10 n-glucose, 1.25 l-aspartic acid and 2 Na-pyruvate bubbled with 95% O2/5% CO2. The slices were recovered at 32°C for 1 h in normal artificial cerebrospinal fluid (ACSF) (in mM: 125 NaCl, 25 NaHCO3, 2.5 KCl, 1.25 NaH2PO4, 2.5 CaCl2, 1.3 MgCl2, 10 n-glucose) and thereafter maintained at room temperature. Extracellular field recordings were performed to monitor LTD induction. Stimulation and recording pipettes were pulled from borosilicate glass capillaries (Harvard Apparatus) using a micropipette electrode puller (Narishige). Field excitatory postsynaptic potentials (fEPSPs) were recorded in the stratum radiatum of the hippocampal CA1 using pipettes filled with ACSF (1 MΩ). The Schaffer collateral pathway was
References

1. Johnson-Venkatesh EM, Umemori H. 2010 Secreted factors as synaptic activators. *Eur. J. Neurosci.* 32, 181–190. (doi:10.1111/j.1460-9568.2010.07338.x)

2. Sudhof TC. 2008 Neuregulins and neuron-rein synaptig function to cognitive disease. *Nature* 455, 903–911. (doi:10.1038/nature07456)

3. Siddiqui TJ, Craig AM. 2011 Synaptic organizing complexes. *Curr. Opin. Neurobiol.* 21, 132–143. (doi:10.1016/j.conb.2010.08.016)

4. Shen K, Scheiffele P. 2010 Genetics and cell biology of building specific synapse connectivity. *Ann. Rev. Neurosci.* 33, 473–507. (doi:10.1146/annurev.neuro.051508.135302)

5. Biederer T, Stagi M. 2008 Signaling by synaptogenic molecules. *Curr. Opin. Neurobiol.* 18, 261–269. (doi:10.1016/j.conb.2008.07.014)

6. Dalva MB, McClelland AC, Kayer MS. 2007 Cell adhesion molecules: signalling functions at the synapse. *Nat. Rev. Neurosci.* 8, 206–220. (doi:10.1038/nrn2307)

7. Brose N. 2009 Synaptogenic proteins and synaptic organizers: ‘many hands make light work’. *Neuron* 61, 650–652. (doi:10.1016/j.neuron.2009.02.014)

8. Tallafuss A, Constable JR, Washbourne P. 2010 Organization of central synapses by adhesion molecules. *Eur. J. Neurosci.* 32, 198–206. (doi:10.1111/j.1460-9568.2010.07340.x)

9. Woo J, Kwon SK, Kim E. 2009 The NGL family of synaptic adhesion molecules. *Mol. Cell Neurosci.* 1016/j.mcn.2009.05.008)

10. Williams ME, de Wit J, Ghosh A. 2010 Molecular mechanisms of synaptic specificity in developing neural circuits. *Neuron* 68, 9–18. (doi:10.1016/j.neuron.2010.09.007)

11. Yuzaki M. 2010 Synapse formation and maintenance by C1q family proteins: a new class of secreted synaptogenic organizers. *Eur. J. Neurosci.* 32, 191–197. (doi:10.1111/j.1460-9568.2010.07346.x)

12. Ko J. 2012 The leucine-rich repeat superfamily of synaptic adhesion molecules: LRRTMs and Slitrks. *Mol. Cells* 34, 335–340. (doi:10.1007/s10059-012-0113-3)

13. Krueger DD, Tuffy LP, Papadopoulos T, Brose N. 2012 The role of neurexins and neuroligins in the formation, maturation, and function of vertebrate synapses. *Curr. Opin. Neurobiol.* 22, 412–422. (doi:10.1016/j.conb.2012.02.012)

14. Missler M, Sudhof TC, Biederer T. 2012 Synaptic cell adhesion. *Cold Spring Harb. Perspect. Biol.* 4, a005694. (doi:10.1101/cshperspect.a005694)

15. de Wit J et al. 2009 LRRTM2 interacts with Neurexin1 and regulates excitatory synapse formation. *Neuron* 64, 799–806. (doi:10.1016/j.neuron.2009.12.019)

16. Ko J, Fuccillo MIV, Malenka RC, Sudhof TC. 2009 LRRTM2 functions as a neurexin ligand in promoting excitatory synapse formation. *Neuron* 64, 791–798. (doi:10.1016/j.neuron.2009.12.012)

17. Siddiqui TJ, Pancarooglu R, Kang Y, Rooyakkers A, Craig AM. 2010 LRRTMs and neuroligins bind neurexins with a differential code to cooperate in glutamate synapse development. *J. Neurosci.* 30, 7495–7506. (doi:10.1523/JNEUROSCI.0470-10.2010)

18. Lin JC, Ho WH, Gurney A, Rosenthal A. 2003 The netrin-6 ligand NGL-1 promotes the outgrowth of thalamocortical axons. *Nat. Neurosci.* 6, 1270–1276. (doi:10.1038/nneuro.1389)

19. Kim S et al. 2006 NGL family PSD-95-interacting adhesion molecules regulate excitatory synapse formation. *Nat. Neurosci.* 9, 1294–1301. (doi:10.1038/nneuro.1763)

20. Kwon SK, Woo J, Kim SY, Kim H, Kim E. 2010 Trans-synaptic adhesion between netrin-G ligand-3 (NGL-3) and receptor tyrosine phosphatases LAR, protein-tyrosine phosphatase β (PTPβ), and PTPγ via specific domains regulate excitatory synapse formation. *J. Biol. Chem.* 285, 13 966–13 978. (doi:10.1074/jbc.M109.061127)

21. Woo J, Kwon SK, Choi S, Kim S, Lee J, Dunah AW, Sheng M, Kim E. 2009 Trans-synaptic adhesion between NGL-3 and LAR regulates the formation of excitatory synapses. *Nat. Neurosci.* 12, 428–437. (doi:10.1038/nrn2279)

22. Takahashi H, Artikaiatik P, Pasad T, Bartlett TE, Wang YT, Murphy TH, Craig AM. 2011 Postsynaptic TrkC and presynaptic PTPα function as a bidirectional excitatory synaptic organizing complex. *Neuron* 69, 287–303. (doi:10.1016/j.neuron.2010.12.024)

23. Matsuoka K et al. 2010 Cbln1 is a ligand for an orphan glutamate receptor δ2, a bidirectional synaptic organizer. *Science* 328, 363–368. (doi:10.1126/science.1185152)

24. Uemura T, Lee SJ, Yasumura M, Takeuchi T, Yoshida T, Ra M, Taguchi R, Sakimura K, Mishina M. 2010 Trans-synaptic interaction of GluR62 and Neurexin through Cbln1 mediates synapse formation in the cerebellum. *Cell* 141, 1068–1079. (doi:10.1016/j.cell.2010.04.035)

25. Takahashi H et al. 2012 Selective control of inhibitory synaptic development by Slit3k-PTPs trans-synaptic interaction. *Nat. Neurosci.* 15, 389–398. (doi:10.1038/nn.3040)

26. Yim YS, Kwon Y, Nam J, Yoon H, Lee K, Kim DG, Kim E, Kim CH, Ko J. 2013 Slitrks control excitatory and inhibitory synapse formation with LAR receptor protein tyrosine phosphatases. *Proc. Natl Acad. Sci. USA* 110, 4057–4062. (doi:10.1073/pnas.1209811110)

27. Valnegri P, Montrasio C, Brambilla D, Ko J, Passafaro M, Sala C. 2011 The X-linked intellectual disability protein IL1RAPL1 regulates excitatory synapse formation by binding PTPs and RhoGAP2. *Hum. Mol. Genet.* 20, 4797–4809. (doi:10.1093/hmg/ddr418)

28. Yoshida T, Yasumura M, Uemura T, Lee SJ, Ra M, Taguchi R, Ikawara Y, Mishina M. 2011 IL-1 receptor accessory protein-like 1 associated with mental retardation and autism mediates synapse formation by trans-synaptic interaction with protein tyrosine phosphatase δ. *J. Neurosci.* 31, 13 485–13 499. (doi:10.1523/JNEUROSCI.2136-11.2011)

29. Yoshida T, Shiroshima T, Lee SJ, Yasumura M, Uemura T, Chen X, Ikawara Y, Mishina M. 2012 Interleukin-1 receptor accessory protein organizes neuronal synaptogenesis as a cell adhesion molecule. *J. Neurosci.* 32, 2588–2600. (doi:10.1523/JNEUROSCI.4637-11.2012)

30. Peixoto RT, Kunz PA, Kwon H, Mabb AM, Sabatini BL, Phlipot BD, Ehlers M. 2012 Transynaptic signaling by activity-dependent cleavage of neurelin-1. *Neuron* 76, 396–409. (doi:10.1016/j.neuron.2012.07.006)

31. Suzuki K et al. 2012 Activity-dependent proteolytic cleavage of neurelin-1. *Neuron* 76, 410–422. (doi:10.1016/j.neuron.2012.10.003)

32. Tada T, Sheng M. 2006 Molecular mechanisms of dendritic spine morphogenesis. *Curr. Opin. Neurobiol.* 16, 95–101. (doi:10.1016/j.conb.2005.12.001)

33. Bastrikova N, Gardner GA, Reece JM, Jeromin A, Dudek SM. 2008 Synapse elimination accompanies functional plasticity in hippocampal neurons. *Proc. Natl Acad. Sci. USA* 105, 17 692–17 697. (doi:10.1073/pnas.0803760105)
plasticity. *Adv. Exp. Med. Biol.* 970, 153–171. (doi:10.1007/978-3-7093-0953-8_7)

48. Gundelfinger ED, Frischknecht R, Croquet D, Heine M. 2010 Converting juvenile into adult plasticity: a role for the brain’s extracellular matrix. *Eur. J. Neurosci.* 31, 2156–2165. (doi:10.1111/j.1460-9568.2010.07253.x)

49. Dityatev A, Rusakov DA. 2011 Molecular signals of plasticity at the tetrapartite synapse. *Curr. Opin. Neurobiol.* 21, 353–359. (doi:10.1016/j.conb.2010.12.006)

50. Dityatev A, Schachner M, Sonderegger P. 2010 The dual role of the extracellular matrix in synaptic plasticity and homeostasis. *Nat. Rev. Neurosci.* 11, 735–746. (doi:10.1038/nrn2898)

51. Milward EA, Fitzsimmons C, Szklarczyk A, Konant G. 2007 The matrix metalloproteinases and CNS plasticity: an overview. *J. Neuroimmunol.* 187, 9–19. (doi:10.1016/j.jneurneuroimmunol.2007.04.010)

52. Rivas S, Khreischatiity K, Kaczmarek L, Rosenberg GA, Jaworski DM. 2010 Metzincin proteases and their inhibitors: foes or friends in nervous system physiology? *J. Neurosci.* 30, 15 337–15 357. (doi:10.1523/JNEUROSCI.3467-10.2010)

53. Mizoguchi H, Yamada K, Nakanishi T. 2011 Matrix metalloproteinases contribute to neuronal dysfunction in animal models of drug dependence, Alzheimer’s disease, and epilepsy. *Biochem. Res. Int.* 2011, 681385. (doi:10.1155/2011/681385)

54. Wright JW, Harding JW. 2009 Contributions of matrix metalloproteinases to neural plasticity, habitation, associative learning and drug addiction. *Neural Plasticity* 2009, 579382. (doi:10.1155/2009/579382)

55. Wang XB, Bozdagi O, Nikicztuz JS, Zhai ZW, Zhou Q, Huntley GW. 2008 Extracellular proteolysis by matrix metalloproteinase-9 drives dendritic spine enlargement and long-term potentiation coordinately. *Proc. Natl. Acad. Sci. USA* 105, 19 520–19 525. (doi:10.1073/pnas.0807248105)

56. Nagy V, Bozdagi O, Huntley GW. 2007 The extracellular protease matrix metalloproteinase-9 is activated by inhibitory avoidance learning and required for long-term memory. *Learn. Mem.* 14, 655–664. (doi:10.1101/lm.678307)

57. Bozdagi O, Nagy V, Kwei KT, Huntley GW. 2007 In vivo roles for matrix metalloproteinase-9 in mature hippocampal synaptic plasticity and physiology. *J. Neurophysiol.* 98, 334–344. (doi:10.1152/jn.00202.2007)

58. Nagy V et al. 2006 Matrix metalloproteinase-9 is required for hippocampal late-phase long-term potentiation and memory. *J. Neurosci.* 26, 1923–1934. (doi:10.1523/JNEUROSCI.4359-05.2006)

59. Michaluk P, Mikirosava L, Groc L, Frischknecht R, Croquet D, Kaczmarek L. 2009 Matrix metalloproteinase-9 controls NMDA receptor surface diffusion through integrin α1 signaling. *J. Neurosci.* 29, 6007–6012. (doi:10.1523/JNEUROSCI.5346-08.2009)

60. Gorkiewicz T, Szczeszakiewicz K, Wyrembek P, Michaluk P, Kaczmarek L, Mozrzymas JW. 2010 Matrix metalloproteinase-9 reversibly affects the time course of NMDA-induced currents in cultured rat hippocampal neurons. *Hippocampus* 20, 1105–1108. (doi:10.1002/hipo.20736)

61. Fragnioli A, Papahedonoporto C, Georgopoulos S, Stamatakis A, Stylianos P, Tullibary EC, Tzina AK. 2012 Enhanced neuronal plasticity and elevated endogenous sAPPα levels in mice over-expressing MMP9. *J. Neurochem.* 121, 239–251. (doi:10.1111/j.1471-4159.2011.07637.x)

62. Meighan SE, Meighan PC, Coudhary P, Davis CJ, Olson ML, Zomes PA, Wright JW, Harding JW. 2006 Effects of extracellular matrix-degrading proteases matrix metalloproteinases 3 and 9 on spatial learning and synaptic plasticity. *J. Neurochem.* 96, 1227–1241. (doi:10.1111/j.1471-4159.2005.03565.x)

63. Dziembowska M, Wlodarczyk J. 2012 MMP9: a novel function in synaptic plasticity. *Int. J. Biochem. Cell Biol.* 44, 709–713. (doi:10.1016/j.biocel.2012.01.023)

64. Michaluk P et al. 2011 Influence of matrix metalloproteinase MMP-9 on dendritic spine morphology. *J. Cell Sci.* 124, 3369–3380. (doi:10.1242/jcs.090852)

65. Wilczynski GM et al. 2008 Important role of matrix metalloproteinase 9 in epileptogenesis. *J. Cell Biol.* 180, 1021–1035. (doi:10.1083/jcb.200708213)

66. Meighan PC, Meighan SE, Davis CJ, Wright JW, Harding JW. 2007 Effects of matrix metalloproteinase inhibition on short- and long-term plasticity of Schaffer collateral/CA1 synapses. *J. Neurochem.* 102, 2085–2096. (doi:10.1111/j.1471-4159.2007.04682.x)

67. Bliss TV, Collingridge GL. 1993 A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361, 31–39. (doi:10.1038/361031a0)

68. Szak´czyk A et al. 2008 MMP-7 cleaves the Nr1 NMDA receptor subunit and modifies NMDA receptor function. *FASEB J.* 22, 3757–3767. (doi:10.1096/fj.07-10402)

69. Braemken PR, Lanahan AA, OB R, Roche K, Barnes CA, Huganir RL, Worley PF. 1997 Homer: a protein that selectively binds metabotropic glutamate receptors. *Nature* 386, 284–288. (doi:10.1038/386284a0)

70. Lin KT, Sloniovski 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, 28 969–28 979. (doi:10.1074/jbc.M804041200)

71. 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. (doi:10.1523/JNEUROSCI.3521-05.2006)

72. Conant K, Lim ST, Randall B, Maguire-Zeiss KA. 2012 Matrix metalloproteinase dependent cleavage of cell adhesion molecules in the pathogenesis of CNS dysfunction with HIV and methamphetamine. *Curr. HIV Res.* 10, 384–391. (doi:10.2174/157016212802138733)

73. Conant K, Lonskaya I, Szak´czyk A, Krall C, Steiner J, Maguire-Zeiss K, Lim ST. 2011 Methamphetamine-associated cleavage of the synaptic adhesion...
molecule intercellular adhesion molecule-5. *J. Neurochem.* **118**, 521 – 532. (doi:10.1111/j.1471-4159.2010.07153.x)

74. Michalak P, Kolodziej L, Mioduczewska B, Wilczynski GM, Dzwojew J, Jaworski J, Gorecki DC, Ottersen OP, Kaczmarek L. 2007 β3-dystroglycan as a target for MM-P-9, in response to enhanced neuronal activity. *J. Biol. Chem.* **282**, 16 036 – 16 041. (doi:10.1074/jbc.M70641200)

75. Bajor M, Michalak P, Gulyassy P, Kekesi AK, Juhasz G, Kaczmarek L. 2012 Synaptic cell adhesion molecule-2 and collapsin response mediator protein-2 are novel members of the matrix metalloproteinase-9 degradome. *J. Neurosci.* **122**, 775 – 788. (doi:10.1111/j.1471-4159.2012.07829.x)

76. Conant K, Wang Y, Szklayczyk A, Dudak A, Mattson MP, Lim ST. 2010 Matrix metalloproteinase-dependent shedding of intercellular adhesion molecule-5 occurs with long-term potentiation. *Neuroscience* **166**, 508 – 521. (doi:10.1016/j.neuroscience.2009.12.061)

77. Tai CY, Kim SA, Schuman EM. 2008 Cadherins and synaptic plasticity. *Curr. Opin. Cell Biol.* **20**, 275 – 283. (doi:10.1016/j.ceb.2008.06.003)

78. Lai KO, Ip NY. 2009 Synapse development and plasticity: roles of ephrin/Eph receptor signaling. *Curr. Opin. Neurobiol.* **19**, 275 – 283. (doi:10.1016/j.conb.2009.04.009)

79. Robbins EM, Knupp AJ, Perez de Arce K, Ghosh AK, Fogel AJ, Bourard A, Sudhof TC, Stein V, Biedere J. 2010 SynCAM 1 adhesion dynamically regulates synapse number and impacts plasticity and learning. *Neuron* **68**, 894 – 906. (doi:10.1016/j.neuron.2010.11.003)

80. Dityatev A, Bukalo O, Schachner M. 2008 Modulation of synaptic transmission and plasticity by cell adhesion and regulation molecules. *Neuron Glia Biol.* **4**, 197 – 209. (doi:10.1016/S1740-925X(09)90111-1)

81. Bliss T, Eerting M, Fransen E, Godfried JM, Kauer JA, Kooy RF, Maness PS, Furley AJW. 2000 Long-term potentiation in mice lacking the neural cell adhesion molecule L1. *Curr. Biol.* **10**, 1607 – 1610. (doi:10.1016/S0960-9822(00)00865-4)

82. Wolfe MS. 2009 γ-secretase in biology and medicine. *Semin. Cell Dev. Biol.* **20**, 219 – 224. (doi:10.1016/j.secmdb.2008.12.011)

83. Kopan R, Ilagan MX. 2004 γ-secretase: proteasome of the membrane? *Nat. Rev. Mol. Cell Biol.* **5**, 499 – 504. (doi:10.1038/nrm1406)

84. Haapasaalo A, Kovacs DM. 2011 The many substrates of presenilin1/γ-secretase. *J. Alzheimer’s Dis.* **25**, 3 – 28.

85. Hemming ML, Elias JE, Gygi SP, Selkoe DJ. 2008 Proteomic profiling of γ-secretase substrates and mapping of substrate requirements. *PLoS Biol.* **6**, e257. (doi:10.1371/journal.pbio.0060257)

86. Marambaud P, Wen PH, Dutt A, Shioi J, Takashima A, Simon R, Rubakis NK. 2003 A CBP binding transcriptional repressor produced by the PS1/epilson-cleavage of N-cadherin is inhibited by PS1 FAD mutations. *Cell* **114**, 635 – 645. (doi:10.1016/j.cell.2003.08.008)

87. Ni CY, Murphy MP, Golde TE, Carpenter G. 2001 γ-secretase cleavage and nuclear localization of Ebb-4 receptor tyrosine kinase. *Science* **294**, 2179 – 2181. (doi:10.1126/science.1065412)

88. Ni CY, Yuan H, Carpenter G. 2003 Role of the Ebb-4 carboxyl terminus in γ-secretase cleavage. *J. Biol. Chem.* **278**, 4561 – 4565. (doi:10.1074/jbc.M21004200)

89. Kim DV, Ingano LA, Kovacs DM. 2002 Nectin-1, an immunoglobulin-like receptor involved in the formation of synapses, is a substrate for presenilin/γ-secretase-like cleavage. *J. Biol. Chem.* **277**, 49 976 – 49 981. (doi:10.1074/jbc.M21079200)

90. Schulz GJ, Annaert W, Vandekerckhove J, Zimmermann P, De Strooper B, David G. 2003 Syndecan 3 intamembrane proteolysis is presenilin/γ-secretase dependent and modulates cytotoxic signaling. *J. Biol. Chem.* **278**, 48 651 – 48 657. (doi:10.1074/jbc.M308424200)

91. Meyer EL, Strutz N, Gaining LC, Rogers SW. 2003 Glutamate receptor subunit 3 is modified by site-specific limited proteolysis including cleavage by γ-secretase. *J. Biol. Chem.* **278**, 23 786 – 23 796. (doi:10.1074/jbc.M30160200)

92. Waschbusch D, Born S, Niediek V, Kirchgessner N, Tamboli IV, Walter J, Merkel R, Hoffmann B. 2009 Presenilin 1 affects focal adhesion site formation and cell force generation via e-Src transcriptional and posttranslational regulation. *J. Biol. Chem.* **284**, 10 138 – 10 149. (doi:10.1074/jbc.M60825200)

93. Tomita T, Tanaka S, Morohashi Y, Iwatsubo T. 2006 Presenilin-dependent intamembrane cleavage of ephrin-B1. *Mol. Neurodegener.* **1**, 2. (doi:10.1186/1742-7001-1-2)

94. Georgakopoulos A, Litterst C, Ghiéri E, Baki L, Xu C, Serban G, Rubakis NK. 2006 Metalloproteinase/Preselin1 processing of ephrinB regulates EphB-induced Snr phosphorylation and signaling. *EMBO J.* **25**, 1242 – 1252. (doi:10.1038/sj.emboj.7601031)

95. Inoue E et al. 2009 Synthetic activity prompts γ-secretase-mediated cleavage of EphA4 and dendritic spine formation. *J. Cell Biol.* **185**, 551 – 564. (doi:10.1083/jcb.200809151)

96. Litterst C, Georgakopoulos A, Shioi J, Ghiéri E, Winневski T, Wang R, Ludwig A, Rubakis NK. 2007 Ligand binding and calcium influx induce distinct ectodomain-γ-secretase-processing pathways of EphB2 receptor. *J. Biol. Chem.* **282**, 16 155 – 16 163. (doi:10.1074/jbc.M601499200)

97. Haapasaalo A, Kim DV, Carey BW, Turunen MK,introsion J, Bear MF. 2007 Activation of NMDA receptors promotes dendritic spine development through MPP-mediated ICAM-5 cleavage. *J. Cell Biol.* **178**, 687 – 700. (doi:10.1016/j.jcb.200612079)

98. Grobelyn D, Poncz L, Galyard RE. 1992 Inhibition of human skin fibroblast collagenase, thromlysin, and *Pseudomonas aeruginosa* elastase by peptide
hydroxamic acids. Biochemistry 31, 7152–7154. (doi:10.1021/bi00146a017)

113. Schultz GS et al. 1992 Treatment of alkali-injured rabbit corneas with a synthetic inhibitor of matrix metalloproteinases. Invest. Ophthalmol. Vis. Sci. 33, 3325–3331.

114. Johnson KG, Van Vactor D. 2003 Receptor protein tyrosine phosphatases in nervous system development. Physiol. Rev. 83, 1–24.

115. Gundelfinger ED, Fejtova A. 2012 Molecular organization and plasticity of the cytomatrix at the active zone. Curr. Opin. Neurobiol. 22, 432–433. (doi:10.1016/j.conb.2011.10.005)

116. Jin Y, Garner CC. 2008 Molecular mechanisms of synaptic plasticity. Curr. Opinion. Neurobiol. 18, 1515–1527. (doi:10.1016/j.conb.2005.12.003)

117. Aicher B, Lerch MM, Muller T, Schilling J, Ullrich A. 2006 EGFR signaling leads to downregulation of FPR-LAR via TACE-mediated proteolytic processing. Cell Signal. 18, 1515–1527. (doi:10.1016/j.cellsig.2005.12.003)

118. Ruhe JE, Streit S, Hart S, Ullrich A. 2006 Matrix metalloproteinase-7 disrupts dendritic spine development. J. Neurochem. 97, 44–56. (doi:10.1111/j.1471-4159.2006.03701.x)

119. Bilousova TV, Rusakov DA, Ethell DW, Ethell IM. 2006 Matrix metalloproteinase-7 disrupts dendritic spines in hippocampal neurons through NMDA receptor activation. J. Neurochem. 97, 44–56. (doi:10.1111/j.1471-4159.2006.03701.x)

120. Wright JW, Meighan PC, Brown TE, Wiediger RV, Schlicksupp A, Kirsch J, Kuhse J, Koch K-W. 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. (doi:10.1371/journal.pone.0002681)

121. Jackson KG, Van Vactor D. 2003 Receptor protein tyrosine phosphatases in nervous system development. Physiol. Rev. 83, 1–24.

122. Johnson KG, Van Vactor D. 2003 Receptor protein tyrosine phosphatases in nervous system development. Physiol. Rev. 83, 1–24.