

Receptor compartmentalization and trafficking at glutamate synapses: a developmental proposal

Brigitte van Zundert^{1,2}, Akira Yoshii¹ and Martha Constantine-Paton¹

¹Department of Biology, Department of Brain and Cognitive Science, and McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

²Day Laboratory for Neuromuscular Research, Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02115, USA

This article focuses on NMDA receptor subunit changes that occur in the forebrain and midbrain during development, namely the switch from predominance of NMDA receptors rich in NR2B subunits to that of NMDA receptors rich in NR2A subunits. We review the potential roles in brain plasticity of two membrane-associated guanylate kinases (MAGUKs), SAP102 and PSD95, which form a scaffold for the ion-passing glutamate receptors at the postsynaptic density, and we consider the known functional significance of these molecules in subunit switching. In addition, based on recent analyses of the synaptic location of glutamate receptors, activity-dependent changes in developing visual neurons, and extensive data on MAGUKs, we propose a model of glutamatergic synaptic differentiation. In this model, different NMDA receptor scaffolding and signaling complexes effect the trafficking and synaptic localization of NR2A-rich and NR2B-rich receptors, leading to tangential compartmentalization of these receptors and their movement between synaptic and extrasynaptic compartments.

NMDA receptors have been implicated in synaptogenesis, learning, memory and disease. The NMDA receptor is a complex consisting of two obligatory NR1 subunits (which have eight splice variants) and up to two of four NR2 subunits (NR2A–NR2D). The NR2 subunits confer distinct pharmacological and kinetic properties on the receptor. For over a decade, it has been recognized that the subunit composition of all ionotropic glutamate receptors changes during development and varies in different regions of the mature brain. Because of the widespread importance of NMDA receptors, many investigators have tried to determine whether particular NMDA receptor subunit combinations underlie different functions of the receptor and regulate synaptic plasticity.

Synaptic and extrasynaptic NMDA receptors

Molecular, immunocytochemical and functional data suggest that NR2A, NR2B and NR1 subunits exist *in vivo*

at synaptic sites as diheteromers (e.g. NR1–NR2A or NR1–NR2B) and triheteromers (e.g. NR1–NR2A–NR2B) [1–3]. However, functional extrasynaptic NMDA receptors also exist [4–10]. Like synaptic NMDA receptors, extrasynaptic NMDA receptors can be activated by synaptically released glutamate [11–13]. Insights into the potential importance of extrasynaptic NMDA receptors *in vivo* come from studies showing that some normal neurons have evoked AMPA-receptor-mediated currents, evoked NMDA-receptor-mediated currents and AMPA-receptor-mediated miniature currents [12,13] (Box 1). Studies in retinal ganglion cells [12] and hippocampal neurons [11] indicate that some extrasynaptic NMDA receptor responses result from spillover and temporal summation of glutamate diffusing from synaptic release sites (Boxes 1,2).

Clark and Cull-Candy [13] recently suggested a possible signaling function of receptor compartmentalization into synaptic and extrasynaptic NMDA receptors. Specifically, different combinations of signals are generated when different receptors respond to mild and to intense stimuli delivered through the same pathways (Box 1). The inclusion of extrasynaptic NMDA receptor currents in an evoked response appears to vary according to several parameters, including the location and degree of saturation of neuronal and glial transporters, the activity of transporters [14], and the amount, timing and proximity of glutamate release.

As will be discussed, other recent work suggests that, as synapses mature, synaptic and extrasynaptic NMDA receptors are likely to be associated with different scaffolding molecules of the membrane-associated guanylate kinase (MAGUK). Specifically, at mature synapses, NR2A-rich receptors are linked to postsynaptic density 95 (PSD95) and located principally at the postsynaptic density, whereas NR2B-rich receptors coupled to synapse-associated protein 102 (SAP102) are predominantly located at perisynaptic or extrasynaptic membrane sites. Such a preference of certain MAGUKs for particular NMDA receptor subtypes raises the likelihood that, at least in development, synaptic and extrasynaptic NMDA receptors are structurally linked to different cytoplasmic signaling cascades. If this proves to be generally true then, under spontaneous release conditions, synaptic receptor

Corresponding author: Martha Constantine-Paton (mcpaton@mit.edu).

Available online 5 June 2004

Box 1. Extrasynaptic NMDA receptor currents in wild-type neurons with only synaptic AMPA receptor currents

Recordings from retinal ganglion cells demonstrate that the release of single synaptic vesicles from active sites on the same neuron can either not activate or activate extrasynaptic NMDA receptors at different contacts. Spontaneous release of predominantly single synaptic vesicles from the active zone of different contacts results in generation of currents mediated by receptors restricted to the postsynaptic density, or of currents mediated by both synaptic and extrasynaptic receptors. Isolated spontaneous NMDA receptor currents are not present in these small spontaneous excitatory postsynaptic currents (sEPSCs) [NMDA receptor sEPSC alone, Figure 1a (bottom left) of this box]. They also display no sensitivity to the glutamate transport blocker TBOA, suggesting that glutamate concentrations outside the synaptic cleft are insufficient to activate extrasynaptic receptors. However, large sEPSCs also resulting predominately from single vesicle release have a slow NMDA receptor component that is enhanced by TBOA, indicating that glutamate diffusing from the synaptic cleft is contributing to this slow extrasynaptic NMDA receptor spontaneous current. Evoked excitatory postsynaptic currents (eEPSCs), both large and small, are insensitive to

external Ca^{2+} concentrations that alter the probability of vesicle release. This supports the assumption that different combinations of synaptic and non-synaptic glutamate currents can occur at different synapses of the same neuron in response to the release of one glutamate vesicle. Parameters such as the amount of glutamate in individual vesicles, the local architecture of the active site, and, potentially, the maturity of the synapse affect the contribution of extrasynaptic NMDA receptors to the synaptic response.

Recordings of rat postnatal-day 18 cerebellar stellate neurons illustrate that the presence and size of extrasynaptic NMDA-receptor-mediated currents in synaptic responses can be modulated by the intensity or activation frequency of the same pathway (Figure 1b of this box). Only high-intensity stimulation (30–40V) could evoke slow NMDA-receptor-mediated currents. Low-intensity stimulation produced only AMPA receptor currents antagonized by CNQX (Figure 1b,i of this box). With increasing frequency of stimulation (Figure 1b,ii of this box), slow extrasynaptically generated NMDA receptor currents increased in amplitude as well as in charge transfer (smooth curves).

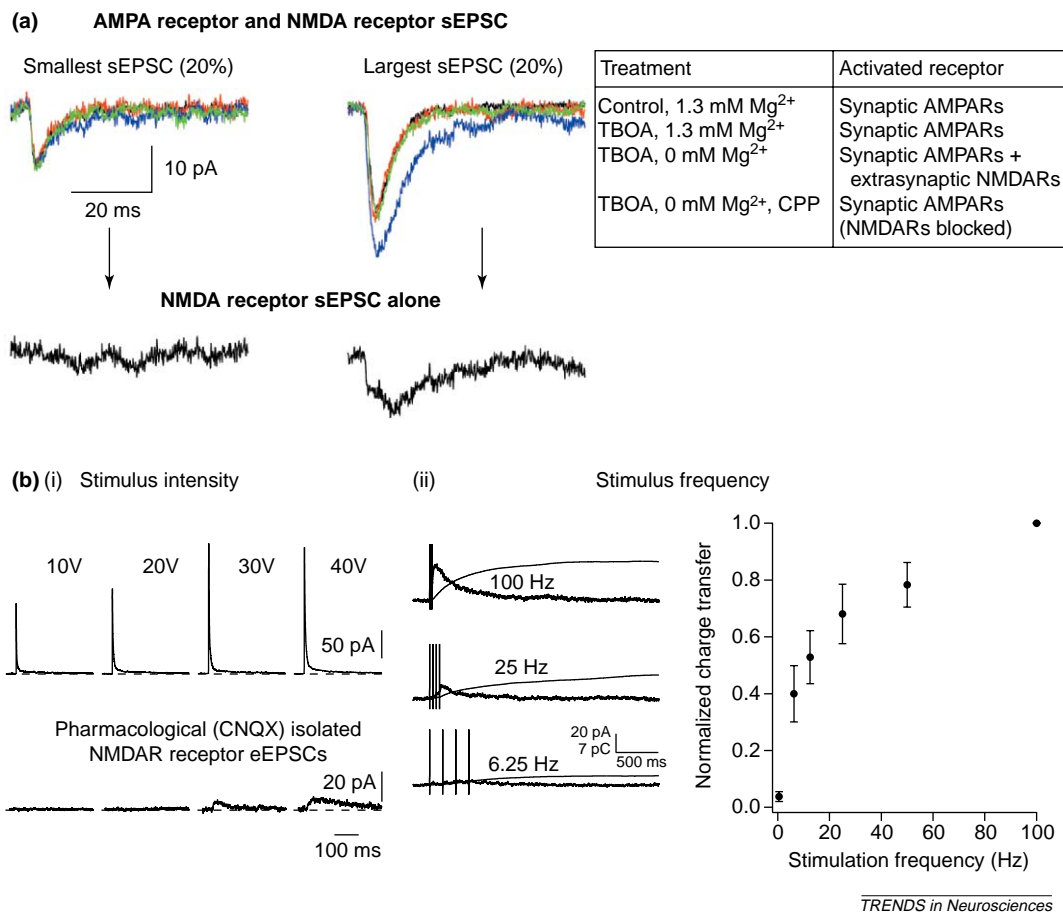


Figure 1. Extrasynaptic whole-cell patch-clamp NMDA receptor currents in wild-type neurons with only synaptic AMPA receptor currents. **(a)** Using whole-cell patch-clamp techniques and rat postnatal day (P)17–22 retinal slices, spontaneous excitatory postsynaptic currents (sEPSCs) were recorded from retinal ganglion cells at -80 mV. **(b)** Increasing intensity or frequency of electrical stimuli applied in slices to parallel fibers reveals an extrasynaptically mediated NMDA receptor current in rat P18 cerebellar stellate neurons held at $+50$ mV. **(i)** Effect of stimulus intensity. **(ii)** Effect of stimulus frequency: charge transfer (normalized to 100 Hz) with respect to lower stimulating frequencies. Abbreviations: AMPAR, AMPA receptor; CPP, 3-(R)-2-carboxypiperazin-4-propyl-1-phosphonic acid; NMDAR, NMDA receptor; TBOA, threo-beta-benzyloxyaspartate. Panel (a) modified with permission from Ref. [12], and (b) modified with permission from Ref. [13], both © (2002) the Society for Neuroscience.

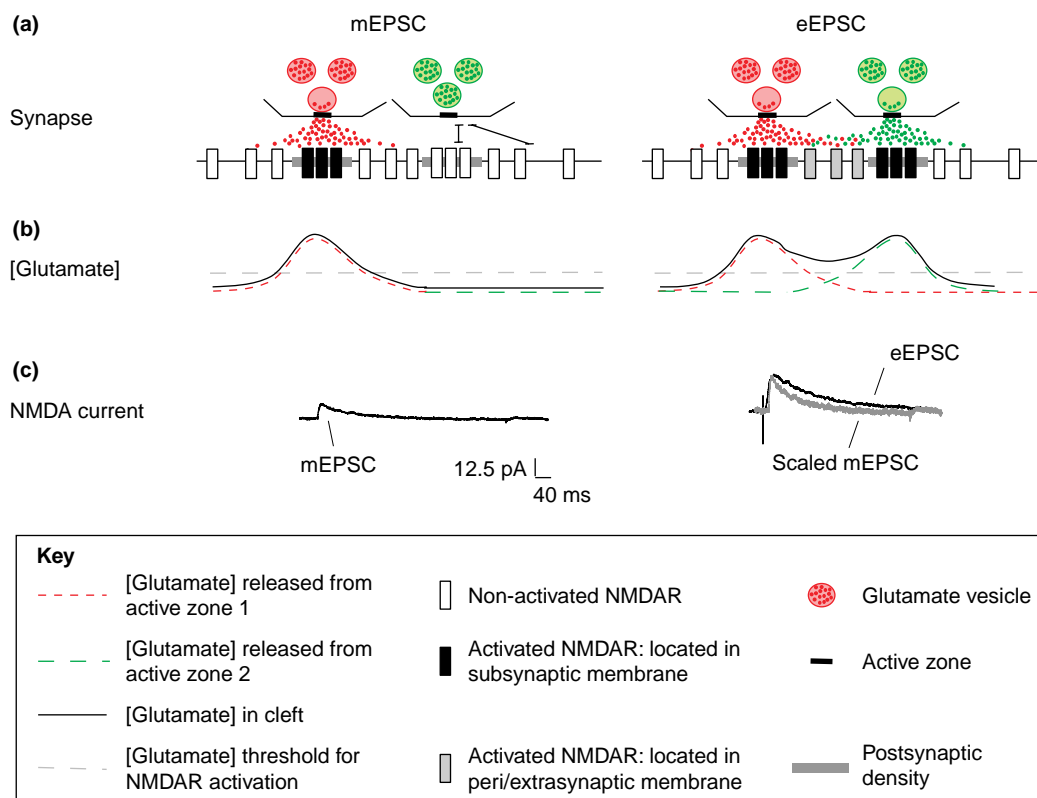
complexes and their downstream signal transducers will dominate the postsynaptic response. By contrast, multi-vesicular release, correlated converging activity and/or high-frequency input will produce diffusion of glutamate out of the synaptic cleft and result in the activation of both

synaptic and extrasynaptic signal transduction cascades (Boxes 1,2). Intensive work on AMPA receptors has indicated that some AMPA receptor subtypes are also initially inserted into the extrasynaptic membrane [15]. However, extrasynaptic AMPA receptors are unlikely to be

Box 2. Recording of spontaneous activity and evoked stimulus activate different sets of receptors

Evoked responses, because they usually involve more than one release site, generate currents that are mediated by activation of synaptic and extrasynaptic NMDA receptors. Glutamate diffusion following stimulation causes simultaneous release of glutamate from multiple synaptic vesicles (Figure 1a of this box). Neighboring synapses driven by the same pathway are shown. The spatial summation of glutamate molecules diffusing from multiple synapses allows extrasynaptic glutamate to reach concentrations ($[Glutamate]$) that activate extrasynaptic NMDA receptors (Figure 1b of this box). Owing to the delay caused by diffusion of glutamate from the synaptic cleft, the activation

of extrasynaptic NMDA receptors is delayed compared with the activation of synaptic NMDA receptors. Scaled traces from a superior colliculus neuron (Figure 1c of this box) show that extrasynaptic glutamate results in evoked excitatory postsynaptic currents (eEPSCs) with slower decay times than the scaled miniature EPSCs (mEPSCs) from the same cell. If the glutamate transporters that remove glutamate from the extracellular space become saturated or are experimentally blocked, the effective distance of activating concentrations of glutamate will increase producing larger and longer extrasynaptic NMDA receptor currents.



TRENDS in Neurosciences

Figure 1. Recording of spontaneous activity and evoked activity reflect current through different sets of receptors. (a) Glutamate diffusion following stimulation. (b) Spatial summation of glutamate molecules diffusing from multiple synapses. (c) Scaled traces from a superior colliculus neuron. Abbreviations: eEPSC, evoked excitatory postsynaptic current; mEPSC, miniature excitatory postsynaptic current; NMDAR, NMDA receptor.

activated by concentrations of glutamate diffusing from active sites because, compared with NMDA receptors, the glutamate binding affinity of AMPA receptors is low and AMPA receptors display fast and complete desensitization [16].

Different MAGUKs show preferences for binding to particular NMDA receptor subunits

The hypothesis that the PSD95 family of MAGUKs compartmentalizes and controls the distribution of vertebrate ionotropic glutamate receptors was advanced in 1992 with the isolation of a 95 kDa protein from the postsynaptic density [17]. Since then, the literature on associations and possible functions of the PSD95 family of molecules has grown exponentially [3]. In addition to binding NMDA receptor heteromers via the C-terminal tails of NR2 subunits [18–20], members of the PSD95

family bind K^+ channels at various plasma membrane sites, AMPA receptors in the endoplasmic reticulum [21], and stargazin–AMPA-receptor complexes on the plasma membrane [22]. The kainate receptor subunits GluR6 and KA1 have also been shown to bind to PSD95 family members [23]. PSD95 directly binds various proteins associated with signal transduction [e.g. synaptic GTPase-activating protein (SynGAP), ErbB, fyn, neuronal nitric oxide synthase (nNOS), spine-associated Rap-guanosine-triphosphatase-activating protein (SPAR) and kalirin], cell adhesion (e.g. neuroligin and syndecan) and Ca^{2+} homeostasis (e.g. Ca^{2+} -ATPases 2a and 4b), and with other scaffolding proteins [e.g. guanylate kinase domain-associated proteins (GKAP), Cript and A-kinase-anchoring proteins (AKAP)]. Several domains of the PSD95 family of molecules, namely the PDZ1–PDZ3, src homology 3 (SH3) and guanylate kinase (GK) domains, are

involved in binding different molecules in this list [3,24,25]. The PDZ domains are known to hold the NMDA receptor and the stargazin–AMPA-receptor complex on the plasma membrane. Besides PSD95 itself, which is also known as SAP90, the two other MAGUK family members important for binding glutamate receptors at the synapse are PSD93 (also known as chapsyn 110) and SAP102 [26–29]. These MAGUKs are highly homologous. This makes it difficult to determine whether they have different binding partners, using two-hybrid screens (where tertiary and quaternary structural information is lost) or in overexpression studies (where specificity due to binding affinities and cellular compartmentalization can be masked).

Using biochemical and immunogold electron-microscopic analyses, Sans *et al.* [30] demonstrated that, coincident with early NR2B subunit expression, SAP102 predominates in neonatal hippocampal neurons, where it is located at the postsynaptic membrane apposed to presynaptic release sites. By contrast, expression of both PSD95 and the NR2A subunit gradually increase during the second and third postnatal weeks. Moreover, immunoprecipitations in juvenile hippocampus showed that PSD95 and PSD93 preferentially pull down NR2A subunits, whereas SAP102 coimmunoprecipitations are enriched in NR2B subunits [30]. A similar temporal expression pattern for NMDA receptor subunits (Figure 1b) with SAP102 and PSD95 (Figure 1c), but not with PSD93 (Figure 1d), was found in the developing superficial visual layers of the superior colliculus and visual cortex [31,32]. In these layers, SAP102 and NR2B-rich receptors predominate early in development but expression of NR2A and PSD95 increases during the juvenile period in close correlation with increasing intensity and temporal patterning of activity from the major visual input: the contralateral retina (Figure 1a). Importantly, both hippocampal and visual neurons still show significant expression of SAP102 in adulthood (Figure 1c). Therefore, the simple model of SAP102 as the fetal and neonate NMDA receptor scaffold, and of PSD95 as the mature NMDA receptor scaffold, does not hold, at least in these regions of the brain.

NMDA receptor currents and synaptic change

Biophysical analyses of the effects of NR2 subunit changes on NMDA receptor channel function indicate that heteromers containing NR2B subunits produce channels with longer open times and longer-lasting series of opening and closing, than do heteromers containing NR2A subunits. Long-open-time channels sum to produce whole cell NMDA receptor currents with longer decay times [1,2,33,34]. In association with increased NR2A subunit expression, whole-cell currents driven by NMDA receptors in young neurons gradually decrease in decay time [4,31,35–37]. These experiments, and the demonstration that activity in the visual cortex causes synaptic NMDA receptor currents to shorten [35], led to the hypothesis that long NMDA receptor currents mediated by NR2B-rich receptors might allow dynamic synaptic changes, whereas NR2A-rich receptors displaying short currents might increase temporal resolution but limit synaptic plasticity [38]. The simplified linkage of NR2B-rich receptors with synaptic plasticity

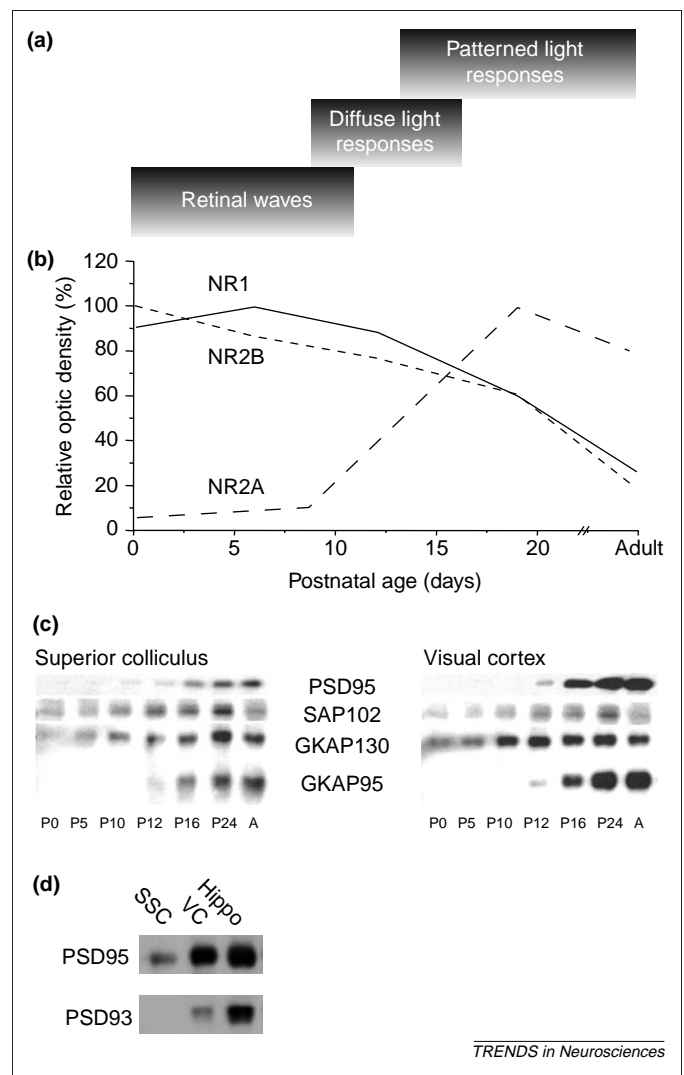


Figure 1. Changes in retinal activity and correlated changes in expression of NMDA receptor subunits, SAP102 and PSD95 in visual neuron dendrites during rodent postnatal development. **(a)** Retinal waves are followed by photoreceptor-driven activity through closed eyelids, then by highly patterned and more intense activity after eye opening. **(b)** In visual superficial layers of the superior colliculus, immediately postsynaptic to the retinal projection, levels of the NMDA receptor NR2A subunit begin to increase when photoreceptors begin to drive the retinal output. Over the same period, levels of the NR1 and NR2B subunits begin to decrease. Each time point is shown as a percentage relative to the maximal level of expression (100%) of that subunit. Data are from quantitative immunoblotting experiments [31]. **(c)** Immunoblots using protein from dendritic fractions of visual neurons of the superior colliculus and cortex. The NMDA-receptor-binding membrane-associated guanylate kinases (MAGUKs) postsynaptic density protein 95 (PSD95) and guanylate kinase-associated protein 95 (GKAP95) exhibit large increases in expression between postnatal day (P)12 and P16. By contrast, the NMDA-receptor-binding MAGUKs synapse-associated protein 102 (SAP102) and GKAP130 are present from P0; their levels increase and gradually then drop in the adult animal. Modified, with permission, from Ref. [32] © (2003) National Academy of Sciences, USA. **(d)** Immunoblots from the superficial visual layers of the superior colliculus (SSC) and the visual cortex (VC) of adult rats reveal that PSD95 is the predominant mature glutamate receptor scaffolding protein in these areas. The synaptic MAGUK PSD93 is expressed at much lower levels in these visual centers than in the hippocampus (Hippo).

and NR2A-rich receptors with loss of plasticity, however, is no longer tenable. For example, in visual cortex, the switch from long decay time, NR2B-mediated currents to short decay time, NR2A-dominated receptor currents occurs before, and not after, the critical period for ocular dominance plasticity. Recently, prolonged dark rearing of rats

with admission to light at postnatal day (P) 21 has been used to show that this dramatic shift from no light stimulation to pattern vision is accompanied by a rapid increase in the expression of NR2A subunits in visual cortical dendrites [39]. Moreover, a return to dark rearing for only four days after the onset of pattern visual experience results in a drop in dendritic NR2A subunit expression to background levels [40].

The rodent visual pathway is especially well suited to studies of activity-dependent synaptogenesis *in vivo*. It is well established that early activity in the retina is not driven by photoreceptors but by cholinergic synapses that mediate slow waves of activity within the amacrine and ganglion cell layer [41,42]. This activity is transmitted into the brain, where it refines the laminar segregation in the dorsal lateral geniculate nucleus [43,44] and the ephrin–Eph receptor-guided topographic distribution of the retinal projection within the superior colliculus [45]. The latter event requires NMDA receptor function [46]. In the beginning of the second postsynaptic week (~P8), photoreceptor differentiation and the formation of the photoreceptor to bipolar cell to ganglion cell pathway matures. This glutamatergic pathway begins to drive retinal output and central visual neurons [47,48]. The event is coincident with a rapid elimination of the remaining topographically inappropriate retinal ganglion cell projections to the superior colliculus [49], appearance of the first small amounts of PSD95 and NR2A subunits in visual dendrites [32,50] (Figure 1b,c), and a rapid activity-dependent decrease in NMDA-receptor-mediated current decay time, which is triggered by calcineurin activity but also requires the NR2A subunit in the heteromeric synaptic NMDA receptor [51] (M. Townsend *et al.*, unpublished). During the ensuing three to four days, between the onset of photoreceptor-driven visual activity through closed eyelids and eye opening, diffuse pattern vision influences CNS visual activity [52]. However, after eye opening and the onset of true patterned vision, significant changes in glutamate synapses have been documented. For example,

Chen and Regher [53] explored changes in synaptic currents within the dorsal lateral geniculate nucleus at various intervals after eye opening. They found increases in AMPA:NMDA receptor ratios, AMPA receptor quantal content, numbers of contacts per axon and numbers of inputs per neuron, and decreases in silent synapses. Presumably, under the influence of the increased and newly patterned activity driven by the visual world, new synapses are made and existing contacts are rearranged and potentiated in the weeks following eye opening (W. Lu and M. Constantine-Paton, unpublished).

The superficial visual layers of the superior colliculus in rodents represent a major visual center in which synaptic development can be studied in the context of changing retinal input. Another advantage of the superior colliculus is that neurons directly postsynaptic to the retinal inputs can be studied with whole-cell patch-clamp techniques and the superficial layers containing these neurons can be readily removed for biochemical and molecular analyses. Western blotting of dendritically enriched (synaptoneurosomes) proteins from the superior colliculus shows a significant upregulation of PSD95 between P12 and P16, whereas SAP102 expression increases gradually from birth but is somewhat reduced in adulthood (Figure 1). Moreover, the two GKAP splice variant families, GKAP130 and GKAP95, are known to bind PSD95 family MAGUKs and the Shank protein at the postsynaptic density [54], and appear in dendritic fractions correlated with the expression of particular MAGUKs. The expression pattern of GKAP130 is associated with SAP102, whereas GKAP95 expression and localization closely follows PSD95 (Figures 1c, 2a,b). Notably, in yeast two-hybrid screens, the tail of the GKAPs binds to dynein light chain 2 (DLC2), which seems to be shared by the actin molecular motor myosin Va and the microtubule motor dynein [55]. This could explain the observation that levels of both PSD95 and GKAP95 rapidly and significantly increase in both visual cortex and superior colliculus neuron dendrites within two hours of

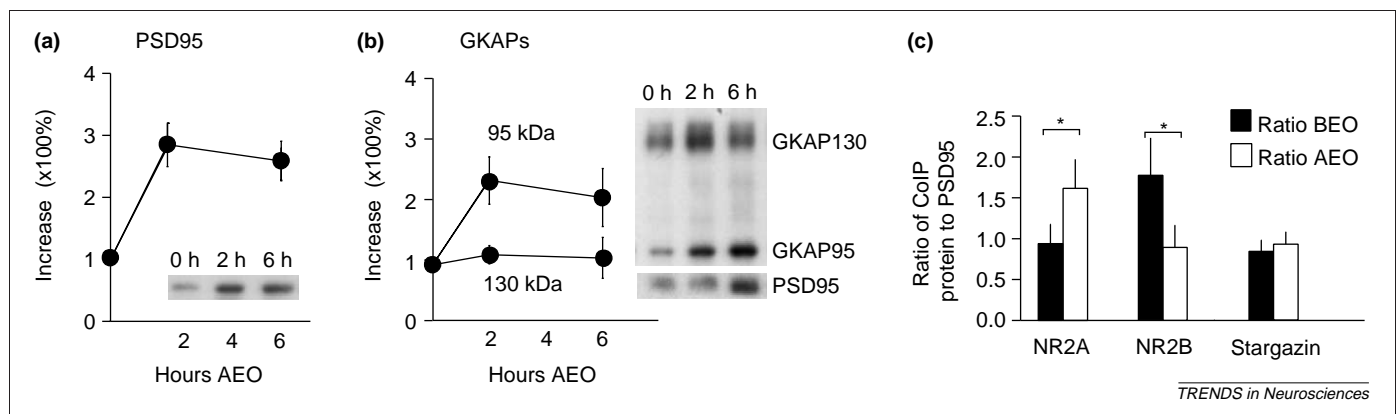


Figure 2. Rapid increases in expression of PSD95 and GKAP95 in dendritic fractions from superficial visual layers of the superior colliculus are time-locked to eye opening. (a) Levels of postsynaptic protein 95 (PSD95) in dendritic fractions from superior colliculus increase two hours after eye opening (AEO) at postnatal day (P)13. Similar results were obtained in visual cortex [32]. (b) Guanylate kinase-associated protein 95 (GKAP95) shows an increase in expression similar to PSD95, whereas GKAP130 levels remain constant. Data are relative to levels of PSD95 and GKAPs in dendritic fractions taken from littermates whose eyelids remained closed. (c) Before eye opening (BEO), PSD95 immunoprecipitations pull down more NMDA receptor NR2B subunits from dendritic fractions; however, more NR2A is coimmunoprecipitated with PSD95 in these dendrites six hours after eye opening. Similar proportions of stargazins (AMPA-receptor-binding and stabilizing molecules) are coimmunoprecipitated with dendritic PSD95 before, and six hours after, eye opening. The coimmunoprecipitated (CoIP) subunit band optical density relative to the PSD95 band density in the CoIP is shown for both the NR2A and the NR2B subunit in dendritic protein taken BEO and AEO. Modified, with permission, from Ref. [32] © (2003) National Academy of Sciences, USA.

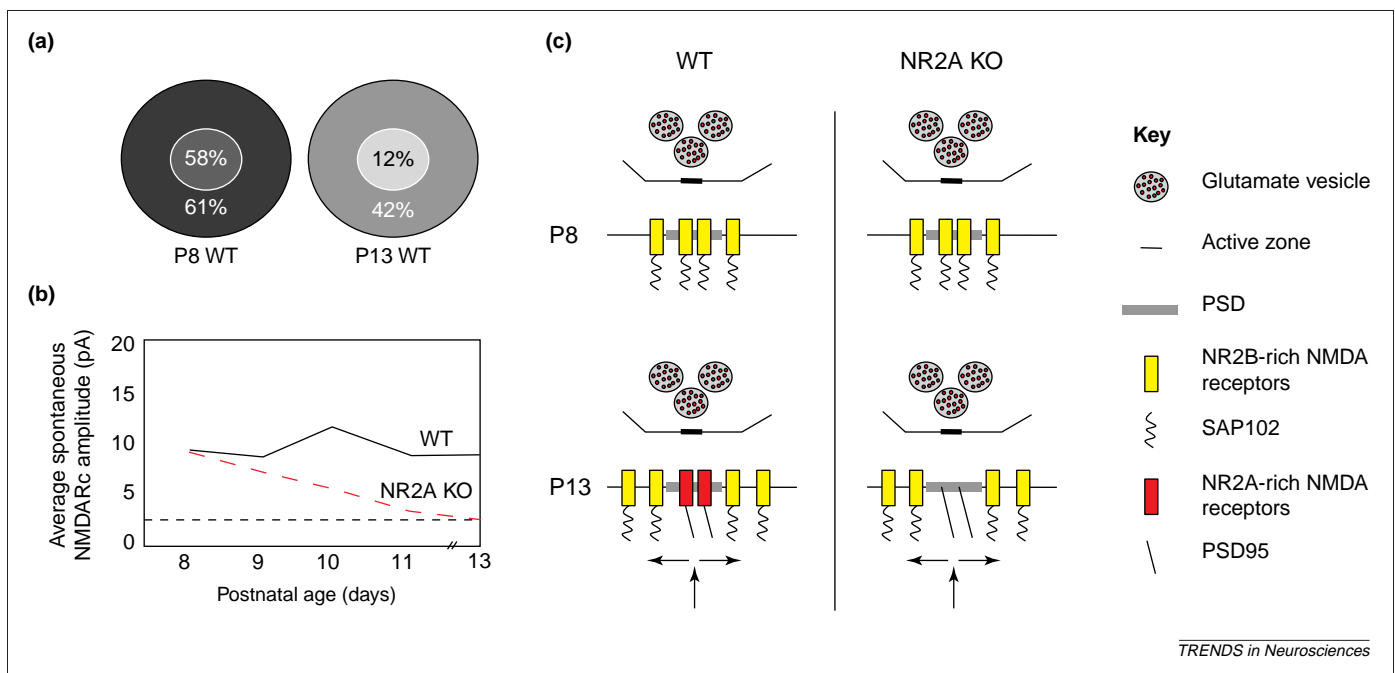


Figure 3. Displacement of synaptic NR2B-rich NMDA receptors by centrally inserted NR2A-rich receptors. **(a)** A postsynaptic membrane viewed from the synaptic cleft. The inner circle represents the postsynaptic density; the outer circle (of arbitrary radius) represents the regions in which extrasynaptic NMDA receptors contributing to the decay time of NMDA-receptor-evoked currents are localized. In superior colliculus neurons of wild-type (WT) mice at postnatal day (P)8, on average 58% of the spontaneous (mostly miniature) NMDA receptor current is lost when ifenprodil (an antagonist selective for NR2B-rich receptors) is applied. Roughly the same amount (61%) of the evoked NMDA receptor current is lost in the presence of ifenprodil at this age. However, by P13, application of ifenprodil reduces spontaneous NMDA receptor currents by ~12%; the reduction in NMDA receptor evoked currents is ~42%, approximately three times greater than the reduction produced in spontaneous NMDA receptor currents by ifenprodil. Thus, the contribution of NR2B-rich NMDA receptors to any synaptic event in visual neurons of the colliculus decreases rapidly with postnatal age. Moreover, as the neurons mature, the contribution of NR2B-rich NMDA receptors to currents originating at the center of the synapse decreases much faster than the NR2B-rich contribution to evoked currents. These data suggest that NR2A-rich receptors are added preferentially to the center of the synapse and that the contribution of NR2B-rich receptors to evoked currents in older animals is similar to the NR2B-rich receptor contribution to the spontaneous and miniature currents of younger animals [50]. **(b)** In wild-type mice, the amplitudes of spontaneous (mostly miniature) NMDA receptor currents (NMDARc) recorded in visual superior colliculus neurons remain relatively constant over the second postnatal week. However, in NR2A-knockout (NR2A KO) mice, the spontaneous NMDA receptor currents disappear into background levels (~3 pA, dotted line) by P13. **(c)** A central insertion model for postsynaptic density protein 95 (PSD95) trafficking to the synapse as a result of increasing activity. The model accounts for the developmental observations in both wild-type (a) and NR2A-knockout mice (b). A synapse-associated protein 102 (SAP102)–NR2B-rich NMDA receptor complex is constitutively expressed across the young postsynaptic membrane by the end of the first postnatal week (left). As afferent activity increases, PSD95 is trafficked to the postsynaptic density via guanylate kinase-associated protein 95 (GKAP95) and microtubule–actin transport (Figure 4) where, via its palmitoylation sites [67], it is inserted into the membrane. This insertion displaces the previously synaptic SAP102-bound NR2B-rich receptors laterally to extrasynaptic membrane. In the NR2A-knockout mice (right), NMDA receptor spontaneous currents are initially normal. However, during the second postnatal week, increased activity recruits the PSD95–GKAP95 complex to the postsynaptic density. As in the wild type animal, the PSD95 complex is inserted into the postsynaptic membrane at the center of the synapse, displacing the SAP102–NR2B receptor complexes laterally. Because PSD95 cannot bind significant numbers of NR1–NR2B heteromers (the only available heteromers in the absence of NR2A; Figure 4), the lateral displacement of earlier NMDA receptors results in the loss of spontaneous and miniature NMDA receptor currents. Nevertheless, NMDA-receptor-evoked currents can still be initiated by glutamate diffusing from synaptic clefts and binding to the now extrasynaptic NR2B-rich receptors [50]. Modified, with permission, from Ref. [50] © (2003) National Academy of Sciences, USA.

eye opening (Figure 2a,b). In addition, immunoprecipitation analyses show that, six hours after eye opening, dendritic PSD95 is associated with significantly more NR2A subunits than in littermates with their eyelids closed (Figure 2c).

Developmental electrophysiological characterizations of glutamate synaptic currents in the superior colliculus of wild-type and NR2A-knockout mice [56] place the observations on rapid PSD95 trafficking to dendrites with eye opening [32] into a broader context [50]. These data suggest that the amount of synaptic PSD95 and the speed of its addition to synaptic regions can be graded according to the amount of incoming activity and that, through PSD95, trafficking activity alters receptor composition and receptor position relative to presynaptic release sites. In addition, early in the second postnatal week, mouse and rat [31,51] superior colliculus neuron miniature and evoked NMDA receptor currents have roughly the same proportions of NR2B-rich NMDA receptors (Figure 3a).

However, during the next five days, the miniature currents lose NR2B-rich NMDA receptor contributions more rapidly than do the evoked NMDA-receptor-mediated currents (Figure 3a). This suggests that different mechanisms are involved in the NR2B versus NR2A subunit switch in the center of superior colliculus synapses, when compared with the subunit switch in more peripheral NMDA receptors that contribute predominantly to evoked NMDA-receptor-mediated current decay times. Studies on glutamate synaptic transmission in the superior colliculus of NR2A-knockout mice [50] reveal an unexpected loss of miniature NMDA-receptor-mediated currents over the same five-day period (Figure 3b). This disappearance is coincident with the early increase in PSD95 and NR2A subunit expression in dendritic fractions, caused by the development of activity in the photoreceptor to retinal ganglion cell pathway. Nevertheless, NMDA-receptor-evoked currents, as well as AMPA-receptor-mediated miniature and evoked currents,

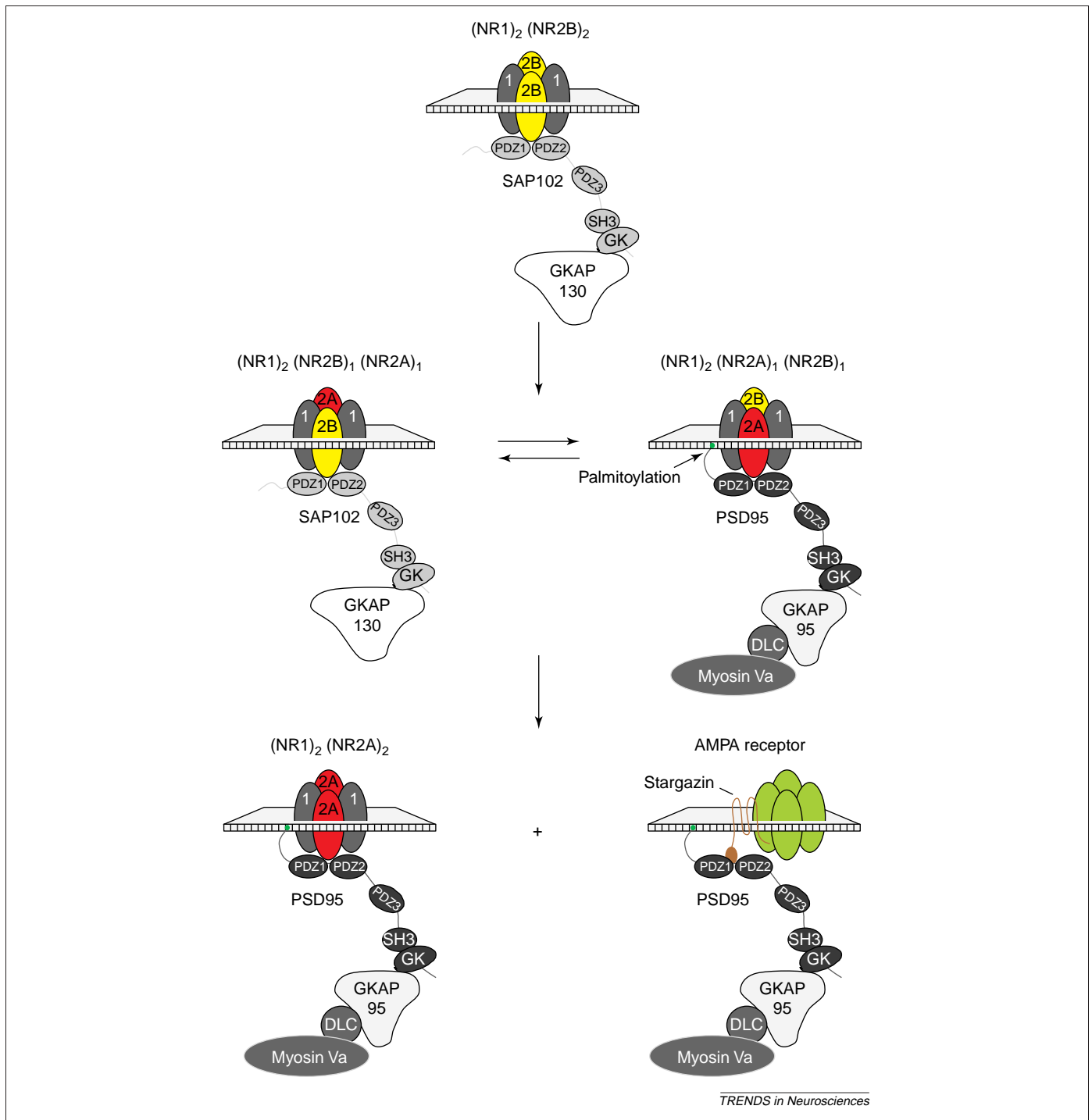


Figure 4. Proposed relationships between di-subunit-containing and tri-subunit-containing NMDA receptor tetramers, scaffolding complexes and trafficking proteins. The composition of these proposed molecular units is based on two assumptions: (i) that NR2B predominantly binds the NMDA receptor heteromer to synapse-associated protein 102 (SAP102), whereas NR2A predominantly binds the heteromer to postsynaptic density protein 95 (PSD95); and (ii) that the two different-sized guanylate kinase-associated protein (GKAP) splice variant families are associated specifically with one of these membrane-associated guanylate kinases (MAGUKs). Downward arrows depict the sequence of appearance of the complexes at the postsynaptic density. Thus, diheteromeric NR1–NR2B-containing complexes are supported by a scaffold of SAP102–GKAP130 and diheteromeric NR1–NR2A receptors are held by PSD95. PSD95, in turn, binds to GKAP95. Through GKAP95, PSD95 is associated with the molecular motor dynein light chain 2 (DLC2). Like the better understood DLC1, DLC2 is assumed to bind vesicle-associated cargo and form a complex with dynein and myosin Va. The developmental switch from NR2B-rich to NR2A-rich NMDA receptors would also involve triheteromers that could be held by either SAP102 or PSD95. To simplify the diagrams in Figures 3(c) and 5(b), the triheteromers are not depicted but they would be localized between the NR2A-rich and the NR2B-rich receptors. PSD95 also binds stargazin and can therefore act as a scaffold for the AMPA-receptor–stargazin complex at the postsynaptic density. Whether PSD95 holds different signaling molecules when associated with AMPA receptors or NMDA receptors is unknown. Abbreviations: GK, guanylate kinase domain; SH3, src homology 3 domain.

are still present in the NR2 subunit knockout mice and the rise times of the NMDA-receptor-evoked currents increase significantly [50]. Thus, synapses in the NR2A-knockout mice come to resemble, by P13, those in normal retinal

ganglion cells [12] and cerebellar interneuron synapses [13], in that they lack NMDA-receptor-mediated miniature currents but retain AMPA-receptor-mediated currents and NMDA-receptor-evoked currents.

A model for regulation of synaptic plasticity by two MAGUK complexes

These analyses of glutamate receptor regulation in the colliculus and visual cortex, together with the data regarding MAGUKs, suggest that different NMDA receptor scaffolding and signaling complexes effect the trafficking and synaptic localization of NR2A-rich and NR2B-rich NMDA receptors. The data also suggest that with increased retinal activity, PSD95 is inserted into the center of the postsynaptic density and displaces the NR2B–SAP102 complexes, which were initially located at the postsynaptic density, to the perisynaptic, and, finally to the extrasynaptic, membrane (Figure 3c).

We propose that the neonate NMDA receptor complex is rich in the NR2B subunit, which binds the entire receptor to SAP102, and that this complex is held at the synapse via GKAP130 (Figure 4). This neonate complex is probably constitutively inserted into the synaptic membrane [57,58]. However, with increases in afferent activity, postsynaptic neurons respond by synthesizing PSD95 and inserting it into the center of the postsynaptic density using vesicular transport. A vesicle-associated PSD95–GKAP95 complex could bind DLC2 in the soma, be transported to dendrites on microtubules by combining with a dynein family motor, and then be transported to synaptic sites by transfer of DLC2 to myosin Va-mediated transport along actin

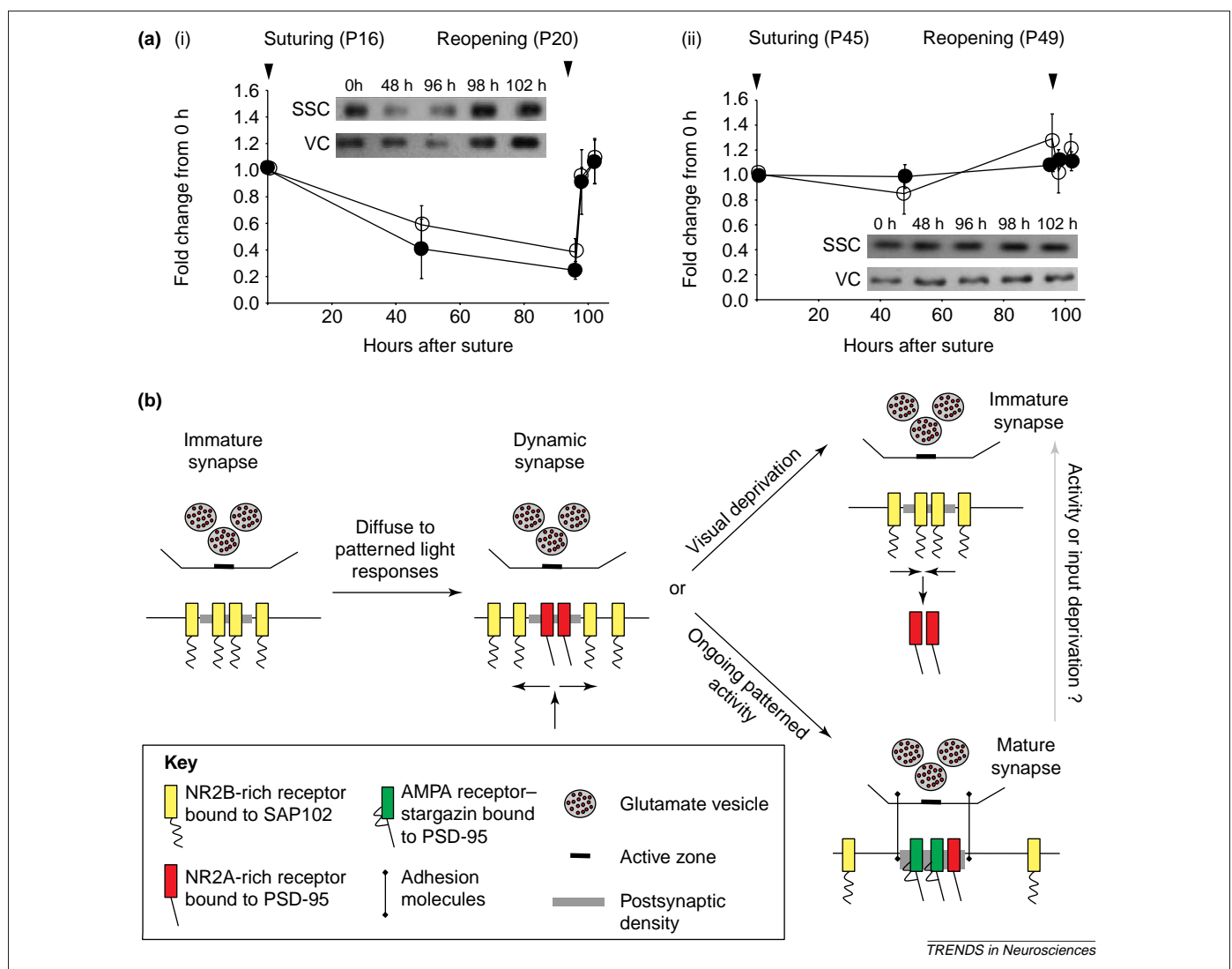


Figure 5. Age-dependent dynamics of PSD95 at the synapse. **(a)** In juvenile visual neurons, deprived of pattern vision by lid suture at postnatal day (P)16 (i), the expression of dendritic postsynaptic protein 95 (PSD95) decreases within four days to levels equivalent to those before eye opening. However, high PSD95 dendritic levels rapidly return within two to six hours after eye reopening. By contrast, in young adult (P45) rats, the same deprivation and eye reopening protocol does not change levels of dendritic PSD95 (ii). Abbreviations: SSC, superficial visual layers of superior colliculus; VC, visual cortex. Modified, with permission, from Ref. [32] © (2003) National Academy of Sciences, USA. **(b)** A developmental proposal for involvement of NMDA receptors and their scaffolding molecules in synaptic plasticity. Immature synapses contain NR2B-rich receptors that are displaced to extrasynaptic membrane when diffuse light responses through closed eyelids initiate insertion of NR2A-rich receptors that are bound to PSD95 at the postsynaptic density. After eye opening, ongoing patterned activity leads to the maturation of synapses characterized by increased numbers of synaptic AMPA receptors, reduced density of extrasynaptic NR2B-rich receptors and, gradually, a reduction of NMDA receptors at the postsynaptic density. Stabilization of these mature synapses might be increased by the development of adhesion complexes between the postsynaptic density and presynaptic terminals. In young animals, the dynamic synapse can rapidly lose PSD95, and extrasynaptic NR2B-rich receptors bound to synapse-associated protein 102 (SAP102) will move back into the postsynaptic density so that they are ready to begin the stabilization process once more. In fully mature synapses deprived of normal activity and/or inputs, it might be possible to induce the juvenile removal and reinsertion of PSD95. However, because of stabilization, it could take longer to disassemble the PSD95 complex and allow new SAP102-bound NR2B-rich receptors back into postsynaptic densities. Other disruptive effects of inactivity during such a prolonged process could mask this plastic potential.

filaments. That the PSD95 complex binds NMDA receptors by the NR2A cytoplasmic tail is indicated by the NR2A-knockout mouse data and by earlier reports utilizing mice in which NR2A subunits lacked their PSD95-binding C-terminal [59]. In wild-type animals, the specificity of the relationship between PSD95 and NR2A is likely to be masked because NMDA receptors also exist as triheteromers containing both NR2A and NR2B subunits. The triheteromers can be held by either SAP102 or PSD95 (Figure 4). Thus, the selectivity of NR2 subunits for particular MAGUKs, the developmental progression of MAGUK to NMDA receptor binding, and the activity-dependent transport of PSD95 specifically to the postsynaptic density, constitute the basis of the central insertion model for NMDA receptor trafficking (Figure 3c). This model also accounts for the differences in subunit content between NMDA receptors mediating miniature and evoked NMDA receptor current decay times in progressively older wild-type superior colliculus neurons (Figure 3a).

Recent studies suggest that a second crucial function of PSD95 is to stabilize AMPA receptors by binding stargazin-AMPA-receptor complexes to the postsynaptic density after NMDA receptor currents initiate insertion of AMPA receptors [22]. This mechanism is easily encompassed into the proposed model: in neurons with NMDA receptor miniature currents, different PSD95 molecules bind stargazin and the NR2A-subunit tail [60] (Figure 3c). Overexpression of PSD95 in cortical and hippocampal neurons results in a more pronounced AMPA receptor expression at synapses mimicking NMDA-dependent long-term potentiation (LTP) [61,62]. Also, recent evidence from our laboratory indicates that the increased synaptic expression of PSD95 in visual neurons upon eye opening is, by 12 hours after eye opening, associated with naturally occurring LTP (W. Lu and M. Constantine-Paton, unpublished).

If this proposal for glutamate receptor dynamics is supported by future experiments, three further questions will deserve investigation. First, which NMDA-receptor-mediated currents and signals – those from the center of the synapses, those from the surrounding extrasynaptic membrane, or an activity-weighted contribution from both populations – are responsible for NMDA-receptor-initiated insertion or removal of AMPA receptors from the synapse? Second, are the same currents responsible for the structural changes in connectivity in which the NMDA receptor has been implicated? Third, does this mechanism of compartmentalized NMDA receptors and signaling complexes have anything to do with the loss of both structural and functional synaptic plasticity as the brain ages?

A suggestion that the last hypothesis could indeed be the case comes from biochemical analyses of PSD95 changes in both superior colliculus and visual cortical dendrites when eyes are reshut in increasingly older animals. Thus, when rat pup eyelids are closed at P16 after three days of pattern vision, the expression of PSD95 in visual system dendrites falls to baseline levels in four days. However, PSD95 can be rapidly reinstated within two to six hours of reopening of the eyes at this age [32] (Figure 5a). The timing of the appearance and disappearance of PSD95

from visual neuron dendrites is remarkably similar to that described for NR2A in visual cortex [39,40]. However, when the same eye closing and reopening procedure is applied to older rats (P45), PSD95 levels in visual neuron dendrites do not change (Figure 5a). Thus at least the rate, if not the existence, of these activity-mediated PSD95 dynamics at synapses decreases with age. It might well be that the ability to maintain PSD95 trafficking into and out of the synapse is essential to glutamate-mediated synaptic plasticity.

Regardless of whether the proposed models are correct or wrong (Figures 3c, 4b), developmental studies that reveal synaptic changes occurring within intact, relatively normal brains, should produce important new insights concerning the mechanisms of brain plasticity during learning and during progressive brain pathologies, where many of the same molecular mechanisms are likely to be involved [63–66]. Developmental studies provide a very predictable timeline of receptor regulation, around which many different kinds of molecular and mechanistic studies can be based.

Acknowledgements

Work in our laboratories is supported by the National Institute of Neurological Disorders and Stroke grant R01NS3290 (to M.C.P.), the National Eye Institute grants R01EY014074 and R03EY014420 (to M.C.P.) and The Pierre L. de Bourknecht Amyotrophic Lateral Sclerosis Research Fund (to B.Z.). We would like to thank Drs Cull-Candy and Diamond for kindly providing figures for Box 1.

References

- Dingledine, R. *et al.* (1999) The glutamate receptor ion channels. *Pharmacol. Rev.* 51, 7–61
- Cull-Candy, S. *et al.* (2001) NMDA receptor subunits: diversity, development and disease. *Curr. Opin. Neurobiol.* 11, 327–335
- Sheng, M. and Kim, M.J. (2002) Postsynaptic signaling and plasticity mechanisms. *Science* 298, 776–780
- Stocca, G. and Vicini, S. (1998) Increased contribution of NR2A subunit to synaptic NMDA receptors in developing rat cortical neurons. *J. Physiol.* 507, 13–24
- Rumbaugh, G. and Vicini, S. (1999) Distinct synaptic and extrasynaptic NMDA receptors in developing cerebellar granule neurons. *J. Neurosci.* 19, 10603–10610
- Tovar, K.R. and Westbrook, G.L. (1999) The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses *in vitro*. *J. Neurosci.* 19, 4180–4188
- Sattler, R. *et al.* (2000) Distinct roles of synaptic and extrasynaptic NMDA receptors in excitotoxicity. *J. Neurosci.* 20, 22–33
- Hardingham, G.E. *et al.* (2002) Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nat. Neurosci.* 5, 405–414
- Li, B. *et al.* (2002) Differential regulation of synaptic and extrasynaptic NMDA receptors. *Nat. Neurosci.* 5, 833–834
- Aarts, M. *et al.* (2002) Treatment of ischemic brain damage by perturbing NMDA receptor-PSD-95 protein interactions. *Science* 298, 846–850
- Rusakov, D.A. and Kullmann, D.M. (1998) Extrasynaptic glutamate diffusion in the hippocampus: ultrastructural constraints, uptake, and receptor activation. *J. Neurosci.* 18, 3158–3170
- Chen, S. and Diamond, J.S. (2002) Synaptically released glutamate activates extrasynaptic NMDA receptors on cells in the ganglion cell layer of rat retina. *J. Neurosci.* 22, 2165–2173
- Clark, B.A. and Cull-Candy, S.G. (2002) Activity-dependent recruitment of extrasynaptic NMDA receptor activation at an AMPA receptor-only synapse. *J. Neurosci.* 22, 4428–4436
- Auger, C. and Attwell, D. (2000) Fast removal of synaptic glutamate by postsynaptic transporters. *Neuron* 28, 547–558

- 15 Passafaro, M. *et al.* (2001) Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. *Nat. Neurosci.* 4, 917–926
- 16 Trussell, L.O. and Fischbach, G.D. (1989) Glutamate receptor desensitization and its role in synaptic transmission. *Neuron* 3, 209–218
- 17 Cho, K.O. *et al.* (1992) The rat brain postsynaptic density fraction contains a homolog of the *Drosophila* discs-large tumor suppressor protein. *Neuron* 9, 929–942
- 18 Kornau, H.C. *et al.* (1995) Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science* 269, 1737–1740
- 19 Niethammer, M. *et al.* (1996) Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. *J. Neurosci.* 16, 2157–2163
- 20 Bassand, P. *et al.* (1999) Differential interaction of the tSXV motifs of the NR1 and NR2A NMDA receptor subunits with PSD-95 and SAP97. *Eur. J. Neurosci.* 11, 2031–2043
- 21 Sans, N. *et al.* (2001) Synapse-associated protein 97 selectively associates with a subset of AMPA receptors early in their biosynthetic pathway. *J. Neurosci.* 21, 7506–7516
- 22 Chen, L. *et al.* (2000) Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. *Nature* 408, 936–943
- 23 Garcia, E.P. *et al.* (1998) SAP90 binds and clusters kainate receptors causing incomplete desensitization. *Neuron* 21, 727–739
- 24 Kennedy, M.B. (2000) Signal-processing machines at the postsynaptic density. *Science* 290, 750–754
- 25 Tomita, S. *et al.* (2001) PDZ protein interactions regulating glutamate receptor function and plasticity. *J. Cell Biol.* 153, F19–F24
- 26 Brenman, J.E. *et al.* (1996) Cloning and characterization of postsynaptic density 93, a nitric oxide synthase interacting protein. *J. Neurosci.* 16, 7407–7415
- 27 Kim, E. *et al.* (1996) Heteromultimerization and NMDA receptor-clustering activity of Chapsyn-110, a member of the PSD-95 family of proteins. *Neuron* 17, 103–113
- 28 Lau, L.F. *et al.* (1996) Interaction of the *N*-methyl-D-aspartate receptor complex with a novel synapse-associated protein, SAP102. *J. Biol. Chem.* 271, 21622–21628
- 29 Muller, B.M. *et al.* (1996) SAP102, a novel postsynaptic protein that interacts with NMDA receptor complexes *in vivo*. *Neuron* 17, 255–265
- 30 Sans, N. *et al.* (2000) A developmental change in NMDA receptor-associated proteins at hippocampal synapses. *J. Neurosci.* 20, 1260–1271
- 31 Shi, J. *et al.* (1997) Temporal correlations between functional and molecular changes in NMDA receptors and GABA neurotransmission in the superior colliculus. *J. Neurosci.* 17, 6264–6276
- 32 Yoshii, A. *et al.* (2003) Eye opening induces a rapid dendritic localization of PSD-95 in central visual neurons. *Proc. Natl. Acad. Sci. U. S. A.* 100, 1334–1339
- 33 Hollmann, M. and Heinemann, S. (1994) Cloned glutamate receptors. *Annu. Rev. Neurosci.* 17, 31–108
- 34 McBain, C.J. and Mayer, M.L. (1994) *N*-methyl-D-aspartic acid receptor structure and function. *Physiol. Rev.* 74, 723–760
- 35 Carmignoto, G. and Vicini, S. (1992) Activity-dependent decrease in NMDA receptor responses during development of the visual cortex. *Science* 258, 1007–1011
- 36 Hestrin, S. (1992) Developmental regulation of NMDA receptor-mediated synaptic currents at a central synapse. *Nature* 357, 686–689
- 37 Flint, A.C. *et al.* (1997) NR2A subunit expression shortens NMDA receptor synaptic currents in developing neocortex. *J. Neurosci.* 17, 2469–2476
- 38 Scheetz, A.J. and Constantine-Paton, M. (1994) Modulation of NMDA receptor function: implications for vertebrate neural development. *FASEB J.* 8, 745–752
- 39 Quinlan, E.M. *et al.* (1999) Rapid, experience-dependent expression of synaptic NMDA receptors in visual cortex *in vivo*. *Nat. Neurosci.* 2, 352–357
- 40 Quinlan, E.M. *et al.* (1999) Bidirectional, experience-dependent regulation of *N*-methyl-D-aspartate receptor subunit composition in the rat visual cortex during postnatal development. *Proc. Natl. Acad. Sci. U. S. A.* 96, 12876–12880
- 41 Wong, R.O. *et al.* (1993) Transient period of correlated bursting activity during development of the mammalian retina. *Neuron* 11, 923–938
- 42 Feller, M.B. *et al.* (1996) Requirement for cholinergic synaptic transmission in the propagation of spontaneous retinal waves. *Science* 272, 1182–1187
- 43 Mooney, R. *et al.* (1996) Thalamic relay of spontaneous retinal activity prior to vision. *Neuron* 17, 863–874
- 44 Grubb, M.S. *et al.* (2003) Abnormal functional organization in the dorsal lateral geniculate nucleus of mice lacking the beta2 subunit of the nicotinic acetylcholine receptor. *Neuron* 40, 1161–1172
- 45 McLaughlin, T. *et al.* (2003) Retinotopic map refinement requires spontaneous retinal waves during a brief critical period of development. *Neuron* 40, 1147–1160
- 46 Simon, D.K. *et al.* (1992) *N*-methyl-D-aspartate receptor antagonists disrupt the formation of a mammalian neural map. *Proc. Natl. Acad. Sci. U. S. A.* 89, 10593–10597
- 47 Bansal, A. *et al.* (2000) Mice lacking specific nicotinic acetylcholine receptor subunits exhibit dramatically altered spontaneous activity patterns and reveal a limited role for retinal waves in forming ON and OFF circuits in the inner retina. *J. Neurosci.* 20, 7672–7681
- 48 Wong, W.T. *et al.* (2000) Developmental changes in the neurotransmitter regulation of correlated spontaneous retinal activity. *J. Neurosci.* 20, 351–360
- 49 Simon, D.K. and O'Leary, D.D. (1992) Development of topographic order in the mammalian retinocollicular projection. *J. Neurosci.* 12, 1212–1232
- 50 Townsend, M. *et al.* (2003) Developmental loss of miniature *N*-methyl-D-aspartate receptor currents in NR2A knockout mice. *Proc. Natl. Acad. Sci. U. S. A.* 100, 1340–1345
- 51 Shi, J. *et al.* (2000) Activity-dependent induction of tonic calcineurin activity mediates a rapid developmental downregulation of NMDA receptor currents. *Neuron* 28, 103–114
- 52 Akerman, C.J. *et al.* (2002) Visual experience before eye-opening and the development of the retinogeniculate pathway. *Neuron* 36, 869–879
- 53 Chen, C. and Regehr, W.G. (2000) Developmental remodeling of the retinogeniculate synapse. *Neuron* 28 (3), 955–966
- 54 Naisbitt, S. *et al.* (1999) Shank, a novel family of postsynaptic density proteins that binds to the NMDA receptor/PSD-95/GKAP complex and cortactin. *Neuron* 23, 569–582
- 55 Naisbitt, S. *et al.* (2000) Interaction of the postsynaptic density-95/guanylate kinase domain-associated protein complex with a light chain of myosin-V and dynein. *J. Neurosci.* 20, 4524–4534
- 56 Ito, I. *et al.* (1996) Age-dependent reduction of hippocampal LTP in mice lacking *N*-methyl-D-aspartate receptor epsilon 1 subunit. *Neurosci. Lett.* 203, 69–71
- 57 Barria, A. and Malinow, R. (2002) Subunit-specific NMDA receptor trafficking to synapses. *Neuron* 35, 345–353
- 58 Sans, N. *et al.* (2003) NMDA receptor trafficking through an interaction between PDZ proteins and the exocyst complex. *Nat. Cell Biol.* 5, 520–530
- 59 Steigerwald, F. *et al.* (2000) C-Terminal truncation of NR2A subunits impairs synaptic but not extrasynaptic localization of NMDA receptors. *J. Neurosci.* 20, 4573–4581
- 60 Losi, G. *et al.* (2003) PSD-95 regulates NMDA receptors in developing cerebellar granule neurons of the rat. *J. Physiol.* 548, 21–29
- 61 Beique, J.C. and Andrade, R. (2003) PSD-95 regulates synaptic transmission and plasticity in rat cerebral cortex. *J. Physiol.* 546, 859–867
- 62 Stein, V. *et al.* (2003) Postsynaptic density-95 mimics and occludes hippocampal long-term potentiation and enhances long-term depression. *J. Neurosci.* 23, 5503–5506
- 63 Brown, R.H. Jr and Robberecht, W. (2001) Amyotrophic lateral sclerosis: pathogenesis. *Semin. Neurol.* 21, 131–139
- 64 Zeron, M.M. *et al.* (2002) Increased sensitivity to *N*-methyl-D-aspartate receptor-mediated excitotoxicity in a mouse model of Huntington's disease. *Neuron* 33, 849–860
- 65 Moghaddam, B. and Jackson, M.E. (2003) Glutamatergic animal models of schizophrenia. *Ann. N. Y. Acad. Sci.* 1003, 131–137
- 66 Dunlop, J. *et al.* (2003) Impaired spinal cord glutamate transport capacity and reduced sensitivity to riluzole in a transgenic superoxide dismutase mutant rat model of amyotrophic lateral sclerosis. *J. Neurosci.* 23, 1688–1696
- 67 El-Husseini, A.E. *et al.* (2000) Ion channel clustering by membrane-associated guanylate kinases. Differential regulation by *N*-terminal lipid and metal binding motifs. *J. Biol. Chem.* 275, 23904–23910