NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders

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Abstract | The number and subunit composition of synaptic *N*-methyl-D-aspartate receptors (NMDARs) are not static, but change in a cell- and synapse-specific manner during development and in response to neuronal activity and sensory experience. Neuronal activity drives not only NMDAR synaptic targeting and incorporation, but also receptor retrieval, differential sorting into the endosomal–lysosomal pathway and lateral diffusion between synaptic and extrasynaptic sites. An emerging concept is that activity-dependent, bidirectional regulation of NMDAR trafficking provides a dynamic and potentially powerful mechanism for the regulation of synaptic efficacy and remodelling, which, if dysregulated, can contribute to neuropsychiatric disorders such as cocaine addiction, Alzheimer's disease and schizophrenia.

Long-term potentiation (LTP). Activity-dependent strengthening of synaptic transmission that is long-lasting (usually more than one hour). Commonly induced by brief, high-frequency stimulation, LTP is widely believed to be a key cellular mechanism involved in learning and memory.

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N-methyl-D-aspartate receptors (NMDARs) are glutamate-gated ion channels that are pivotal in the regulation of synaptic function in the CNS. NMDARs are heteromeric assemblies of NR1, NR2 and NR3 subunits, which co-translationally assemble in the endoplasmic reticulum (ER) to form functional channels with differing physiological and pharmacological properties and distinct patterns of synaptic targeting[1–3]. Additional molecular diversity arises through alternative RNA splicing of the NR1 subunit (for a review, see REF. 4). Each NMDAR subunit contains a large amino (N)-terminal extracellular domain, three membrane-spanning domains, a re-entry, or 'hairpin', loop that forms the pore-lining region (membrane domain 2) and an intracellular carboxy (C)-terminal domain. NMDARs are highly permeable to Ca^{2+}, and Ca^{2+} influx through NMDARs is essential for synaptogenesis, experience-dependent synaptic remodelling and long-lasting changes in synaptic efficacy such as long-term potentiation (LTP) and long-term depression (LTD)[5].

The identity of the NR2 subunit determines many of the biophysical and pharmacological properties of NMDARs, and can also influence NMDAR assembly, downstream signalling, receptor trafficking and synaptic targeting[1]. The NR2A subunit confers a lower affinity for glutamate, distinctly faster kinetics, greater channel open probability and more prominent Ca^{2+}-dependent desensitization than does the NR2B subunit, which confers slower channel kinetics and reduced open probability. The NR2C and NR2D subunits are characterized by low conductance openings and reduced sensitivity to Mg^{2+} block[1]. The NR3 subunit confers reduced Ca^{2+} permeability and reduced surface expression[1]. Since most central NMDARs are NR1/NR2 assemblies, this article will focus on the role of NR1 and NR2 subunits in receptor trafficking.

Synaptic NMDARs are localized to postsynaptic densities (PSDs), where they are structurally organized (and spatially restricted) in a large macromolecular signalling complex of synaptic scaffolding and adaptor proteins which physically links the receptors to kinases, phosphatases and other downstream signalling proteins and to group I metabotropic glutamate receptors (mGluRs) (FIG. 1). mGluRs are G-protein-coupled receptors that are implicated in synaptic plasticity, excitability and neuronal connectivity[6]. PSD protein of 95 kDa (PSD-95; also known as synapse-associated protein-90) and synapse-associated protein-102 (SAP-102) are synaptic scaffolding proteins and members of the large PSD-95 family of modular PDZ-containing proteins that anchor NMDARs in the PSD (for a review, see REF. 7). In addition to their role in synaptic anchoring, PDZ proteins are important in the intracellular trafficking and synaptic delivery of NMDARs.

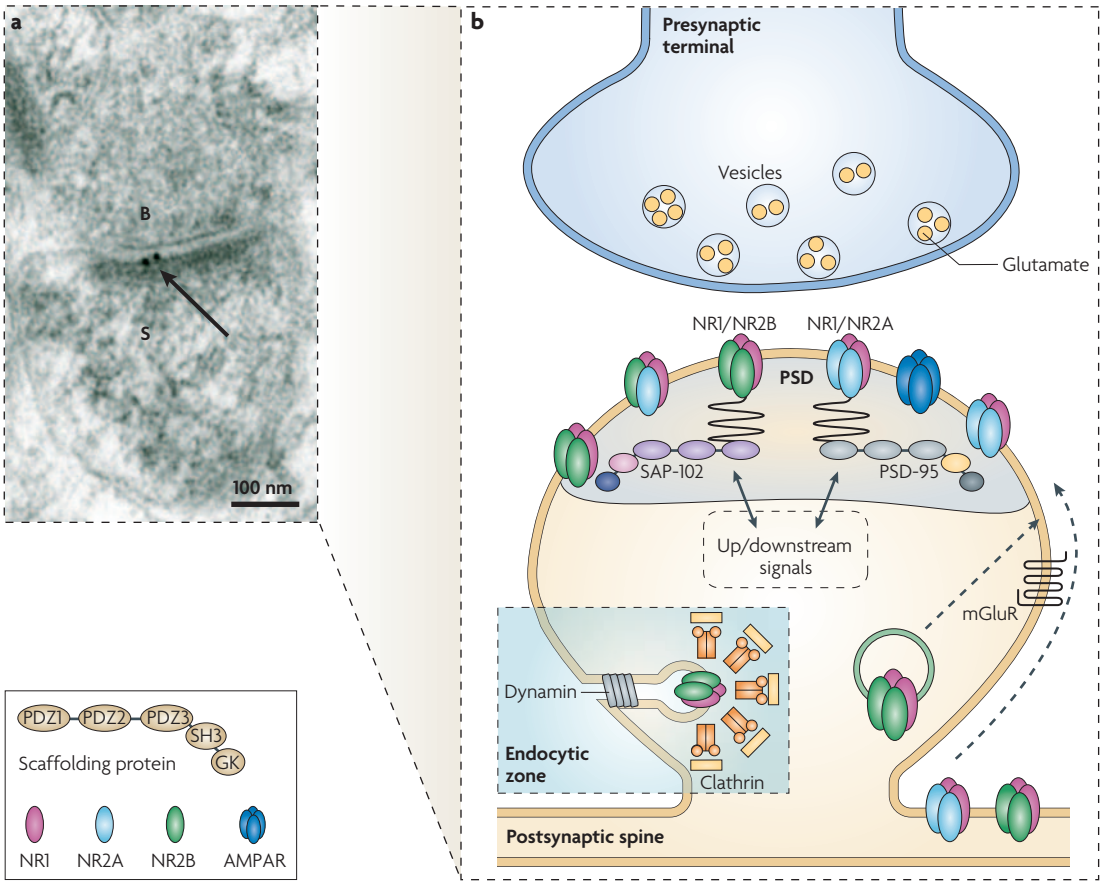

Figure 1 | **The NMDAR macromolecular signalling complex at excitatory synapses. a** | Electron micrograph showing the localization of NR2A subunits in the postsynaptic density (PSD) on dendritic spines (S) in the rat hippocampal CA1 region. Arrow indicates NR2A molecules immunolabelled with gold particles. **b** | Synaptic NMDARs (*N*-methyl-D-aspartate receptors) and AMPARs (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors) are localized to the PSD (grey area), where they are structurally organized and spatially restricted in a large macromolecular signalling complex comprising scaffolding and adaptor proteins. These patterns physically link the receptors to signalling proteins and to group I mGluRs, which localize to the perisynaptic region. At mature synapses NMDARs are predominantly composed of NR1/NR2A or NR1/NR2A/NR2B assemblies, which associate with the scaffolding proteins PSD protein of 95 kDa (PSD-95) and synapse-associated protein of 102 kDa (SAP-102). These scaffolding proteins link NMDARs directly or indirectly to a number of signalling proteins, such as protein kinase A (PKA) and PKC, which are thought to be important in the regulation of NMDAR number and function at synaptic sites (upstream/downsteam signalling molecules). NMDARs internalize via clathrin-coated pits at specialized endocytic zones located tangential to the PSD[57,58]. Dynamin is localized at the neck of these pits, assisting fission. B, synaptic bouton; mGluR, metabotropic glutamate receptor. Panel **a** reproduced, with permission, from REF. 149 © (2005) Elsevier Science.

Long-term depression
(LTD). A long-lasting suppression of synaptic strength that is elicited by specific patterns of synaptic stimulation (for example, low-frequency stimulation). LTD is typically dependent on NMDA-receptor activation, and is widely believed to be a means of information storage in the brain.

Open probability
The fraction of time that a single channel remains open when fully activated by ligand or voltage.

Postsynaptic density
(PSD). An electron-dense specialization of excitatory postsynaptic membranes that contains a high concentration of glutamate receptors, ion channels, kinases, phosphatases and associated signalling and cytoskeletal proteins.

Ubiquitin–proteasome system
Ubiquitin is a 76 amino-acid protein that serves as a tag to mark proteins destined for degradation. Proteins tagged by a polyubiquitin chain are targeted to the proteasome, a large, multimeric barrel-like complex that acts by proteolysis to degrade proteins.

New research provides evidence that synaptic NMDAR number and subunit composition are not static, but change dynamically in a cell-specific and synapse-specific manner during development and in response to neuronal activity or sensory experience. Whereas blocking neuronal activity promotes alternative RNA splicing and accelerates forward trafficking of NMDARs to the synapse, chronic activity drives receptor internalization and degradation through the ubiquitin–proteasome system. Here we review the literature describing dynamic trafficking of NMDARs to and from the synapse, the role of such trafficking in synaptic plasticity and remodelling, and its dysregulation in neuropsychiatric disorders such as cocaine addiction, chronic alcohol abuse, schizophrenia and Alzheimer's disease.

Targeting of NMDARs to synapses

Delivery of NMDAR packets to nascent synapses. Normal NMDAR activity requires accurate delivery and targeting to the synapse. At nascent synapses, assembled NMDARs are targeted selectively to the postsynaptic side of glutamatergic synapses[8] and appear (together with α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs)) 1–2 hours after initial axodendritic contact[9].

Time-lapse imaging of fluorescently tagged receptor subunits in rat cortical neurons at young ages *in vitro* show that NMDAR and AMPAR subunits are present in distinct mobile transport packets that are recruited rapidly and independently to sites of axodendritic contact[10,11] (FIG. 2). At the time of synaptogenesis, NMDARs are transported along microtubules more

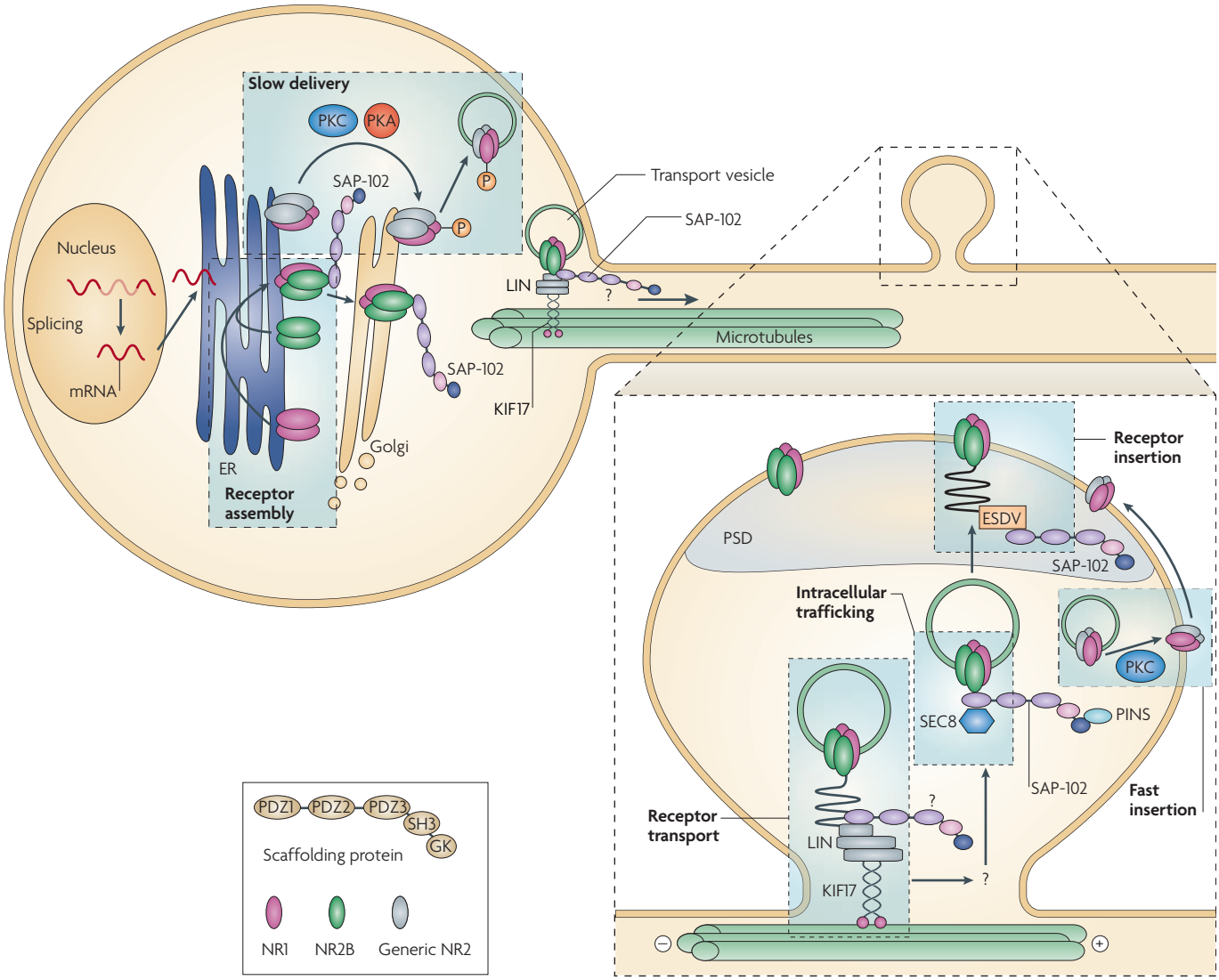

Figure 2 | **NMDAR assembly and transport to dendritic spines.** Assembled NMDARs (*N*-methyl-D-aspartate receptors) exit the endoplasmic reticulum (ER) and proceed through the Golgi network to the neuronal surface. Packets of nascent receptors are transported along microtubule tracks from the cell body to synaptic sites as part of a large, vesicle-associated macromolecular complex that contains the assembled receptor and synapse-associated protein-102 (SAP-102)[10,11]. The NR2B subunit links through adaptor proteins (LIN2, LIN7 and LIN10) to the motor protein kinesin family member 17 (KIF17)[14,15]. It is thought that during forward trafficking from the ER, SAP-102 links the NR2B subunit to SEC8, a component of the exocyst complex; association with the exocyst enhances basal delivery of receptors to the plasma membrane[44]. The number of NMDARs stabilized at the cell surface represents a balance between clathrin-mediated internalization and insertion, which is facilitated by scaffolding proteins such as SAP-102. Activation of protein kinase C (PKC) and PKA regulates NMDAR trafficking by phosphorylating NR1 and promoting ER export and slow delivery of NMDARs to the plasma membrane over a period of hours[36,46]. On a shorter timescale, namely within minutes, PKC enhances NMDA channel opening and rapidly inserts NMDARs at the cell surface[47]. Dashed boxes indicate critical steps in receptor assembly, transport, intracellular trafficking, slow delivery and insertion at synapses.

Exocyst complex
A macromolecular multimeric protein complex involved in directing cargo-loaded vesicles to sites of fusion in the plasma membrane; it is often concentrated at sites of active secretion and cell growth.

Plus-end-directed motor
Plus-end-directed motors transport cargo from the minus to the plus end of microtubules (in the anterograde direction, or from the neuronal cell body out into the neuronal process).

rapidly than AMPARs, at a mean velocity of ~4 μm min^{-1}. NMDAR packets are vesicle-associated protein complexes containing the scaffolding protein SAP-102 and early endosomal antigen 1 (EAA1)[11]. Before synapse formation, NMDARs cycle in and out of the dendritic plasma membrane during their long journey from the cell body to distal dendrites[11]. By contrast, at older ages *in vitro*, when the majority of synapses have formed, NMDAR subunits are recruited more gradually in the form of clusters containing a small number of receptors to nascent synapses[12]. Thus, dendritic transport and synaptic recruitment of NMDARs might occur via distinct mechanisms at differing ages *in vitro*.

Kinesin KIF17, a plus-end-directed motor, transports NMDAR-containing vesicles together with the adaptor protein LIN10 (also known as MINT1 or XII) along microtubules in dendrites to nascent synapses (for a review, see REF. 13). KIF17 interacts through its N-terminal domain with the PDZ domain of LIN10, which binds through intermediate adaptor

Extrasynaptic receptors
A receptor population located in a region of the dendritic or somatic membrane outside of the postsynaptic density and that is not activated by a single pulse of neurotransmitter release.

Excitatory postsynaptic potentials
(EPSPs). A transient postsynaptic membrane depolarization caused by presynaptic release of neurotransmitter.

proteins (LIN2 and LIN7) to the NR2B subunit (FIG. 2). Interestingly, the genes encoding KIF17 and NR2B are coregulated[14,15]. In neurons, KIF17 colocalizes with NR2B and moves along dendrites in anterograde and retrograde directions at an average speed of ~0.76 μm sec^{-1} (REFS 14,15). In addition, overexpression of KIF17 enhances working memory and spatial learning in transgenic mice, which indicates a role for KIF17-dependent transport in synaptic plasticity[16].

Subunit-specific rules for NMDAR synaptic targeting. At immature synapses, targeting of nascent NMDARs is tightly regulated and follows subunit-specific rules. At nascent synapses of hippocampal neurons in culture, NMDARs are primarily NR1–NR2B heterodimers and are outnumbered 3:1 by NMDA extrasynaptic receptors[17]. During postnatal development of mammalian forebrain, there is an increase in the expression and subsequent number of NR2A subunits[18–20], which assemble to form NR1–NR2A heterodimers and NR1–NR2A–NR2B heterotrimers[17] (FIG. 1). The heterotrimeric receptors are identified by their lower affinity for ifenprodil compared with pure NR1/NR2B receptors[17,21]. One hypothesis is that synapses form promiscuously and that input-specific activity promotes fine-tuning of the NMDAR phenotype. As NR1–NR2A channels display the fastest decay kinetics, this conversion in synaptic NMDAR subunit composition results in the shortening of NMDAR-mediated synaptic currents and profoundly affects synaptic plasticity[22] (see below). Surprisingly, despite extensive research on NMDARs over the past two decades, we have little understanding of the number, subunit composition and function of synaptic versus extrasynaptic NMDARs in mature neurons (BOX 1).

Synaptic scaffolding proteins such as PSD-95, SAP-97, SAP-102 and PSD-93 (also known as chapsyn-110) are important in ensuring the accurate targeting of nascent NMDARs and the exchange of NR2A for NR2B[7]. PSD-95 promotes NMDAR clustering[23], its surface expression[24–26] and the targeting of NR2A versus NR2B to synapses[27]. PDZ proteins directly bind NMDARs via PDZ recognition motifs, contained at the distal end of the long, C-terminal tail of the NR2 subunits[25,28]. Accordingly, mice expressing C-terminally truncated NR2A and NR2B subunits exhibit improper localization of synaptic NMDARs[29,30]. PSD-95 also couples synaptic NMDARs to downstream signalling molecules such as neuronal nitric oxide synthase (nNOS)[31]. In addition, PSD-95 enhances NMDA channel opening[25] and reduces the desensitization of NMDA responses[32]. A structural and functional homologue of PSD-95, PSD-93 is expressed abundantly in the spinal cord and forebrain. Mice deficient in PSD-93 exhibit reduced surface expression of NR2A and NR2B, lower NMDA excitatory postsynaptic potentials at synapses of the spinal cord and anterior cingulate cortex and blunted NMDAR-dependent persistent pain in response to peripheral nerve injury[33]. Unexpectedly, NMDA excitatory postsynaptic currents (EPSCs) at CA1 synapses of mice deficient in both PSD-93 and PSD-95 are only modestly reduced even after knockdown of SAP-102 (REF. 34), suggesting potential redundancy among modular (PDZ-containing) synaptic scaffolding proteins or the existence of PDZ protein-independent synaptic targeting of NMDARs. Further studies are warranted to fully understand the role of PDZ proteins in NMDAR targeting and function.

Regulation of NMDARs by proteasomal degradation. The number and subunit composition of synaptic NMDARs are regulated not only by stimulus-dependent gene expression and protein synthesis, but also by activity-dependent protein degradation by the ubiquitin–proteasome system[35]. This method of regulated protein degradation is crucial for synapse growth and development, synaptic transmission and plasticity at mammalian synapses, and can mediate remodelling of protein composition of synaptic structures. Synaptic activity accelerates the turnover rate of a subset of PSD proteins (such as NR1, NR2B and SAP-102); by contrast, the turnover rate of other proteins (such as NR2A, mGluR1 and PSD-95) is relatively stable[35]. As a consequence, the relative abundance of NR2A and PSD-95 increases in active synapses and decreases relative to the abundance of NR2B and SAP-102 in inactive synapses (FIG. 3). Therefore, ubiquitin-based protein degradation is crucial to the homeostatic control of synaptic NMDAR strength, and provides a link between synaptic activity, protein turnover and the reorganization of PSDs.

Cycling of NMDA receptors

NMDAR insertion into the plasma membrane. Newly synthesized NMDARs are delivered to the synapse after synthesis in the ER. Localization signals that regulate delivery of NMDARs to, and retrieval from, the plasma membrane have been identified in the C-terminal tails of the NR1 subunits[26,28,36–41] (FIG. 4a). Longer NR1 subunits are retained in the ER owing to the presence of an ER

Box 1 | Extrasynaptic NMDARs: commuters at rush hour?

Relatively little is known about the composition, number, function and/or downstream signalling pathways of synaptic versus extrasynaptic NMDARs. It was once thought that NR2A- and NR2B-containing receptors exclusively segregated to synaptic (NR2A) and extrasynaptic (NR2B) compartments, but new findings cast doubt on this notion[17,140]. During postnatal development, there is a switch in synaptic NMDAR phenotype from NR2B- to primarily NR2A-containing[17,21]. However, the subunit composition and relative fraction of extrasynaptic NMDARs in mature neurons has remained elusive. Whereas it was once thought that synaptic and extrasynaptic NMDARs coupled to different downstream signalling cascades and differentially influenced neuronal survival[141,142], and that they could exert different and even opposing actions on AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor) trafficking[143] and synaptic plasticity[144,145], at present there is little consensus on these matters (for examples, see REFS 146–148).

The recent finding of stable endocytic zones in regions lateral to the synapse/PSD of mature neurons[57,58] suggests a requirement for lateral diffusion of receptors away from the synapse (where they are tightly anchored) to the perisynaptic region (where they are more mobile) in order for endocytosis to proceed. Although not yet identified, it is possible that specialized exocytic zones are also organized laterally to synapses. If so, extrasynaptic NMDARs might be likened to commuters at rush hour; that is, passing through on their way to their final destination at synaptic sites or in endocytic vesicles from which they are sorted for recycling versus degradation. A prediction of this model is that the search for a unique molecular phenotype, function and/or downstream signalling for extrasynaptic NMDARs might be in vain.

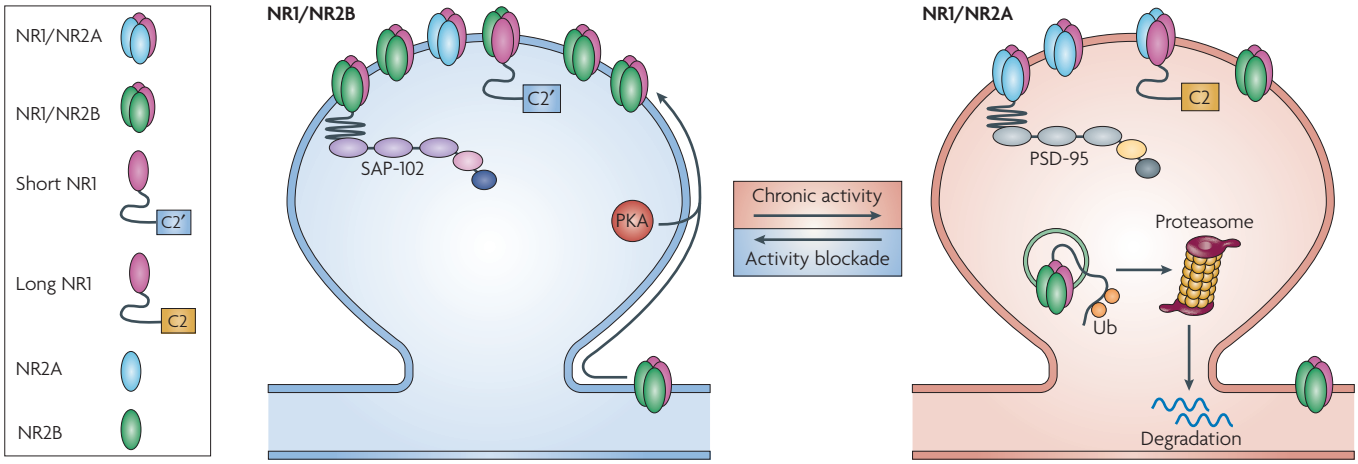

Figure 3 | **Synaptic activity regulates the molecular composition of the postsynaptic density.** Neuronal activity bidirectionally remodels the molecular composition of the postsynaptic density (PSD). Activity blockade promotes localization of NMDARs (*N*-methyl-D-aspartate receptors) to synaptic sites via protein kinase A (PKA) and enhances the levels of the NR2B subunit and synapse-associated protein-102 (SAP-102) in the PSD[35] (left). In addition, activity blockade promotes splicing out of the C2 exon cassette and splicing in of the C2′ cassette of the NR1 subunit[38]. Chronic activity enhances the levels of NR2A and PSD protein of 95 kDa (PSD-95) at synaptic sites[35] (right). Chronic neuronal activity promotes ubiquitylation (Ub) and proteasomal degradation of the NR1 subunit, the NR2B subunit and SAP-102, shifting the balance from mostly NR2B- and SAP-102-containing to predominantly NR2A- and PSD-95-containing complexes[35].

retention signal (RRR) in the alternatively spliced C1 cassette. ER retention signals retain unassembled receptors in the ER as a quality control mechanism. During receptor assembly, binding of the NR2 subunit masks the NR1 retention signal, and promotes forward trafficking of the heterodimeric receptors through the secretory pathway to the cell surface. Accordingly, unassembled NR1 subunits show little or no surface expression and are retained in the ER; once assembled with NR2 subunits, they are efficiently delivered to the cell surface of heterologous cells[42] and neurons[43] and targeted to dendritic spines[21]. Only a short tail of 3–4 amino acids (HFLY) directly following transmembrane domain 4 of NR2 is required to overcome the ER retention of NR1 (REFS 40,41) (FIG. 4b).

The association with modular synaptic scaffolding proteins also promotes forward trafficking of nascent receptors from the ER to the postsynaptic membrane. SAP-102 directly binds through a PDZ domain to SEC8, a member of the exocyst complex[44] (FIG. 2). In developing mammalian neurons, the exocyst complex localizes to the tips of growing neurites and areas of active exocytosis. The co-association of SAP-102 with the NR2B subunit enables the exocyst complex to recognize newly synthesized NMDARs as vesicular cargo and transport them in a pre-assembled state to the synapse[44].

In addition, SAP-102 binds via its SH3 and GK domains to the mammalian homologue of *Drosophila melanogaster* partner of inscuteable (PINS), a modular protein with multiple protein–protein interaction motifs[45]. PINS belongs to a conserved family of proteins that directly binds the G protein inhibitory subunit $G\alpha_i$ and mediates G-protein signalling. Interaction of SAP-102 with NR2B and PINS occurs early in the secretory pathway; ultimately, NMDARs are delivered together with SAP-102 and PINS to dendritic spines[45] (FIG. 2).

PDZ domain
A modular protein interaction domain that is specialized for binding to carboxy-terminal peptide motifs of other proteins. Scaffolding and adaptor proteins that contain PDZ-domains mediate the assembly of large molecular complexes at specific subcellular sites, such as synapses. PDZ domains are named after the proteins in which these sequence motifs were originally identified (PSD-95, discs large, zona occludens 1).

Regulated receptor insertion. The insertion of NMDARs at the cell surface is tightly regulated during development and in response to synaptic activity and sensory experience. Phosphorylation is a well-known mechanism for regulating receptor trafficking and in the case of NMDARs there is good evidence for a role of protein kinase A (PKA) and PKC. Phosphorylation of NR1 near the RRR ER retention signal by PKC (Ser890 and Ser896) and PKA (Ser 897) promotes NMDAR trafficking to the plasma membrane over a number of hours as shown in non-neuronal cells[36,46]. The delayed appearance of receptors at the cell surface suggests a requirement for forward trafficking of NMDARs through the secretory pathway. On a much shorter timescale, namely minutes, activation of PKC enhances NMDA channel opening and delivers new channels to the surface of hippocampal neurons[47]. The rapid channel insertion has been shown to occur through SNARE-dependent exocytosis[47]. This form of PKC potentiation does not involve direct phosphorylation of receptor subunits, but rather a receptor anchoring and/or trafficking protein[48]. Association of NMDARs with PSD-95 also enhances the surface expression of NMDARs but occludes PKC potentiation of channel activity[49]. Blocking neuronal activity promotes synaptic clustering of NMDARs[50]; on removal of the neuronal block, PKC mediates dispersal of NMDARs from synaptic sites[51]. In addition to PKC, the activation of group I mGluRs[52], the insulin receptor[53] and dopamine D1 and D2 receptors[54–56] also promote the insertion of NMDARs in heterologous cells.

NMDAR endocytosis. Endocytosis is also tightly regulated and governed by subunit-specific rules. Endocytosis is a fundamental mechanism by which neurons regulate intercellular signalling, synapse maturation and synaptic strength, and it occurs by the

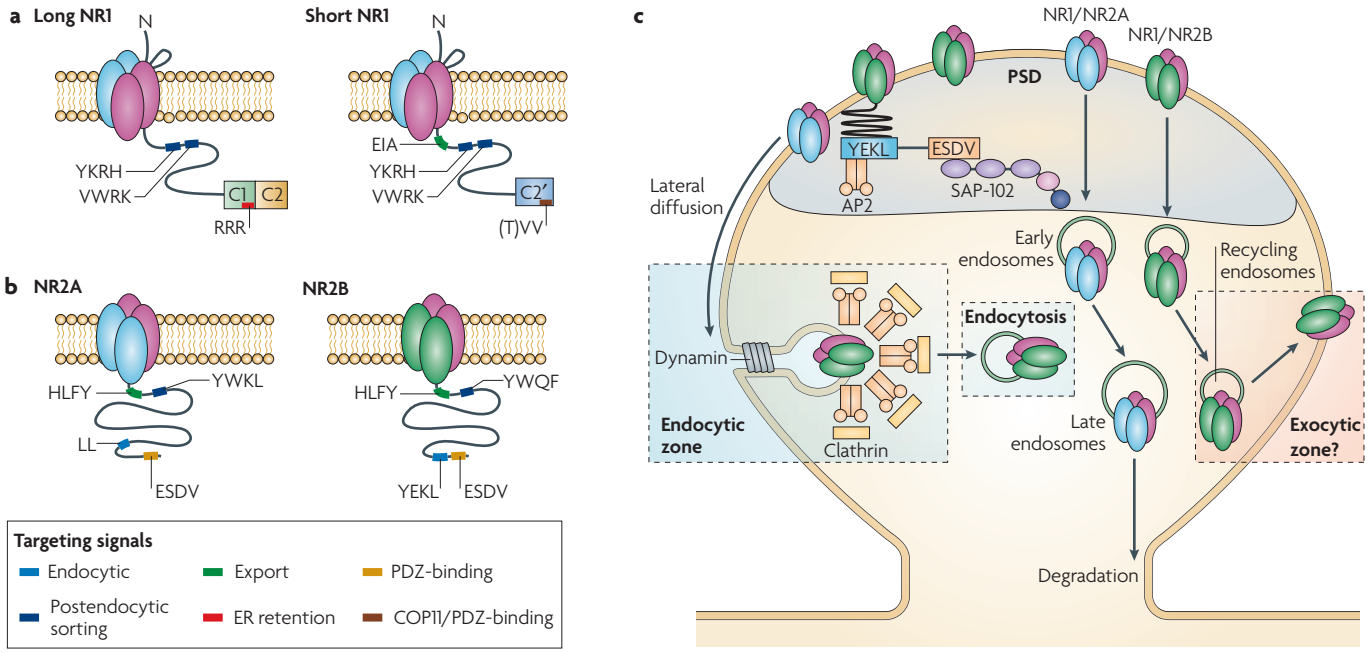

Figure 4 | **NMDAR subunit targeting signals. a** | Trafficking signals intrinsic to NR1 subunits regulate NMDAR (*N*-methyl-D-aspartate receptor) trafficking. An endoplasmic reticulum (ER) retention motif (RRR) in the C1 cassette retains NMDARs in the ER. Short NR1 splice variants lacking the C1 and/or C2 cassette exhibit higher surface expression. Two sorting signals (YKRH and VWRK) in the NR1 subunit target NMDARs to the late endosomal–lysosomal pathway for degradation. **b** | The NR2A/B subunits contain an ER export signal (HLFY), which promotes receptor delivery to the cell surface. Membrane-proximal signals in NR2A (YWKL) and NR2B (YWQF) carboxy (C)-tails direct NMDARs to late endosomes for degradation. Internalization signals in NR2A (LL)[24] and NR2B (YEKL)[26] bind the clathrin adaptor protein AP2 and initiate clathrin-dependent endocytosis. The PDZ recognition motif (ESDV) in NR2A/B C-tails mediates the association of NMDARs with scaffolding proteins such as synapse-associated protein-102 (SAP-102), which promotes receptor delivery to the cell surface[25,28]. **c** | NMDAR internalization is thought to occur by lateral diffusion of receptors away from the synapse to endocytic zones[57,58]. Following endocytosis, NR2A-containing NMDARs are preferentially targeted for degradation, whereas NR2B receptors are targeted for recycling. PSD, postsynaptic density.

assembly of clathrin coats and the budding of clathrin-coated vesicles from the neuronal plasma membrane. Under basal conditions, internalization of NMDARs occurs through the clathrin-coated pit pathway and is mediated primarily by interactions between tyrosine-based internalization motifs contained within the NR2 distal C termini and the clathrin adaptor protein 2 (AP2), which is thought to deliver cargo such as NMDARs into clathrin-coated vesicles by lateral migration (FIG. 4). NR2A and NR2B contain distinct internalization motifs in their distal C termini, which regulate endocytosis at somewhat different rates[24]. Whereas the more robust internalization of NR2B receptors is mediated by an interaction between the YEKL motif and the AP2 complex[26], the relatively slower internalization of NR2A receptors is mediated by a dileucine (LL) motif[24].

NMDAR subunits also contain conserved membrane-proximal endocytic motifs — two independent signals in the NR1 subunit (YKRH and VWRK) and one each in the NR2A and NR2B subunits (YWKL and YWQF, respectively) — which are necessary and sufficient to drive the internalization of NMDARs[39] (FIG. 4). Whereas membrane-proximal and distal signals contribute in an additive manner to endocytosis, they exert distinct effects on postendocytic trafficking. Distal C-terminal motifs in NR2B direct receptors to recycling endosomes; by contrast, membrane-proximal signals on both the NR1 and NR2A/B subunits direct internalized receptors to late, degradative endosomes[39]. The presence of specialized endocytic zones in regions lateral to the synapse and PSD of mature neurons has been documented[57,58], a finding that suggests a requirement for lateral diffusion of receptors away from the synapse (where they are tightly anchored) to the perisynaptic region (where they are more mobile) for endocytosis to proceed. The spatial organization of clathrin, AP2 and dynamin (a GTPase involved in the scission of clathrin-coated vesicles from the plasma membrane) indicates that NMDARs might bind AP2 before they migrate to lateral sites of endocytosis. Although not yet identified, it is possible that specialized exocytic zones are also organized laterally to synapses and that nascent receptors are inserted perisynaptically, followed by lateral diffusion to the PSD, where they are anchored by synaptic scaffolding proteins. Such lateral movement is well established for the constitutive cycling of AMPARs into and out of synaptic sites[59], and the findings discussed above suggest that NMDARs are laterally mobile in synaptic plasticity and spine morphogenesis.

Regulated receptor endocytosis. In mammalian neurons, activity-dependent phosphorylation of the YEKL motif of NR2B at Tyr1472 by the non-receptor tyrosine kinase Fyn suppresses clathrin-mediated endocytosis of NMDARs[28,60]. Fyn-dependent phosphorylation of Tyr1472 is also required for proper localization of NR2B-containing receptors at synapses in the hippocampus[28] and amygdala[61]. Mice with an NR2B Tyr1472Phe knock-in mutation exhibit mislocalization of NR2B and impaired NMDAR-mediated signalling through calcium/calmodulin-dependent kinase II (CaMKII), weakened LTP at synapses of the amygdala and reduced fear-related learning[61]. Collectively, these findings implicate Fyn-dependent phosphorylation in NMDAR trafficking and synaptic plasticity.

Stabilization of NR2A receptors at the cell surface depends on tyrosine phosphorylation[62]. Tyrosine dephosphorylation triggers NMDAR internalization. Repeated application of the NMDAR agonist glutamate over several minutes promotes dephosphorylation of Tyr842 in the C-terminal tail of the NR2A subunit and induces rapid, use-dependent rundown of NMDARs independent of ion flux[62], consistent with a ligand-induced conformational change that promotes receptor internalization. Moreover, glutamate triggers Ca^{2+}-dependent, preferential loss of extrasynaptic NMDARs[63]. Similarly, repetitive application of the co-agonist glycine primes NMDARs for clathrin-dependent endocytosis even in the absence of ion flux[64]. Glycine primes synaptic as well as extrasynaptic NMDARs for internalization. Although the physiological significance of priming remains to be determined, it might maintain homeostasis under conditions of high neuronal firing or insult by reducing NMDAR number. Under normal conditions, the number of NMDARs would be stabilized at the cell surface of neurons because ambient glycine is just below the concentration for priming. At times of high neuronal firing, as occurs during seizures or in response to neuronal insults such as ischaemia, extracellular glycine rises above the set point and engages the priming mechanism.

Lateral mobility of NMDARs

NMDARs not only cycle in and out of synaptic sites, but also move laterally in the plasma membrane between synaptic and extrasynaptic sites in an activity- and phosphorylation-dependent manner. A prevailing view is that, whereas AMPAR number and function are dynamically modified and tightly regulated in response to neuronal activity and/or experience, NMDARs are less mobile[65] (for an early suggestion of NMDAR mobility see, REF. 66). This view is reinforced by findings of an elaborate macromolecular signalling complex in which scaffolding and adaptor proteins link NMDARs to downstream signalling proteins and the cytoskeleton, and the high resistance of NMDARs to detergent extraction[7]. However, the view that NMDARs are tightly locked and stably situated in the synapse has been challenged. Using autaptic cultures of embryonic rat hippocampal neurons, one study reported that NMDARs move laterally within the plasma membrane into and out of synapses[67]. More recently, studies involving single-particle and molecule tracking have shown that NMDARs exhibit lateral mobility between synaptic and extrasynaptic domains within the plasma membrane with basal diffusion rates comparable to those of AMPARs[68,69]. These studies further show that NR2A-containing NMDARs diffuse laterally more slowly within the synapse ($\sim 2 \times 10^{-4}$ μm^2 sec^{-1}) than NR2B-containing receptors ($\sim 500 \times 10^{-4}$ μm^2 sec^{-1})[69]. Therefore, lateral mobility of NMDARs decreases with neuronal maturation in parallel with the progressive inclusion of NR2A into synaptic NMDARs[69]. Whereas AMPAR lateral mobility is markedly enhanced by neuronal activity, NMDAR diffusion is unaltered[68]. Given that endocytic zones are localized tangentially to the synapse, lateral diffusion of NMDARs is likely to be a crucial step in receptor endocytosis (FIG. 4).

Activity-dependent changes in synaptic NMDARs

LTP of NMDAR-mediated currents. New research indicates that activity-dependent alterations in NMDAR trafficking might provide a dynamic and potentially powerful mechanism for regulating synaptic efficacy and remodelling. LTP and LTD are cellular processes that are involved in learning and memory. NMDAR-dependent LTP at the Schaffer collateral–CA1 synapse (Sch–CA1 synapse) is perhaps the best-characterized form of synaptic plasticity. The prevailing view is that NMDARs function as the trigger of LTP and LTD and that the primary expression mechanism of synaptic plasticity involves alterations in the number, phosphorylation state[5,70,71] and/or subunit composition[72] of synaptic AMPARs. Owing to its voltage-dependent block by Mg^{2+}, the NMDAR functions as a coincidence detector of presynaptic and postsynaptic firing and is well suited, therefore, to be the trigger of LTP. Following depolarization of the postsynaptic membrane and relief of Mg^{2+} block, the NMDAR-mediated rise in postsynaptic Ca^{2+} activates kinases, notably CaMKII, PKA, PKC and mitogen-activated protein kinase (MAPK), and protein phosphatases. Activated CaMKII phosphorylates the AMPA-type glutamate receptor 1 (GluR1) subunit, which, in turn, promotes synaptic incorporation of GluR1-containing AMPARs, thereby increasing AMPAR number and channel conductance[5,65]. By contrast, LTD and depotentiation involve dephosphorylation of GluR1 and retrieval of AMPARs from synaptic sites.

The notion that LTP can also involve an increase in NMDA EPSCs at synapses of the hippocampus has been around for nearly 16 years[73–77]. However, evidence that alterations in NMDAR number and/or subunit composition contribute to the expression mechanism of LTP is relatively recent[78,79]. The contribution of NMDAR trafficking to LTP was first demonstrated at Sch–CA1 synapses of the adult hippocampus[78]. At these synapses, NMDAR-mediated Ca^{2+} influx triggers LTP of synaptic NMDAR responses (LTP_{NMDAR}) by a mechanism involving activation of PKC and the tyrosine kinase Src, followed by rapid synaptic incorporation of NR2A-containing NMDARs[78] (FIG. 5). The mechanism is consistent with observations that PKC enhances channel opening and promotes the insertion of NMDAR channels in hippocampal neurons[47].

Clathrin-mediated endocytosis
A form of receptor-mediated endocytosis, in which invagination of the endocytic vesicle is driven by the clathrin coat.

Autaptic cultures
Cultures in which hippocampal neurons are plated at an exceedingly sparse density so that each cell is physically isolated from other cells and makes synaptic connections only with itself.

Schaffer collateral–CA1 synapse
(Sch–CA1 synapse). Synapses formed by excitatory afferents from the CA3 to CA1 pyramidal cells in the hippocampus. LTP at this synapse is one of the most well-characterized forms of synaptic plasticity in the brain.

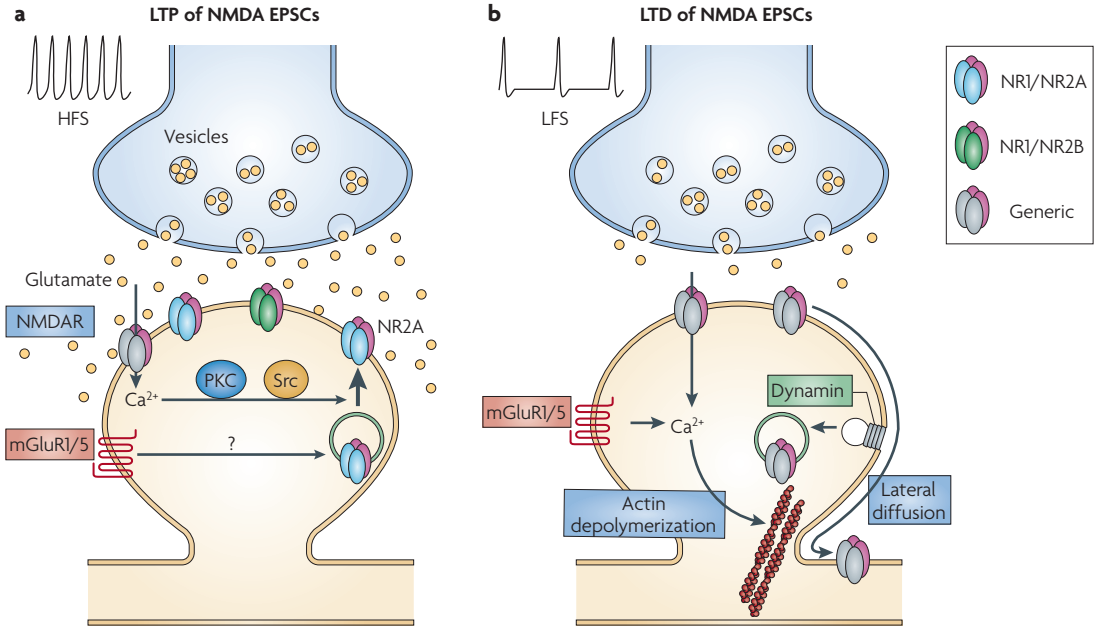

Figure 5 | **Long-term potentiation and long-term depression involve activity-dependent insertion or internalization of NMDARs. a** | In the hippocampal CA1 of adult animals, high-frequency stimulation (HFS) and long-term potentiation (LTP) promote rapid insertion of NR2A-containing NMDARs (*N*-methyl-D-aspartate receptors) and an increase in NMDA field excitatory postsynaptic potentials (fEPSPs) at CA1 synapses through a protein kinase C (PKC)- and Src-dependent pathway[78] (blue box). HFS-induced activation of mGluR5 can also induce LTP of NMDARs[76,80], but in this case a role of NMDAR insertion has not been explored (red box). **b** | Homosynaptic long-term depression of synaptic NMDA EPSCs (LTD_{NMDAR}) occurs by retrieval of NMDARs from synaptic sites. Low frequency stimulation (LFS) of Schaffer collaterals can induce LTD_{NMDAR} in a manner that is dependent on metabotropic glutamate receptors (mGluRs)[93] or NMDARs[88]. mGluR-triggered LTD is associated with enhanced internalization of NMDARs[93] (red box). NMDAR-triggered LTD promotes actin depolymerization and lateral diffusion of NMDARs away from the synapse site[88] (blue boxes). LFS of connected pairs of CA3 pyramidal neurons induces LTD_{NMDAR} by stimulating dynamin-dependent internalization of NMDARs[92] (green box).

LTP_{NMDAR} also occurs at synapses of the visual cortex. Stimulation of pairs of connected pyramidal neurons in the visual cortex induces synchronous firing and enhances AMPA EPSCs over an order of minutes; the AMPA LTP is followed by a delayed potentiation of NMDA EPSCs over 30–60 minutes, which ultimately restores the AMPA:NMDA ratio to ~1 (REF. 79). These results are consistent with temporally distinct mechanisms of potentiation of AMPA versus NMDA currents, possibly through regulated receptor trafficking. Group I mGluR activation can also function as a trigger for synaptic plasticity involving enhanced efficacy of NMDA EPSCs[76]. Group I mGluRs (mGluR1 and mGluR5) are localized to the perisynaptic region of spines, where they are strategically poised for modulating NMDARs. At the medial perforant path–dentate granule cell synapse, high frequency stimulation (HFS) in the presence of blockers of AMPA and GABA (γ-aminobutyric acid) receptors, or application of the mGluR agonist ACPD (1-amino-1,3-cyclopentanedicarboxylic acid) activates mGluR5 and triggers LTP of NMDA EPSCs[76]. Similarly, HFS-induced LTP of NMDA EPSCs at the Sch–CA1 synapse is mGluR5-dependent[80]. These observations are consistent with findings that co-stimulation of mGluR5 and NMDARs selectively potentiates NMDA currents in neurons[81,82]. In hippocampal slices, the degree and even the direction of the plasticity are sensitive to changes in the intracellular Ca^{2+} concentration. Intensifying intracellular Ca^{2+} buffering converts the HFS-elicited response from LTP to LTD of NMDA EPSCs[77].

Acute administration of oestradiol to ovariectomized adult female rats, a paradigm that mimics hormone replacement therapy in postmenopausal women, increases the magnitude of LTP at Sch–CA1 synapses by a two-step process. First, it produces an increase in NMDA synaptic currents by synaptic incorporation of NR2B-containing NMDARs, leading to an increase in LTP magnitude, NMDA:AMPA ratio and spine density[83,84]. Second, AMPA EPSCs are increased, bringing the NMDA:AMPA ratio to basal levels and re-establishing homeostatic balance in excitatory transmission in the face of increased spine density[84]. These studies document clear evidence for LTP of NMDAR and make a clear case for diverse expression mechanisms in LTP that are age-, synapse- and stimulus-specific.

LTD of NMDAR-mediated currents. NMDAR trafficking is also implicated in LTD. In the hippocampus, low-frequency stimulation (LFS) of Sch–CA1 synapses induces homosynaptic LTD that is critically dependent on NMDAR activation[85] and a rise in postsynaptic Ca^{2+} (REF. 86). The same trigger, namely LFS, can induce simultaneous LTD of AMPA and NMDA currents at CA1 synapses, but these involve different

Metaplasticity
A higher-order plasticity than synaptic plasticity, metaplasticity ('plasticity of plasticity') refers to the phenomenon whereby previous synaptic activity (for example, prolonged changes in overall network activity over long time periods) or other external stimuli can influence (the occurrence of) subsequent synaptic plasticity (process or event).

Ocular dominance columns
In the mature primary visual cortex of mammals, most neurons respond predominantly to visual inputs from one eye or the other. Ocular dominance columns arise from the spatially organized, alternating columns of cells that receive sensory information from one eye or the other.

Orientation selectivity
Property of visual cortex neurons that enables the detection of bars and edges within visual images and the encoding of their orientations. As the cortex is organized in columns, neurons that belong to the same column share the same orientation tuning.

Barrel
A cylindrical column of neurons found in the rodent neocortex. Each barrel receives sensory input from a single whisker follicle, and the topographical organization of the barrels corresponds precisely to the arrangement of whisker follicles on the face.

signal transduction pathways and distinct expression mechanisms[87,88]. Unlike LTD of AMPA EPSCs, which involves the binding of AMPARs to AP2 and dynamin-mediated receptor endocytosis, LTD of synaptic NMDA responses (LTD_{NMDAR}) at CA1 synapses requires actin depolymerization, a finding consistent with a role for lateral diffusion of NMDARs between synaptic and extrasynaptic sites[88] (FIG. 5) (for a different view, see REFS 89,90). Unlike LTD_{NMDAR} at CA1 synapses, LTD_{NMDAR} at synapses between pairs of interconnected CA3 neurons is dynamin-dependent and involves NMDAR endocytosis[91,92]. In addition to LFS, application of the mGluR group I agonist 3,5-dihydrophenylglycine (DHPG) induces chemical LTD_{NMDAR} at CA1 synapses in hippocampal slices and internalization of NMDARs in hippocampal neurons in culture[93].

Activity-dependent alterations in NMDAR synaptic strength are of great interest in that they involve potentiation of the trigger itself and, as such, would be expected to critically influence metaplasticity, experience-dependent plasticity and synaptic pruning. Although there are clear examples of LTP and LTD of NMDA EPSCs, the actual changes in NMDA EPSCs are for the most part relatively modest[87,88,92]. Moreover, only a small number of these studies document a requirement for receptor trafficking. By contrast, there has been an explosion of studies that document a role for AMPAR trafficking in LTP and LTD of AMPA EPSCs. Historically, potentiation and depression of NMDA responses might have been overlooked owing to the smaller current amplitudes and greater variability of NMDA versus AMPA responses, the voltage-dependent inhibition of NMDA responses by Mg^{2+} at physiological (resting) membrane potentials and the relative immobility of NMDARs relative to that of AMPARs. Another factor could be the somewhat higher induction threshold required to achieve LTP of NMDA versus AMPA responses[94,95]. Further studies are warranted to investigate the mechanisms by which NMDAR trafficking contributes to synaptic plasticity.

Experience-dependent regulation of NMDAR subunits. During the early postnatal period (weeks 2–3 postpartum in rodents), sensory experience shapes and refines synaptic connections between thalamocortical relay neurons and their targets in the primary sensory cortex. This 'critical period' is characterized by heightened sensitivity of the neural circuitry in the visual cortex to experience-dependent, NMDAR-dependent modifications[96,97]. As an example, synapses of lateral geniculate nucleus neurons onto pyramidal neurons in visual cortex layer IV undergo remodelling, develop ocular dominance columns and acquire orientation selectivity during this period[96,97]. Also during this period, NMDA EPSCs in the visual cortex become faster owing to progressive inclusion of NR2A subunits into synaptic NMDARs; the replacement of NR1–NR2B receptors by NR2A-containing receptors coincides with closure of the critical period for ocular dominance plasticity[98].

Visual experience and deprivation can rapidly and reversibly alter NMDAR subunit composition and kinetics at synapses of the visual cortex, and can modify the duration of the critical period[99,100]. For example, rearing rodents in the dark delays synaptic incorporation of NR2A-containing NMDARs in area IV of the visual cortex and postpones closure of the critical period[99,101]. Returning the animal to the light promotes the rapid (within hours) synaptic incorporation of NR2A receptors and closure of the critical period[99,101]. These findings are consistent with a role for receptor trafficking in the recovery process.

At synapses of visual cortex pyramidal cells, NMDAR activation functions not only as the trigger, but also as the substrate of alterations in synaptic efficacy. At these synapses, NMDAR signalling is thought to be a trigger for metaplasticity, in that the signalling modifies the threshold for induction of LTP and LTD[102,103]. Visual deprivation, by rearing animals in the dark, reduces the threshold for induction of LTP in layer III of the visual cortex, possibly owing to the prolonged presence of NR2B-containing NMDARs at synaptic sites[104]. Consistent with this, overexpression of NR2A-containing NMDARs reduces the magnitude of LTP in area CA1 of the hippocampus[105], and overexpression of NR2B enhances CA1 LTP and hippocampal-based cognition[106]. Accordingly, targeted deletion of the *Nr2a* gene abolishes visual-experience-dependent modification of the threshold for induction of LTP or LTD[107]. These findings support a model whereby NR2B-dominant synapses are more plastic than NR2A-containing synapses (for a different view, see REFS 108–110). Given that alterations in synaptic NMDAR strength underlie metaplasticity, experience-dependent changes in NMDAR phenotype would be expected to qualitatively affect synaptic plasticity and remodelling of local circuitry.

Alterations in NMDAR synaptic strength are implicated in other sensory modalities — for example, olfaction. The initial phase of olfactory rule learning requires the activation of NMDARs and involves a change in NMDAR subunit composition at synapses of the olfactory cortex[111]. Sensory experience can produce synapse-specific suppression of NMDA responses in the olfactory bulb[112]. The selective reduction in NMDAR synaptic strength increases the AMPA:NMDA ratio and raises the threshold for LTP induction. Moreover, sensory deprivation by whisker trimming during postnatal development alters the composition of NMDAR subunits at synapses of the barrel cortex[113]; however, its impact on the AMPA:NMDA ratio is less clear[113,114]. Experience-driven synaptic incorporation of NMDAR subunits therefore appears to be important in the maturation and plasticity of cortical maps.

Homeostatic plasticity. Neuronal activity maintains the weight of synaptic strength (and neuronal excitability) in an optimal range for information transfer — a process known as homeostatic plasticity or synaptic scaling[115]. For example, blocking neuronal activity can upregulate AMPAR and/or NMDAR function. Alterations in

Silent synapse
An excitatory synapse containing functional NMDARs (*N*-methyl-D-aspartate receptors) but lacking AMPARs (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors). At the resting potential of the cell, NMDARs are blocked by extracellular Mg^{2+}. To activate NMDARs, synaptically released glutamate must activate AMPARs, leading to Na^{+} influx and depolarization of the neuronal membrane, which in turn relieves block of NMDARs by Mg^{2+}. The proportion of silent synapses at central synapses decreases during mammalian postnatal development.

network activity levels can concomitantly scale up or down AMPA and NMDA miniature EPSCs (mEPSCs), thereby maintaining the NMDA:AMPA ratio[116]. The notion that bidirectional, activity-dependent alterations in NMDA synaptic strength contribute to homeostatic plasticity is relatively recent. Chronic blockade of neuronal activity by the sodium channel blocker tetrodotoxin and/or the NMDAR antagonist D(-)-2-amino-5-phosphonovaleric acid (D-AP5) enhances the expression of NR2A and NR2B subunits and increases the synaptic NMDAR number and the spine density[50,117] of hippocampal neurons in a protein kinase A (PKA)-dependent manner[118] (FIG. 3). Chronic activity blockade also increases spine density in projection neurons of the lateral geniculate nucleus *in vivo*[119] and generates silent synapses by increasing the NMDA:AMPA ratio[120].

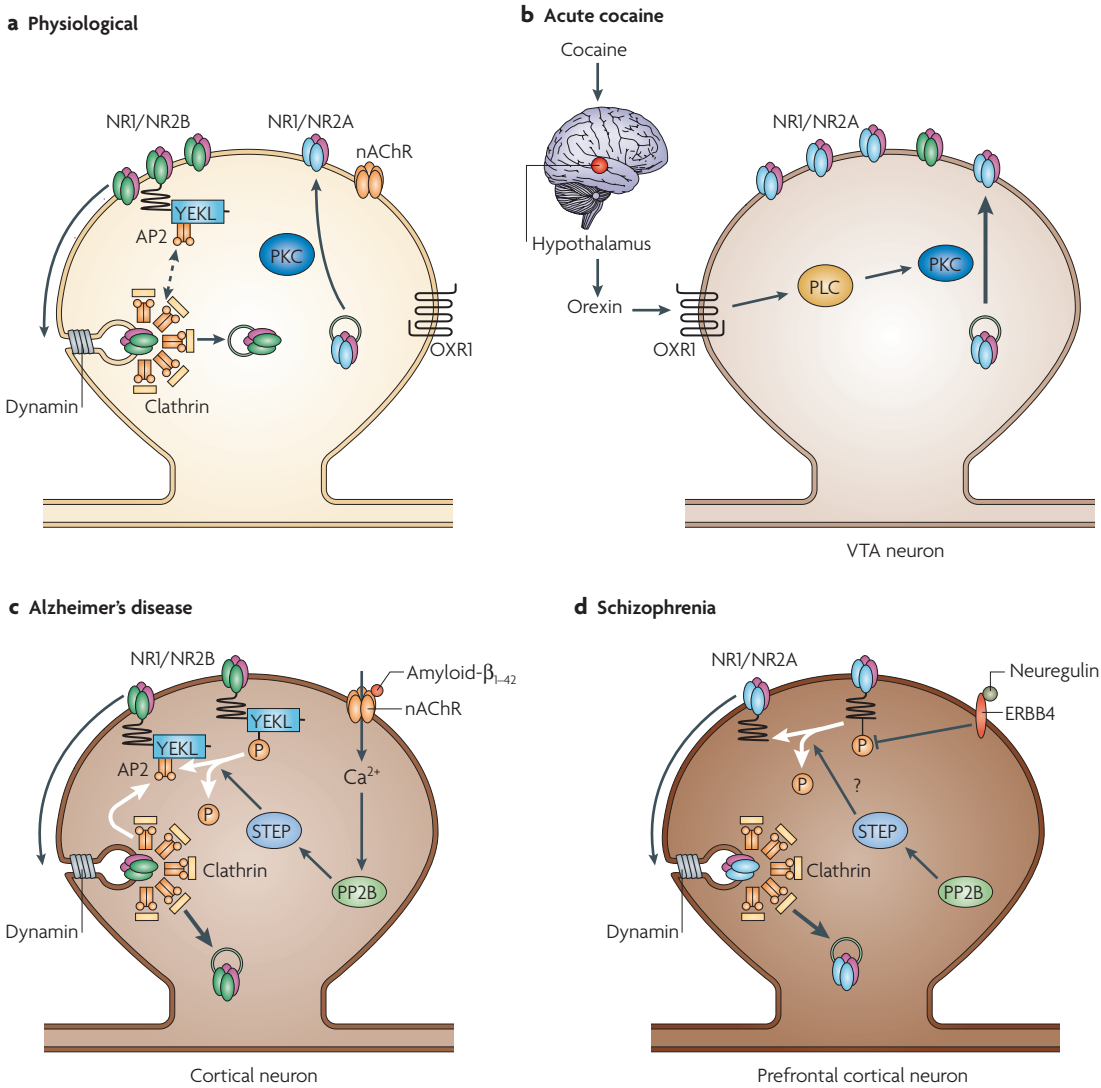

Figure 6 | **Dysregulation of NMDAR trafficking in neuropsychiatric disorders. a** | Under physiological conditions, dephosphorylation of Tyr1472 in the NR2B subunit promotes NMDAR (*N*-methyl-D-aspartate receptor) internalization via clathrin-coated pits. Activation of protein kinase C (PKC) enhances NMDAR insertion. **b** | Acute cocaine administration activates orexin/hypocretin-containing neurons in the hypothalamus. On its release, orexin binds to OXR1 receptors in the ventral tegmental area (VTA) and stimulates signalling by phospholipase C (PLC) and PKC. This promotes insertion of NMDARs at VTA synapses, leading to long-term potentiation of synaptic NMDA excitatory postsynaptic currents and behavioural sensitization to cocaine[123]. **c** | The toxic peptide amyloid-β_{1-42}, implicated in the aetiology of Alzheimer's disease, binds to α7-containing nicotinic acetylcholine receptors (nAChRs) and promotes Ca^{2+} influx[129]. Ca^{2+} activates the protein phosphatase PP2B, which dephosphorylates and activates the striatal-enriched tyrosine phosphatase (STEP). STEP dephosphorylates Tyr1472 in the NR2B subunit and promotes NMDAR internalization via the clathrin pathway[129]. **d** | Two molecules genetically linked to schizophrenia, neuregulin and PP2B regulate NMDAR trafficking. In a hypothetical model, activation of the ERBB4 receptor by neuregulin suppresses tyrosine phosphorylation of NR2A[135], which promotes NMDAR internalization. In a second model, PP2B dephosphorylates and activates STEP[129], which dephosphorylates tyrosine residues on NR1 and/or NR2 subunits and promotes NMDAR internalization. OXR1, orexin receptor 1. Anatomical image modified, with permission, from REF. 150 © (1996) Appleton & Lange.

Coatomer protein complex II (COPII). The coat protein COPII forms carrier vesicles that mediate intracellular transport of newly synthesized proteins from exit sites of the endoplasmic reticulum to the *cis* face of the Golgi apparatus.

In addition to altering NMDAR number and subunit composition, activity regulates NMDA synaptic strength by modifying alternative RNA splicing of a cassette encoding the most distal part of the NR1 C terminus[38]. Chronic activity promotes splicing in of the C2 cassette of the NR1 subunit, whereas activity blockade favours splicing in of the C2′ cassette[38]. The C2′ cassette contains a coatomer protein complex II (COPII) recognition motif, which mediates binding of nascent NMDA receptors to COPII; association of receptors with COPII promotes ER exit and accelerates forward trafficking to synaptic sites[38]. Consequently, short NR1 splice forms containing the C2′ cassette exhibit greater NMDAR surface expression. These findings provide a mechanistic link between synaptic activity and surface delivery of NMDARs, but how alterations in activity are detected by the transcriptional splicing machinery is so far unknown.

A theme that emerges is that whereas activity or sensory blockade favours incorporation of NMDARs and the scaling up of synaptic strength, chronic activity or sensory experience favours endocytosis of NMDARs and the scaling down of synaptic activity. Synaptic scaling is thought to be a mechanism crucial to the long-term stability of neuronal function. An increase in the number of NMDARs not only enhances the synaptic strength, but also the strength of the trigger, thereby enhancing the probability that synaptic plasticity will occur. This form of homeostasis, unlike classic synaptic scaling, produces qualitative as well as quantitative changes in synaptic responses and plasticity.

Neuropsychiatric disorders

NMDAR trafficking and drugs of abuse. NMDAR trafficking is also essential in the plasticity associated with the rewarding and addictive effects of drugs of abuse such as cocaine. These behaviours are mediated by alterations in the brain reward circuit, which involves glutamatergic projections from the prefrontal cortex to dopamine neurons in the ventral tegmental area (VTA), the main target in the mesolimbic midbrain[121,122]. Acute cocaine injection enhances NMDA EPSCs at glutamatergic synapses onto VTA dopamine neurons by promoting the rapid insertion of primarily NR2A-containing NMDARs, a mechanism thought to underlie behavioural sensitization[123]. Acute cocaine administration activates orexin- and hypocretin-containing neurons in the lateral hypothalamus that project to the VTA. On release, orexin acts by binding to OXR1 receptors to promote, through phospholipase C and PKC signalling, the rapid insertion of primarily NR2A-containing NMDARs at the synapses of the VTA[123] (FIG. 6). The increase in synaptic NMDAR number is associated with the potentiation of NMDAR-mediated EPSCs and a reduced AMPA:NMDA ratio that lasts from minutes to hours[123], consistent with the generation of silent synapses[120]. By contrast, repeated cocaine administration enhances AMPAR-mediated EPSCs, increases the AMPA:NMDA ratio and occludes LTP at VTA synapses[123]. In addition, repeated cocaine exposure *in vivo* facilitates LTP induction (increases the susceptibility to LTP) at excitatory inputs onto VTA dopamine neurons by reducing $GABA_A$ receptor-mediated inhibition[124]. These quantitative and qualitative alterations in synaptic efficacy are thought to be vital to the acquisition of cocaine sensitization, and might underlie drug craving and compulsive drug-seeking behaviour.

Ethanol also acts through regulated NMDAR trafficking to modify neural circuitry. Although the mechanism(s) by which ethanol exerts its addictive effects is as yet unclear, ethanol is known to alter GABA-mediated neuron function and to enhance VTA dopaminergic neuron firing[121]. Ethanol regulates the AMPA:NMDA ratio in midbrain dopaminergic neurons, consistent with plastic modifications of excitatory transmission as a common pathway to drug addiction[125]. In the hippocampus, acute ethanol administration promotes selective internalization of NR2A through H-Ras and the inhibition of Src, thereby changing the synaptic NMDAR subunit composition from a mixture of NR2A- and NR2B receptors to essentially pure NR2B receptors[126]. By contrast, chronic ethanol administration promotes synaptic incorporation of NMDARs (but not AMPARs), with little or no effect on total cellular NMDAR abundance, and enhances NMDA mEPSCs, with little or no effect on NMDA-elicited whole-cell currents[127].

NMDAR trafficking in Alzheimer's disease. Dysregulation of NMDAR trafficking is also implicated in Alzheimer's disease. Accumulation of amyloid-β, a small secreted peptide with a high propensity to form aggregates, is a central causative factor in Alzheimer's disease[128]. Elevated levels of amyloid-β reduce glutamatergic transmission and inhibit synaptic plasticity[128]. The NMDAR internalization occurs through high-affinity binding of amyloid-β_{1-42} to the α7-nicotinic acetylcholine receptor, enhanced α7-mediated Ca^{2+} influx and activation of the serine–threonine phosphatase PP2B (also known as calcineurin), a Ca^{2+}-sensitive enzyme that regulates NMDAR transmission and synaptic plasticity. PP2B dephosphorylates and activates striatal-enriched tyrosine phosphatase (STEP), which dephosphorylates the NR2B subunit at Tyr1472 and promotes internalization of NR2B-containing NMDARs[129] (FIG. 6). Consistent with this, amyloid-β_{1-42} reduces NMDA EPSCs in organotypically cultured hippocampal slices[130].

NMDAR trafficking in schizophrenia. NMDAR hypofunction is implicated in the behavioural manifestations (social withdrawal, increased motor stereotypy and locomotor activity) of schizophrenia in humans and animal models[131,132]. These observations raise the possibility that dysregulation of NMDAR trafficking might contribute to the aetiology of schizophrenia. Consistent with this, activation of neuregulin 1, a growth factor genetically linked to schizophrenia in humans[133], promotes rapid internalization of NMDARs from the cell surface by a clathrin-dependent mechanism in prefrontal pyramidal neurons[134]. Overactivation of the ERBB4 receptor by neuregulin suppresses tyrosine phosphorylation of NR2A in the prefrontal cortex of patients with schizophrenia[135] and could suppress NMDAR activity, eliciting schizophrenic-like symptoms. Another candidate

schizophrenia gene, PP2B γ-subunit (*PPP3CC*)[136,137], promotes NMDAR internalization via STEP[129,138] (FIG. 6). These studies link dysregulation of NMDAR trafficking to the behavioural manifestations of schizophrenia and implicate proteins that regulate NMDAR trafficking as potential therapeutic targets for intervention in this mental disorder.

Concluding remarks

Recent findings provide evidence that synaptic NMDAR number and subunit composition are not static, but are dynamically remodelled in a cell- and synapse-specific manner during development and in response to neuronal activity and sensory experience. Activity drives not only NMDAR synaptic targeting and incorporation, but also receptor retrieval, differential sorting of receptors into the endosomal–lysosomal pathway and lateral diffusion between synaptic and extrasynaptic sites. Whereas activity blockade promotes alternative RNA splicing of the NR1 subunit and accelerates forward trafficking of NMDARs, chronic neuronal activity drives subunit-specific receptor internalization and degradation through the ubiquitin–proteasome system. Activity-dependent insertion and retrieval of NMDARs mediate some forms of LTP and LTD and can regulate metaplasticity at central synapses. Emerging studies implicate dysregulation of NMDAR trafficking in neuropsychiatric disorders such as cocaine addiction, chronic alcohol abuse, schizophrenia and Alzheimer's disease.

Despite recent progress in our understanding of the dynamics of NMDAR synaptic incorporation and trafficking, many questions remain unanswered. By analogy to AMPARs, do phosphorylation and dephosphorylation regulate insertion and/or retrieval of NMDARs from synaptic sites? Are receptor-anchoring proteins and/or trafficking proteins crucial to the regulated insertion of NMDARs in LTP and might their dysregulation be implicated in neuropsychiatric disorders? Where and how are NMDARs recycled and degraded during synaptic plasticity? What is the number, subunit composition, potential role of splicing and function of extrasynaptic NMDARs in the regulation of synaptic strength, plasticity and/or neuronal survival? Increasing evidence documents the localization of NMDARs at presynaptic axon terminals and supports their role in several forms of presynaptic plasticity[139], but little is known about targeting or trafficking of these receptors. Time-lapse imaging of NMDAR channels during and after induction of LTP and LTD will shed light on the cellular dynamics of NMDAR trafficking. Given that NMDARs mediate the rise in postsynaptic Ca^{2+}, neuronal firing pattern and synaptic plasticity, the dynamic regulation of the number and composition of synaptic NMDARs is expected to have profound implications for neuronal activity and survival.

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Competing interests statement
The authors declare competing financial interests: see web version for details.

DATABASES
The following terms in this article are linked online to:
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ERRATUM

NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders

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In FIG. 4a of the above article, the EIA motif and the associated green box indicated in the panel "short NR1" should have been deleted. Please refer to the corrected figure below.

In addition, some of the text describing reference 67 and reference 68 was misplaced.

The highlighted text for reference 67, Tovar, K. R. & Westbrook, G. L., should read: the first report that NMDARs can move laterally within the plasma membrane between synaptic and extrasynaptic sites.

The highlighted text for reference 68, Groc, L. *et al*. should read: this study used single-molecule tracking and time-lapse live imaging of neurons to measure the rate of mobility of synaptic NMDARs.