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Homeostatic plasticity and NMDA receptor trafficking

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Learning, memory and brain development are associated with long-lasting modifications of synapses that are guided by specific patterns of neuronal activity. Such modifications include classical Hebbian plasticities (such as long-term potentiation and long-term depression), which are rapid and synapse-specific, and others, such as synaptic scaling and metaplasticity, that work over longer timescales and are crucial for maintaining and orchestrating neuronal network function. The cellular mechanisms underlying Hebbian plasticity have been well studied and involve rapid changes in the trafficking of highly mobile AMPA receptors. An emerging concept is that activity-dependent alterations in NMDA receptor trafficking contribute to homeostatic plasticity at central glutamatergic synapses.

Introduction

Most excitatory synapses exhibit a rich repertoire of plasticity modes that act over timescales ranging from milliseconds to weeks, and that have spatial dimensions ranging from individual synapses to all afferent synapses onto a neuron. Hebbian forms of plasticity alter the strength of specific synapses that exhibit coincident activity. Typically, correlated presynaptic and postsynaptic firing or high-frequency stimulation results in long-term potentiation (LTP) of synaptic strength, whereas low-frequency stimulation or uncorrelated firing yields long-term depression (LTD). Such fast, durable and selective modifications of synaptic strength are necessary for wiring the brain during development and for encoding information in response to experience. However, Hebbian forms of plasticity operate by positive feedback rules that, if left unchecked, tend to destabilize neuronal networks over time by driving neurons towards maximal and minimal action potential firing frequency ranges, which degrade propagating signals in the network and render neurons unable to encode subsequent plastic changes by Hebbian mechanisms [1]. Homeostatic forms of plasticity might provide the global negative feedback necessary to maintain synaptic strength and plasticity within a functional dynamic range, by scaling the strength of all synaptic inputs up or down while preserving their relative weights (synaptic scaling) or by altering the ability of synapses to undergo subsequent Hebbian modifications (metaplasticity) [2,3].

Alterations in synaptic strength can be encoded presynaptically as alterations in the machinery releasing the neurotransmitter glutamate, or postsynaptically by changing the number or function of receptors sensing the glutamate signal. A key advance has been the discovery that glutamate receptors are dynamically transported to and from the postsynaptic membrane. Such dynamic transport operates over a wide range of timescales and responds to diverse stimuli, matching the varied spectrum of plasticity modes. It is now widely accepted that the trafficking of AMPA-type glutamate receptors mediates rapid synaptic modification in the classic Hebbian forms of plasticity, LTP and LTD [4,5]. Several other cellular and molecular changes have been implicated in synaptic homeostasis but one common feature of many forms of homeostatic plasticity is an alteration in the number or complement of NMDA-type glutamate receptors [6–8]. Although the subject of extensive debate, the precise mechanisms triggering these changes and how they translate into persistent synaptic modifications to preserve the stability or plasticity of the network are still poorly understood.

Because homeostatic plasticity often proceeds gradually over hours or days [2,8–10], early studies focused on slow receptor turnover coupled to transcriptional changes as the main mechanism to effect NMDA receptor changes [11–13]. A series of new studies has revealed that NMDA receptors cycle rapidly into and out of synapses, and that regulated trafficking of NMDA receptors, working cumulatively and over longer timescales, can effectively modify the number and composition of synaptic NMDA receptors (reviewed in Refs [14–16]). Here, we consider recently discovered ways (some predicted, others unexpected) in which activity-induced changes in NMDA receptor trafficking can drive non-Hebbian forms of synaptic plasticity. As a primary source of postsynaptic Ca^{2+}, NMDA receptors initiate rapid forms of synaptic plasticity, and the magnitude or duration of NMDA-receptor-mediated Ca^{2+} influx dictates the type and sign of plasticity induced [17,18]. A useful simplified framework has been that small amounts of NMDA-receptor-mediated Ca^{2+} influx produce LTD whereas strong activation of NMDA receptors leads to LTP [18,19]. As will be discussed, chronic activity perturbations bidirectionally modify Ca^{2+} influx through NMDA receptors by adjusting the number of NMDA

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receptors clustered at synapses [6,8] or their NR2 subunit composition [7,20,21], which in turn influences the magnitude and kinetics of NMDA receptor currents [22–24], the Ca^{2+} permeability or voltage-dependence of the receptor [25–27], and coupling of the receptor to scaffolding proteins [21,28]. One could predict that altered Ca^{2+} responses, working in concert with changes in postsynaptic scaffolds tethered to the NMDA receptor, would drive changes in downstream signaling and gene transcription [29]. Subsequent remodeling of synaptic structure and function would then facilitate the long-term maintenance of synaptic modifications.

Homeostatic plasticity and the NMDA receptor

Two main modes of homeostatic plasticity, synaptic scaling and metaplasticity, have been reviewed recently [1,30,31]. We will therefore limit our discussion to the contribution of dynamic changes in postsynaptic elements, particularly NMDA receptors, to these forms of plasticity. Such an approach does not imply an exclusion of other cellular mechanisms.

Synaptic scaling

Synaptic scaling is a homeostatic form of plasticity that tends to restore neuronal activity to 'baseline' levels. It does so by changing the postsynaptic response of all of the synapses of a neuron as a function of activity (Figure 1). For instance, chronic activity blockade of hippocampal, cortical or spinal neurons in culture increases the amplitude of miniature excitatory postsynaptic currents (mEPSCs) [2,8,32], whereas prolonged postsynaptic depolarization or blockade of GABA-mediated inhibition has the opposite effect [8,33] (Figure 1a,b). Synaptic scaling is (i) global, because it alters the strength of the synapses of a neuron in unison, (ii) gradual, because it proceeds over hours to days, and (iii) multiplicative, because it scales synapses proportionally to their initial strength [2]. Synaptic scaling was originally described for AMPA-receptor-mediated postsynaptic currents, but it is now known that NMDA receptor currents can be co-regulated with AMPA receptor currents [8]. Intriguingly, rapid forms of synaptic plasticity that selectively increase the AMPA receptor component of excitatory synaptic transmission are followed by a delayed potentiation of NMDA receptor currents [34]. Thus, information regarding the weighted strength of individual synapses is preserved, as is the relative contribution of both receptor types to synaptic transmission.

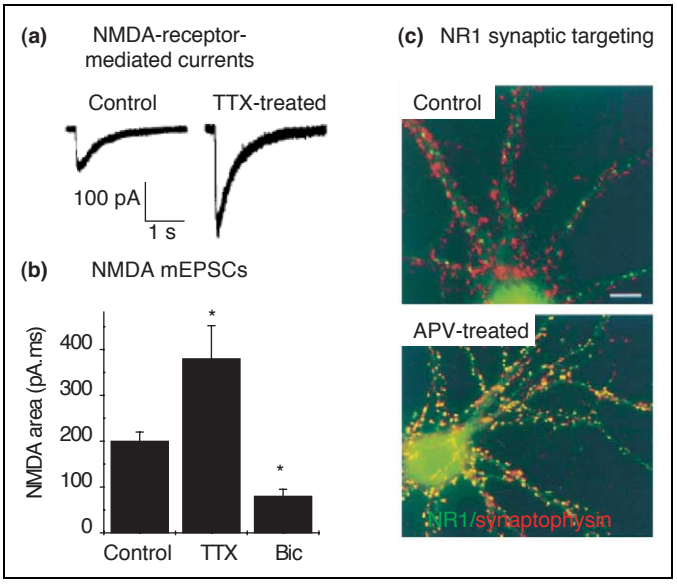

Figure 1. Synaptic scaling: activity regulates the number of synaptic NMDA receptors. **(a)** Activity blockade using tetrodotoxin (TTX) increases the amplitude of NMDA-receptor-mediated currents evoked by glutamate application. **(b)** Bidirectional scaling of NMDA-receptor-mediated mEPSC amplitude after decreasing activity (using TTX) or increasing activity (using bicuculline, Bic). **(c)** Accumulation of NMDA receptors at synapses measured by immunofluorescence. The images show superimposed double labeling for the NMDA receptor subunit NR1 (green) and synaptophysin (red). Activity blockade (here using the NMDA receptor antagonist D-2-amino-5-phosphonovaleric acid, APV) increases the number of synaptically localized NR1 puncta. Yellow indicates colocalization. (a) and (b) reproduced, with permission, from Ref. [8]; (c) reproduced, with permission, from Ref. [6].

NMDA receptor scaling, as for that of AMPA receptors, occurs through changes in the number of receptors clustered at the synapse. Noise analysis suggested that the increased amplitude of NMDA-receptor-mediated mEPSCs caused by activity blockade was due to increased numbers of postsynaptic NMDA receptors rather than changes in single-channel conductance [8]. Further, the decay kinetics and voltage-dependence of NMDA receptor currents are unaffected during scaling, also arguing against changes in NMDA receptor subunit composition [8]. Finally, immunocytochemical studies have provided direct evidence that NMDA receptors accumulate at synapses after chronic activity blockade [6,21,35] (Figure 1c).

These observations were made in culture systems, but a synaptic scaling-like phenomenon was recently described in the developing rodent visual cortex *in vivo*, unveiling differences in the homeostatic regulation of AMPA receptor and NMDA receptor currents. Desai *et al.* [36] found that the steady increase in synaptic drive that occurs during defined times of postnatal development is compensated by a slow, experience-dependent reduction in AMPA receptor mEPSC amplitude. The authors did not examine NMDA receptor currents, but earlier electrophysiological studies in layer 4 of the visual cortex and superior colliculus revealed no changes in the amplitude of NMDA-receptor-mediated synaptic currents during similar periods of development or after activity blockade induced by dark-rearing or intracortical application of tetrodotoxin [22,23]. Also, in CA1 hippocampus, immunogold electron microscopy revealed that the number of NMDA receptors per synapse remained approximately constant during postnatal development [37]. This suggests that mechanisms other than changing the number of postsynaptic NMDA receptors might be recruited to scale NMDA receptor responses during development. For instance, synaptic activity influences parameters such as spine geometry [38] and NMDA receptor subunit composition (discussed later) – modifications that could alter NMDA receptor properties without changing total receptor number.

Metaplasticity

Metaplasticity refers to a higher-order form of synaptic plasticity where prior synaptic activity leads to a persistent change in the direction or magnitude of subsequent

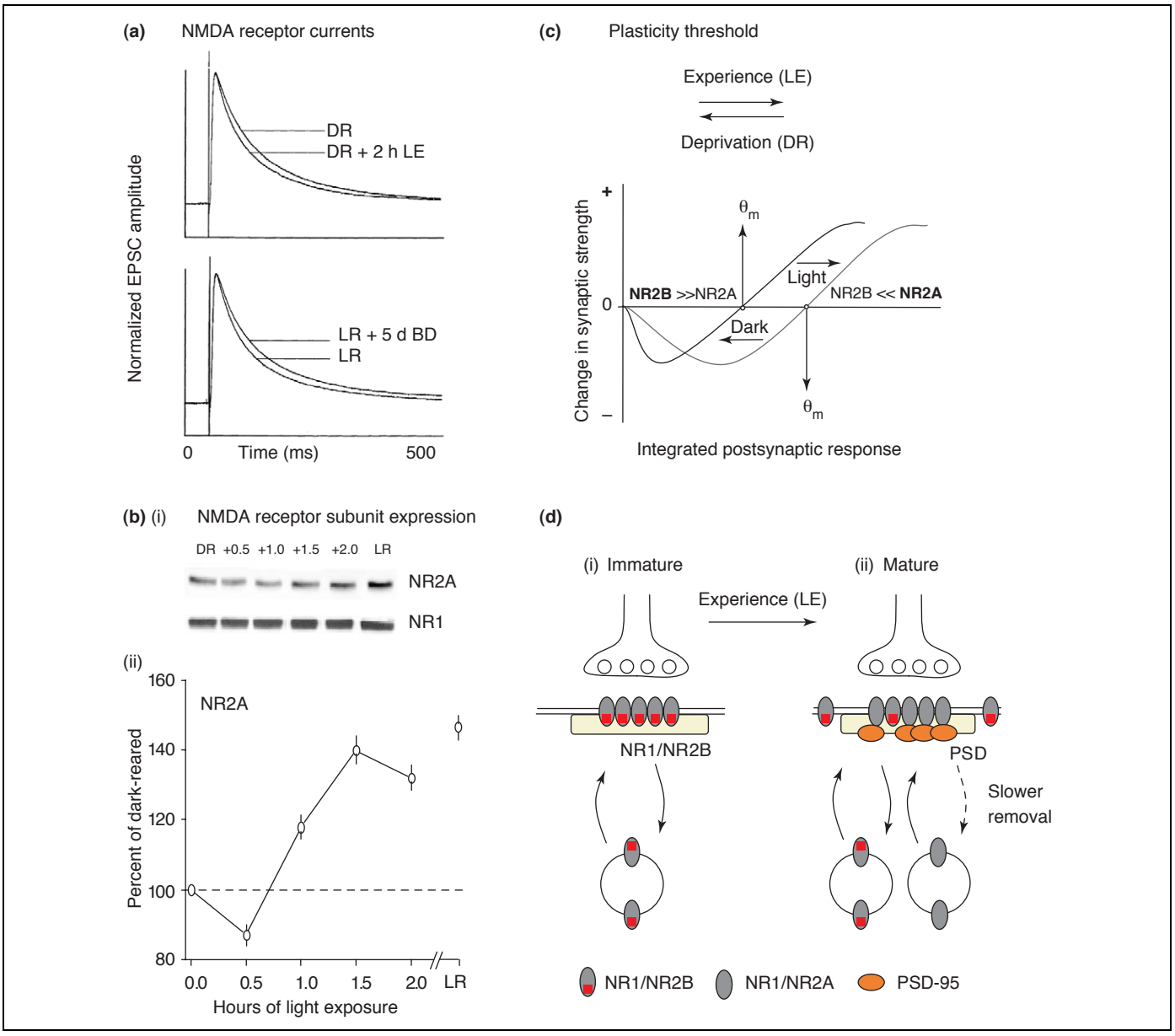

Figure 2. Metaplasticity: experience-dependent changes in NMDA receptor subunit composition could underlie the sliding synaptic modification threshold. **(a)** Visual experience bidirectionally regulates NMDA receptor current duration but not amplitude. Upper traces: overlay of NMDA-receptor-mediated EPSCs from layer 2/3 pyramidal cells in visual cortex of dark-reared (DR) rats before and after 2 h of light exposure (LE). Lower traces: overlay of NMDA-receptor-mediated EPSCs from layer 2/3 pyramidal cells in visual cortex of light-reared (LR) rats before and after 5 d of binocular deprivation (BD). Reproduced, with permission, from Ref. [24] **(b)** Brief light exposure induces a rapid change in synaptic NMDA receptor composition in visual cortex. (i) Immunoblots of NR2A and NR1 proteins in synaptoneurosomes from DR rats or after different periods of LE. (ii) Quantification of NR2A abundance. Reproduced, with permission, from Ref. [7]. **(c)** Model relating the NMDA receptor subunit switch to changes in synaptic plasticity thresholds. Activity mildly above the spontaneous level results in synaptic weakening (LTD) whereas that above the modification threshold (θ_m) leads to synaptic potentiation (LTP). The point of crossover (θ_m) from LTD to LTP is dynamic and can be shifted by changes in the NR2A:NR2B ratio. An increase in this ratio, as seen with LE after dark-rearing, correlates with higher stimulation frequencies required to induce LTP. Also, at these frequencies, NR2A-containing receptors may be more effective at increasing intracellular Ca^{2+} concentrations [116]. Conversely, a fall in NR2A:NR2B ratio would permit LTP induction at lower frequencies. Modified, with permission, from Ref. [24]. **(d)** Proposed model for the role of activity-regulated trafficking of NMDA receptors in NR2B–NR2A subunit replacement at synapses. (i) NR2B-containing receptors cycle rapidly into and out of immature synapses. (ii) As synapses develop, activity increases the synthesis of NR2A, leading to competition of NR2A-containing and NR2B-containing receptors for synaptic insertion. NR2B subunits carry robust internalization signals whereas NR2A removal rates are slower. Activity also increases PSD-95 expression, which preferentially binds NR2A and further limits its internalization rate. The net result is a progressive enrichment of NR2A-containing NMDA receptors, while NR2B-receptors are progressively displaced from synapses through a combination of lateral diffusion and internalization.

activity-dependent plasticity, without affecting actual synaptic efficacy [39,40]. Put another way, metaplasticity is the 'plasticity of plasticity' [31]. It serves a homeostatic role because it ensures that plasticity is kept within a working range (away from saturation). The best-studied examples of metaplasticity are those in which prior activity shifts the threshold for LTP and LTD induction [31,41]. For instance, during development of the visual cortex, high levels of coordinated activity such as those driven by eye opening or light exposure shift the modification threshold to the right – that is, stimulation frequencies that before elicited LTP now produce either no potentiation or LTD [9,42] (Figure 2c). Conversely, rearing animals in complete darkness delays this plasticity shift, promoting LTP over LTD over a range of stimulation frequencies when compared with visually experienced

animals [9,42]. Similar bidirectional and activity-dependent shifts in plasticity thresholds occur in somatosensory, piriform and motor cortices during development and learning [10,43,44]. In most of these examples, metaplasticity is a cell-wide effect. Consistent with a slow time course, developmental shifts in plasticity thresholds happen gradually (over several days) and prolonged times of sensory deprivation are required to restore plasticity [9,10,45]. Other forms of metaplasticity, however, occur as fast as 30 min to 2 h [24,46]. For instance, the use of high-frequency stimulation 'priming' protocols in hippocampal slices triggers a rapid shift in plasticity thresholds, facilitating subsequent induction of LTD and disfavoring LTP [46,47].

At many synapses, both LTP and LTD induction require Ca^{2+} entry through NMDA receptors, with modest elevations in Ca^{2+} levels triggering LTD and larger increases causing LTP. For this reason, altered NMDA receptor function, and particularly changes in Ca^{2+} signals generated by NMDA receptors, have been proposed to cause metaplasticity [48,49]. One mechanism that could account for the shifts in plasticity thresholds is the developmental switch between NR2B-containing receptors, which exhibit currents with slow decay kinetics and allow the temporal summation of Ca^{2+} currents, and NR2A-containing receptors, which have faster kinetics and increased temporal resolution of Ca^{2+} signals (Figure 2). Visual experience and olfactory learning increase the proportion of NR2A-containing receptors at cortical synapses [7,20] and thus shorten the duration of NMDA receptor currents [13,22,24,50], correlating with a shift to higher stimulation frequencies required to produce LTP. Conversely, visual deprivation slows or reverses the switch from NR2B to NR2A [7,20,24] (Figure 2a,b). Tests of this hypothesis in transgenic models have yielded conflicting results: mice overexpressing the immature NR2B subunit showed enhanced hippocampal LTP and learning [51], but mice lacking NR2A displayed no delay in the developmental decrease in the susceptibility to LTP in somatosensory cortex [52]. Further, the difficulty of inducing not only LTP but also LTD increases with age [53,54] and recent studies have found that, in the adult cortex and hippocampus, NMDA receptors containing NR2A mediate LTP whereas activation of NMDA receptors containing NR2B trigger LTD [55,56]. These apparent contradictions probably reflect the heterogeneity of homeostatic mechanisms and NMDA receptor regulation depending on developmental stages and brain areas.

A switch in NMDA receptor subunit composition could govern the balance between LTP and LTD by controlling not only the biophysical properties of NMDA receptors at synapses but also their precise subcellular localization and downstream signaling. For instance, NMDA receptors at mature synapses contain mostly NR2A whereas NR2B-containing receptors are extrasynaptic [50,57]; LTD in adult cortex can be induced only after blockade of glutamate uptake, suggesting that it might result from glutamate spillover onto extrasynaptic NR2B receptors [56]. In addition, synaptic and extrasynaptic NMDA receptors (presumably differing in NR2A and NR2B composition) couple to distinct downstream signaling pathways, including the cAMP-response-element binding protein (CREB) and the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathways. Both CREB and MAPK/ERK pathways sense Ca^{2+} entry through NMDA receptors and translate the transient Ca^{2+} signals into persistent changes by mediating synapse-to-nucleus signaling and activating gene transcription [58,59], which explains their crucial role in long-lasting synaptic plasticity and memory storage [60,61]. However, CREB and MAPK/ERK are differentially sensitive to the spatial and temporal patterns of Ca^{2+} signals, with MAPK/ERK activation relying on Ca^{2+} microdomains localized in the immediate vicinity of the NMDA receptor channel [62,63]. Further, NR2A and NR2B subunits differ in their large C-terminal cytoplasmic domains that mediate binding to scaffolding and signaling proteins. This ability to organize distinct signaling complexes is thought to direct downstream signaling from NMDA receptors to CREB and MAPK/ERK pathways (among others) [15,21,28], thereby triggering different forms of plasticity. Such a notion is supported by the altered or absent synaptic plasticity in mutant mice lacking NR2 subunit C-terminal domains [64–66].

Activity-dependent mechanisms that control the complement of NMDA receptors at synapses

Changes in postsynaptic NMDA receptors could be brought about by a wide variety of mechanisms operating at different levels: transcriptional, post-translational, intracellular trafficking, or a combination of these. Although subunit synthesis determines receptor availability, and as such exerts a first level of control over the numbers and types of NMDA receptors present at synapses [11,67], several trafficking steps following subunit synthesis appear to operate as regulatory checkpoints for the synaptic incorporation of NMDA receptors [68–70]. In the following sections, we will review recent advances in our understanding of NMDA receptor secretory trafficking, exocytic–endocytic cycling and degradation, and we will discuss how each of these steps could be regulated by activity and how they might contribute to the maintenance of synaptic homeostasis.

Coupling between activity and mRNA splicing regulates ER export of NMDA receptors

NMDA receptors are assembled in the endoplasmic reticulum (ER) from the essential NR1 subunit and different NR2 and NR3 subunits, and they exit the ER in vesicles that are transported to the Golgi apparatus. Thereafter, targeting to synapses occurs via post-Golgi transport, probably through a combination of delivery onto the plasma membrane, diffusion within the plane of the membrane, and selective retention at synapses. Several trafficking checkpoints are strategically located along this secretory pathway. The first operates at the level of the ER to recognize and retain unassembled NMDA receptor subunits and ensure the forward trafficking of only certain subunit combinations [71–73]. ER retention and export have been classically considered as mere quality-control mechanisms, but a recent study revealed that ER export is sensitive to neuronal activity

[70]. The mechanism was unexpected: the level of neuronal activity bidirectionally controls mRNA splicing, alternately including or excluding exon 22, one of the three alternatively spliced exons of NR1, from the NR1 pre-mRNA [74]. When activity is blocked, exon 22 is excluded (or more precisely, an alternate splice-acceptor site within exon 22 is utilized). Conversely, increasing neuronal activity results in the selective inclusion of exon 22 in the NR1 pre-mRNA. Exon 22 encodes the distal C-terminal region of NR1 and its inclusion results in the synthesis of NR1 subunits containing the C2 domain at the distal C terminus. By contrast, alternate splice-acceptor-site usage (exclusion) of exon 22 following prolonged activity blockade results in synthesis of NR1 subunits containing the C2′ domain. The C2′ domain contains a divaline-based ER-export motif that enhances the exit of newly synthesized NMDA receptors from the ER by binding the COPII coat complex, thereby accelerating secretory trafficking, surface delivery and synaptic insertion of NMDA receptors (Figure 3). As evidence linking this phenomenon to synaptic activity, disrupting the trafficking of C2′-containing NR1 subunits slows ER export and prevents the synaptic accumulation of NMDA receptors induced by activity blockade [70].

ER export is the rate-limiting step for surface delivery of many integral membrane proteins [75]. As such, affecting the kinetics of ER export presumably increases the size of the intracellular receptor pool readily available for insertion into the plasma membrane, and therefore changes global insertion rates, providing an unanticipated cellular mechanism for synaptic homeostasis (Figure 3). Like synaptic scaling, the coupling between splicing, ER export and synaptic delivery takes time, works globally, and is bidirectionally regulated by activity. Further, the target of the splicing event is the mRNA encoding the ubiquitous NR1 subunit, explaining the simultaneous increase in numbers of NR2A and NR2B clusters measured by immunofluorescence after activity blockade [6].

The sensors and signaling pathways that link activity changes to splicing remain unknown, but likely candidates are protein kinase pathways that regulate both mRNA splicing [76] and NMDA receptor synaptic targeting [77,78] in an activity-dependent manner. Possible kinase targets include RNA-binding proteins [76,79]. In this regard, it is interesting to note that some neuronal splicing factors such as Nova-1 can associate with sets of mRNA encoding multiple synaptic proteins [79]. It is tempting to speculate that activity, by regulating splicing factor association with pre-mRNA, could direct the splicing of a group of exons to scale NMDA receptors in unison with other components of excitatory synapses. In addition to regulating splicing, protein kinases including protein kinase A (PKA) and protein kinase C (PKC) directly and synergistically phosphorylate ER-resident NR1, further facilitating ER exit [80]. Interestingly, PKA is required for inactivity-induced synaptic accumulation of NMDA receptors [77] and PKC promotes surface delivery of NMDA receptors [81]. These results suggest that the

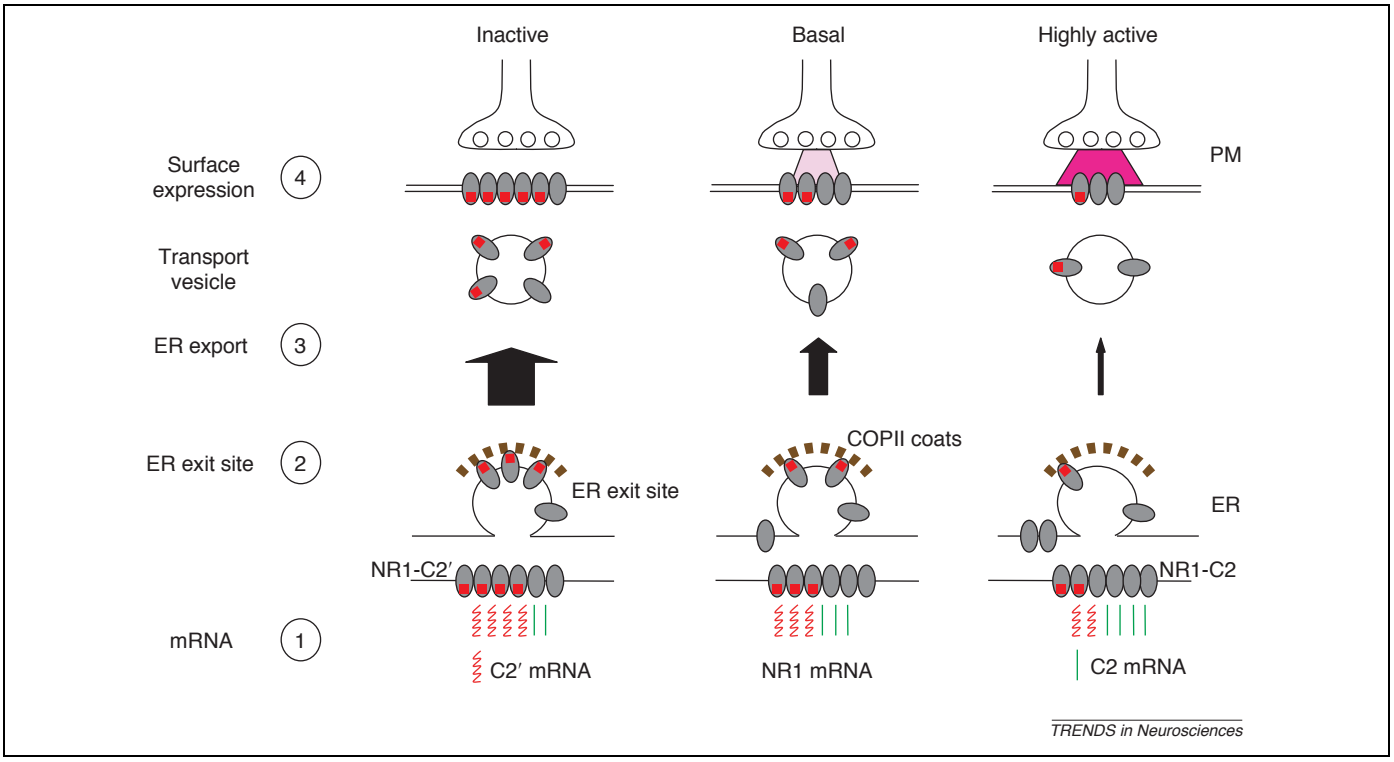

Figure 3. Model for activity-dependent ER export and synaptic targeting of NMDA receptors. Long-term activity manipulations in cultured neurons, such as blockade of action potentials using TTX ('Inactive') or suppression of inhibitory transmission using bicuculline ('Highly active') bidirectionally regulate NR1 pre-mRNA splicing. Prolonged synaptic blockade results in alternate splice-acceptor-site usage at exon 22 of NR1 pre-mRNA, leading to synthesis of NR1 subunits containing the C2′ domain (1). The divaline-based ER export signal in C2′ interacts with COPII coats to recruit the receptor to ER exit sites (2), accelerating ER export (3). The result is an increase in the size of the available NMDA receptor pool, the rate of surface delivery and the total number of synaptic receptors (4). Raising activity levels using bicuculline leads to a precisely opposite sequence of events (inclusion of exon 22, synthesis of NR1 subunits containing the C2 domain rather than the C2′ domain, decreased ER export and decreased surface delivery of NMDA receptors). Abbreviation: PM, plasma membrane.

forward trafficking kinetics of NMDA receptors is tightly tuned to neuronal activity and intracellular signaling events, providing a potential 'online' readout in the form of surface-delivered NMDA receptors. Because a large fraction of endogenous NR1 resides in such an intracellular pool [82,83], it is perhaps not surprising that neurons have evolved mechanisms to regulate their forward trafficking kinetics.

Although these findings emphasize the importance of early trafficking checkpoints in controlling the supply of NMDA receptors to the synapse, it has not been demonstrated that early secretory trafficking can change synaptic receptors rapidly or mediate the NMDA receptor subunit switch thought to underlie metaplasticity. Several studies indicate that increased subunit availability must be coupled to mechanisms operating at later stages of trafficking to enable receptor delivery to the postsynaptic membrane [68,69].

Differential exocytic–endocytic vesicular trafficking of NMDA receptor subunits

Recent work has shown that NMDA receptors undergo rapid insertion into and removal from the neuronal plasma membrane through exocytosis and endocytosis [68,81,84–90]. Thus, analogous to AMPA receptors, the number of NMDA receptors at a given synapse results from a dynamic equilibrium between insertion and removal rates, and this equilibrium is likely to be shifted by activity. In support of this idea, NMDA receptor delivery to the plasma membrane can be enhanced sixfold by PKC, which triggers the rapid insertion of receptor-containing vesicles via SNAP receptor (SNARE)-dependent exocytosis [81]. The effect is probably mediated by phosphorylation of receptor-associated proteins rather than of the receptor itself. One potential target of PKC is soluble *N*-ethylmaleimide-sensitive factor attachment protein (SNAP)-25, which can be phosphorylated in an activity-dependent manner [81]. LTP in adult brain slices can trigger the rapid insertion of NMDA receptors into the neuronal surface [91]. However, NMDA receptor endocytosis is also regulated by activity. For instance, metabotropic glutamate receptor activation [85], ligand binding [86,92] and low-frequency stimulation [93] all promote NMDA receptor downregulation and simultaneously stimulate the association of NMDA receptors with the clathrin adaptor AP-2 [87], suggesting that the downregulation is mediated by clathrin-dependent endocytosis.

Interposing between exocytosis and endocytosis are trafficking events within the bilayer of the plasma membrane. Recent electrophysiological and single-molecule imaging studies have elegantly demonstrated that NMDA receptors undergo rapid synaptic exchange [94] and diffuse within restricted domains of the synaptic and extrasynaptic plasma membranes [95]. Extrasynaptic receptors could provide a highly mobile pool that can be readily transported to synapses to achieve rapid changes in NMDA receptor currents and thereby alter synaptic strength and plasticity. Intriguingly, both the lateral mobility and the synaptic dispersal of NMDA receptors are increased by selective activation of PKC using phorbol esters [78,95], suggesting regulated movement of NMDA receptors between membrane microdomains. Such membrane microdomains could include synapse-associated endocytic zones [96], thereby linking lateral diffusion to endocytosis.

Regulating local trafficking provides a mechanism for controlling not only the abundance but also the subtypes of NMDA receptors placed at synapses, because the incorporation of NMDA receptors into synapses follows different rules depending on subunit composition. Although synaptic insertion of NR2B-containing receptors is constitutive and does not require ligand binding or synaptic activity, their replacement by NR2A-containing receptors is use-dependent [68]. In this regard, recent studies have demonstrated subunit specificity in several steps of NMDA receptor exocytic–endocytic trafficking. First, NMDA receptors containing NR2C or NR2D subunits do not show PKC potentiation [97]. Second, NR2A and NR2B carry different amino acid motifs in their C termini that bind the endocytic adaptor AP-2 with different affinities, resulting in a more robust internalization of NR2B-containing receptors [89]. Indeed, NMDA receptors in early postnatal life, which contain more NR2B than NMDA receptors in the adult, are endocytosed at higher rates [84]. The rate of NMDA receptor endocytosis declines as neurons mature and incorporate more of the NR2A subunit (Figure 2d). Third, NR2A-containing receptors are more strictly localized to synapses than NR2B-containing receptors, which are commonly found in the extrasynaptic membrane [50,57]. This differential distribution might further influence their relative rates of internalization [84,86]. Fourth, the route of post-endocytic sorting of NMDA receptors is differentially encoded within subunits. NR2A endocytic signals direct transport along a degradative pathway to late endosomes, and NR2B preferentially directs receptors through recycling endosomes and back to the plasma membrane [88,89]. It remains to be established whether activity can control sorting of NMDA receptors, as in the case of AMPA receptors [98]. The presence in membrane-proximal C-terminal domains, near a Ca^{2+}/calmodulin-binding site, of a conserved family of endocytic motifs that target NMDA receptors for degradation [88] suggests potential regulation by Ca^{2+} influx and/or by ligand-induced conformational change.

Although local exocytic–endocytic trafficking fulfills many of the requirements for a mechanism mediating homeostatic plasticity, it is intrinsically rapid. Because of this, exocytic–endocytic cycling is ideally suited for driving rapid forms of activity-dependent insertion or removal, such as the fast (within 1 h) NR2B–NR2A switch caused by light exposure or the rapid loss of NMDA receptors that accompanies LTD [7,20,85,93]. However, the developmental NR2B–NR2A switch proceeds slowly, as does scaling. One possibility is that the number of synaptic receptors can be shifted by even small activity-dependent alterations of trafficking rates, prompting slow accumulation or scaling down of receptors. Alternatively, slow changes in synaptic NMDA receptors could reflect the presence of rate-limiting steps upstream from receptor insertion or an inability to remove NMDA-receptor subunits already in place at synapses, perhaps owing to

a lack of specific adaptors or sorting proteins or the need for a specific mechanism triggering synaptic removal. Consistent with this general idea is the finding that ligand binding is required for synaptic removal of NR2B and replacement by NR2A [68]. Such findings suggest that only once synaptic NR2B-containing receptors are removed can NR2A be readily inserted at synapses. Detailed elucidation of subunit-specific endocytic, sorting and scaffolding proteins will enable this hypothesis to be tested.

Activity-dependent control of postsynaptic composition by the ubiquitin–proteasome system

The changes in NMDA receptors that are proposed to mediate synaptic scaling and metaplasticity are often accompanied (or followed) by global remodeling of the postsynaptic density (PSD). Although conventionally viewed as a stable structure, in large part owing to its biochemical rigidity and resistance to detergent solubilization [99,100], the PSD experiences remarkable morphological changes in response to synaptic activity [101–103] and its size can be scaled over long time periods to accommodate more receptors [104]. Further, the molecular components of the PSD undergo continuous and relatively rapid turnover (e.g. taking only a few hours) that is accelerated by activity and slows down in inactive cultures [21]. A large component of this turnover results from the selective removal of existing PSD proteins via ubiquitination and subsequent degradation by the proteasome [21].

An interesting aspect of activity-regulated changes in PSD turnover is that they target coordinated sets of receptors, scaffolding proteins and signaling proteins. For instance, the half-lives of NR1 and NR2B (but not NR2A) subunits, and those of their scaffolding proteins A-kinase-anchoring protein (AKAP)79 and synapse-associated protein (SAP)102, are increased by activity blockade [21]. Remodeling of PSD protein composition by the ubiquitin–proteasome system also alters the coupling of NMDA receptors to downstream signaling pathways such as CREB and MAPK/ERK, predicting changes in plasticity properties [21] (Figure 4). This alteration in signaling might require regulation of postsynaptic proteins, such as Ras-guanine-nucleotide-releasing factor (RasGRF)1 or the GTPase-activating protein SynGAP, that bind NR2 subunits and couple NMDA receptors to MAPK/ERK, perhaps facilitating the subsequent induction of plasticity [105–108]. The exact mechanism is unclear, but is likely to involve the coordinated regulation of ubiquitin regulatory enzymes (E1, E2 and E3) by activity. In this regard, Colledge *et al.* have recently shown that the E3 ubiquitin ligase Mdm2 can ubiquitinate PSD-95 in an NMDA-receptor-dependent fashion, resulting in loss of PSD-95 and synaptic AMPA receptors [109]. Depletion of PSD-95 and a loss of mature dendritic spines also follow activity-induced expression of serum-inducible kinase (SNK), which leads to SNK-dependent phosphorylation and degradation of spine-associated Rap-GTPase-activating protein (SPAR) by the ubiquitin–proteasome system [110]. Because SPAR binds actin and several other proteins potentially involved in spine morphogenesis, the SNK–SPAR pathway could work as a homeostatic regulatory mechanism that destabilizes synaptic connections in response to activity [110].

Thus, ubiquitination-dependent removal and degradation appears a suitable mechanism not only to change the physical space and protein network available for scaling synapses, but also to drive selective receptor replacement by coordinating the exchange of receptor anchors and other trafficking regulatory proteins. In addition, by altering signaling, it provides an attractive way to couple synapse composition to subsequent gene transcription based on activity history. To develop a

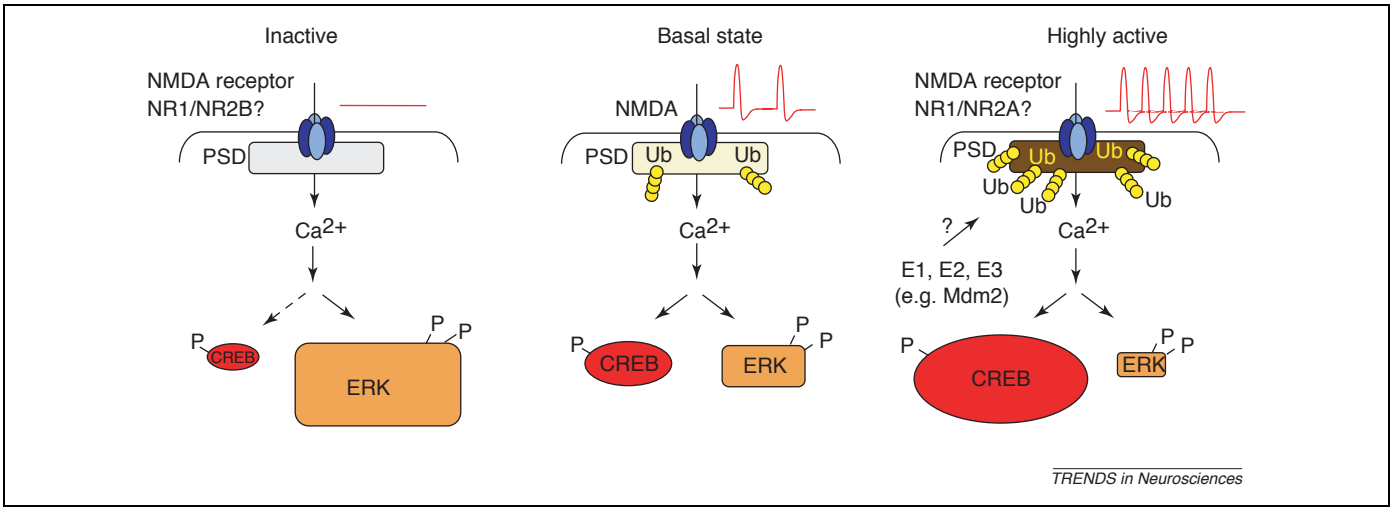

Figure 4. Ubiquitin-dependent remodeling of the PSD. Long-term alterations in neuronal activity alter ongoing ubiquitination and protein turnover in the PSD, causing global changes in its molecular composition (indicated by different shading), including changes in the abundance and subunit composition of NMDA receptors. This remodeling alters the coupling of synaptic NMDA receptors to the downstream signaling pathways of CREB and MAPK/ERK that mediate synaptic plasticity. In this model, inactivity reduces ubiquitin (Ub) conjugation and proteasome-mediated turnover of synaptic components, enhancing the half-life of postsynaptic proteins such as NR1 and NR2B and, in turn, increasing NMDA-receptor-mediated MAPK/ERK phosphorylation (enlarged arrow) and reducing the coupling of NMDA receptors to CREB (broken arrow). By contrast, increased synaptic activity increases the ubiquitination and turnover of specific postsynaptic proteins, perhaps through the activation of ubiquitin-transferring enzymes (E1, E2 and E3) including E3 ubiquitin ligases such as Mdm2. This destabilization and increased turnover decreases the half-life of NR1 and NR2B subunits and associated protein scaffolds, increasing the synaptic abundance of NR2A-containing NMDA receptors and their coupling to the CREB signaling pathway. Model based on results from Ref. [21].

coherent framework for synaptic modification, it will be crucial to map the targets of ubiquitination, ubiquitin ligases and other components of the ubiquitin-regulatory machinery that orchestrate postsynaptic remodeling.

Reconciling trafficking and transcription

It is becoming clear that transcriptional and trafficking mechanisms are intimately linked and work in concert to accomplish the synaptic changes necessary to maintain homeostasis. Synaptic activity influences the transcription of NMDA receptor subunits [111,112]. Because the subunits possess intrinsic molecular determinants that control trafficking, their relative abundance in the receptor complex will, in turn, affect the movement of receptors through internal compartments and into and out of the postsynaptic membrane (Figure 2d). Activity also alters the expression of scaffolding proteins that provide synaptic anchors for receptors (Figure 2d). For instance, the levels of PSD-95, which preferentially binds NR2A subunits, are regulated during development [113], and experience rapidly increases dendritic PSD-95 expression in visual pathways [114]. It will be exciting to determine whether the expression of molecules that control mRNA splicing, receptor degradation and exocytic–endocytic cycling are also subject to activity-dependent regulation. Activity-induced changes in protein expression might also occur locally, near synapses, to enable rapid synaptic change. For instance, the rapid endocytosis of glutamate receptors requires ongoing protein synthesis in some cases [85] or can be blocked by proteasome inhibitors [115], suggesting a need for dendritic synthesis or degradation of proteins that regulate receptor trafficking.

Perspectives

The recent awakening of the field of NMDA receptor trafficking has unveiled multiple mechanisms that control the ultimate density of NMDA receptors at synapses. Much work needs to be done to test the models proposed, starting with a further definition of the cell biological pathways, of which we still know very little, including a determination of the extent to which regulation of NMDA receptor trafficking occurs globally or is spatially restricted near synapses. A quantitative approach, with careful determination of trafficking rates and how they are modulated by varied patterns of activity, must be undertaken. Proteomic approaches should aid the characterization of postsynaptic ensembles and their regulation by activity. Finally, use of genetic animal models to visualize receptor trafficking or disrupt selected trafficking steps *in vivo* will be essential to translate the cell biological and molecular knowledge into a physiological context. It is a fascinating but complicated task, because the mechanisms that determine plasticity are more sophisticated and diverse than originally thought. Ever front and center in synaptic plasticity, the NMDA receptor has become a moving target.

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