

PSD-95 promotes the stabilization of young synaptic contacts

Christine E. Taft and Gina G. Turrigiano

Phil. Trans. R. Soc. B 2014 **369**, 20130134, published 2 December 2013

References

[This article cites 46 articles, 21 of which can be accessed free](#)

<http://rstb.royalsocietypublishing.org/content/369/1633/20130134.full.html#ref-list-1>

Subject collections

Articles on similar topics can be found in the following collections

[neuroscience](#) (377 articles)

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click [here](#)

rstb.royalsocietypublishing.org



Research

Cite this article: Taft CE, Turrigiano GG. 2014 PSD-95 promotes the stabilization of young synaptic contacts. *Phil. Trans. R. Soc. B* **369**: 20130134.
<http://dx.doi.org/10.1098/rstb.2013.0134>

One contribution of 35 to a Discussion Meeting Issue 'Synaptic plasticity in health and disease'.

Subject Areas:

neuroscience

Keywords:

PSD-95, CaMKII, GluA2, synapse stabilization, live imaging

Author for correspondence:

Gina G. Turrigiano
e-mail: turrigiano@brandeis.edu

PSD-95 promotes the stabilization of young synaptic contacts

Christine E. Taft and Gina G. Turrigiano

Department of Biology, Brandeis University, Waltham, MA 02454, USA

Maintaining a population of stable synaptic connections is probably of critical importance for the preservation of memories and functional circuitry, but the molecular dynamics that underlie synapse stabilization is poorly understood. Here, we use simultaneous time-lapse imaging of post synaptic density-95 (PSD-95) and Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) to investigate the dynamics of protein composition at axodendritic (AD) contacts. Our data reveal that this composition is highly dynamic, with both proteins moving into and out of the same synapse independently, so that synapses cycle rapidly between states in which they are enriched for none, one or both proteins. We assessed how PSD-95 and CaMKII interact at stable and transient AD sites and found that both phospho-CaMKII and PSD-95 are present more often at stable than labile contacts. Finally, we found that synaptic contacts are more stable in older neurons, and this process can be mimicked in younger neurons by overexpression of PSD-95. Taken together, these data show that synaptic protein composition is highly variable over a time-scale of hours, and that PSD-95 is probably a key synaptic protein that promotes synapse stability.

1. Introduction

Circuit maturation and experience-dependent plasticity are thought to involve the fine-tuning of synaptic connectivity within specific microcircuits. Presumably neurons must form and then maintain a population of stable contacts with specific synaptic partners in order to ensure proper circuit function, but the molecular mechanisms that underlie this process of synapse stabilization are still poorly understood [1,2]. Further, the ability of a synapse to remain stable is complicated by the recent demonstration that many synaptic proteins are highly dynamic and turn over in both activity-dependent and independent manners [3–7]. Here, we used time-lapse imaging of synapse formation in cultured neocortical neurons to examine the dynamics of association of Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) and postsynaptic density-95 (PSD-95) with sites of axon–dendrite (AD) contact, in order to assess how the localization and amount of these proteins correlates with changes in contact turnover.

Both CaMKII and PSD-95 are localized at excitatory synapses, and have been implicated in promoting synapse stability. CaMKII expression is important for synapse formation and maturation [8–12], and previous work from our laboratory demonstrated that overexpression of constitutively active CaMKII enhances connectivity between active presynaptic partners by increasing new contact formation [13,14]. The scaffold protein PSD-95 is highly abundant at [15], and clusters very early at [16–18] excitatory synapses. Further, PSD-95 is more strongly associated with stable than transient spines [19], and is thought to accelerate synaptic maturation [3,20–22] through coordinated regulation of postsynaptic AMPA-type glutamate receptors (GluARs) [23–25] and neuroligins [26,27]. Synaptic PSD-95 levels increase during development and with time *in vitro* [28,29], over a similar developmental window there is a reduction in synapse turnover [30], and knockdown of PSD-95 increases spine turnover [31]. These studies raise the possibility that CaMKII and/or PSD-95 might play critical roles in stabilizing synaptic contacts.

In the following set of experiments, we investigated the role of CaMKII and PSD-95 in synapse turnover and stability. We used time-lapse imaging of connected pairs of cultured neocortical pyramidal neurons to examine the dynamics of fluorescently tagged CaMKII and PSD-95 at sites of AD contact. One advantage of this approach is

that we can monitor pre- and postsynaptic structures simultaneously, something difficult to do in most slice culture or *in vivo* models. We found that PSD-95 and CaMKII dynamically associate and dissociate from sites of both transient and stable AD contacts, but that these synaptic proteins spend more time at stable contacts. Additionally, stable AD contacts show an increase in the fraction of CaMKII in an active, phosphorylated state. Finally, we studied the effects of overexpressed PSD-95 on the stability of putative synaptic contacts, as assessed by monitoring the dynamics of postsynaptic GluA2 clusters that colocalize with a marker of presynaptic function (FM4-64). We found that overexpression of PSD-95 reduced the turnover rates of pre- and postsynaptic structures, thus promoting the stabilization of putative synaptic contacts. These data suggest that recruitment of PSD-95 into the postsynaptic compartment is one factor that contributes to the stabilization of synaptic contacts.

2. Material and methods

(a) Transfection of cortical cultures

Cultures were prepared as described previously [13,14] from P1 to P3 Long-Evans rat pups, and were transfected between 6 and 9 days *in vitro* (DIV) using the Lipofectamine 2000 (Invitrogen) reagent according to the manufacturer's instructions. Twelve to 16 h prior to the experiment, cultures were co-transfected with green fluorescent protein (GFP) tagged CaMKII (CaMKII-GFP) [13] and Discosoma red-tagged PSD-95 (PSD-95-DsRed) [32], while a separate population of neurons was transfected with cyan fluorescent protein (CFP) in order to visualize axons. In a second set of experiments cultures were transfected at either DIV 6–7 or DIV 14–15 with enhanced yellow fluorescent protein-tagged GluA2 (GluA2-EYFP) [33], alone or in combination with PSD-95-CFP (made by replacing DsRed with CFP in the vector described in [32] for 48 h prior to FM4-64 labelling).

(i) Time-lapse Imaging

Time-lapse imaging was performed as described previously [14,33]. Pyramidal neurons that expressed both CaMKII-GFP and PSD-95-DsRed and were contacted by a CFP-expressing axon from a neighbouring pyramidal neuron were identified. For FM4-64 experiments the dye was added for 2 min in a high K^+ HEPES-buffered solution in the presence of 50 μ M D-2-amino-5-phosphonovaleric acid, 20 μ M 6-cyano-7-nitroquinoxaline-2,3-dione and 10 μ M tetrodotoxin, then washed; at the end of the imaging period, cultures were re-labelled with FM4-64 to label any newly formed presynaptic puncta. Images were acquired every 20 min (2 μ M Z stack, 0.2 μ M step size) on an Olympus IX-70 using a 60 \times 1.25 na oil-immersion objective and a cooled CCD camera (Orca R2, Hamamatsu) controlled by Volocity software (Improvision), as described in [14]; pixel size was 0.11 μ m. AD contacts refer to the physical contact between the fluorescently labelled AD. Puncta contacts were defined as an AD contact with a punctate fluorescent signal more than 25% above the local dendritic background; puncta were considered colocalized if they were within 1 pixel of each other. AD contacts could contain zero, one or multiple CaMKII-GFP and/or PSD-95-DsRed puncta. Stable contacts were defined as those that persisted throughout the 3 h experiment. Average contact lifetime refers to the average lifetime of all contacts, including transient and stable contacts, over the course of the 3 h experiment. Contact gain and loss were quantified as described [14].

(ii) Immunostaining

After transfection with CaMKII-GFP and PSD-95-DsRed, cultures were fixed and stained as described previously [14], using an

epifluorescence microscope equipped with a 60 \times oil-immersion objective (na 1.25) and the appropriate filters. Primary antibodies were anti-VGlu1 (guinea pig, 1:500, Synaptic Systems) or anti-phospho-T286 (mouse, 1:100, Abcam). The total area of the puncta or the total AD contact area was used as a mask and overlaid onto the phospho-T286 signal to allow quantification of signal intensity at these sites. Gained and lost contacts consisted of two types: those where a punctum was formed *de novo* or disappeared, and those where a punctum moved towards or away from an axon. Lost contacts where the punctum disappeared were excluded from this analysis since there was no punctum area to use as a mask. Gained and lost contacts had similar phospho-CaMKII levels so were combined into one category, labelled 'transient'.

(b) Statistics

All data are reported as mean \pm s.e.m. with the *n* referring to the number of neurons or puncta as indicated. Statistical analyses were made with unpaired two-tailed Students *t*-tests, or for multiple comparisons single factor ANOVAs followed by corrected *t*-tests using a Bonferroni correction for multiple comparisons.

3. Results

A number of studies have shown that CaMKII and PSD-95 puncta are dynamic [3,4,7,17,34] but how these dynamics relate to the formation, loss and stabilization of synaptic contacts where both pre- and postsynaptic structures can be followed simultaneously has not been studied. To begin to understand how PSD-95 and CaMKII are distributed at stable and labile AD connections, we first assessed their static localization rates to presynaptic sites in fixed cultures, using either sites of AD contact (figure 1*a*) or the excitatory presynaptic marker VGlu1. We showed previously that these AD contacts have approximately 80% colocalization with Synapsin, GluA2 and FM dyes [14], suggesting that the majority of these sites represent functional synaptic contacts. Consistent with this previous report, here we found a high degree of colocalization between individual markers and either VGlu1 (figure 1*b*) or AD contacts (figure 1*c*). Interestingly, significantly fewer of these putative synaptic sites had PSD-95 and CaMKII together than PSD-95 alone (figure 1*b,c*). These data indicate that although localization rates of individual synaptic markers at synaptic sites are fairly high, only a fraction (about 30%) of these sites have both PSD-95 and CaMKII simultaneously. This suggests that the dynamics of these synaptic proteins are to some degree independent, so that the complement of proteins at existing AD contacts detected at any one time point will be quite variable.

To directly examine the dynamics of PSD-95 and CaMKII at AD contacts, we performed time-lapse imaging on cultured rat visual cortical neurons sparsely transfected with PSD-95-DsRed and CaMKII-GFP, that were contacted by an axon expressing CFP from a neighbouring pyramidal neuron. This allowed us to follow the formation and loss of AD contacts, as well as the movement of PSD-95 and CaMKII puncta, into and out of these sites of contact. We first looked at the stability of AD contacts and the stability of PSD-95 or CaMKII puncta at these contact sites (referred to as puncta contacts); we found that AD contacts are significantly more stable, and have longer contact lifetimes, than PSD-95 or CaMKII puncta contacts (figure 1*d,e*). Further, PSD-95 puncta contacts were more stable and

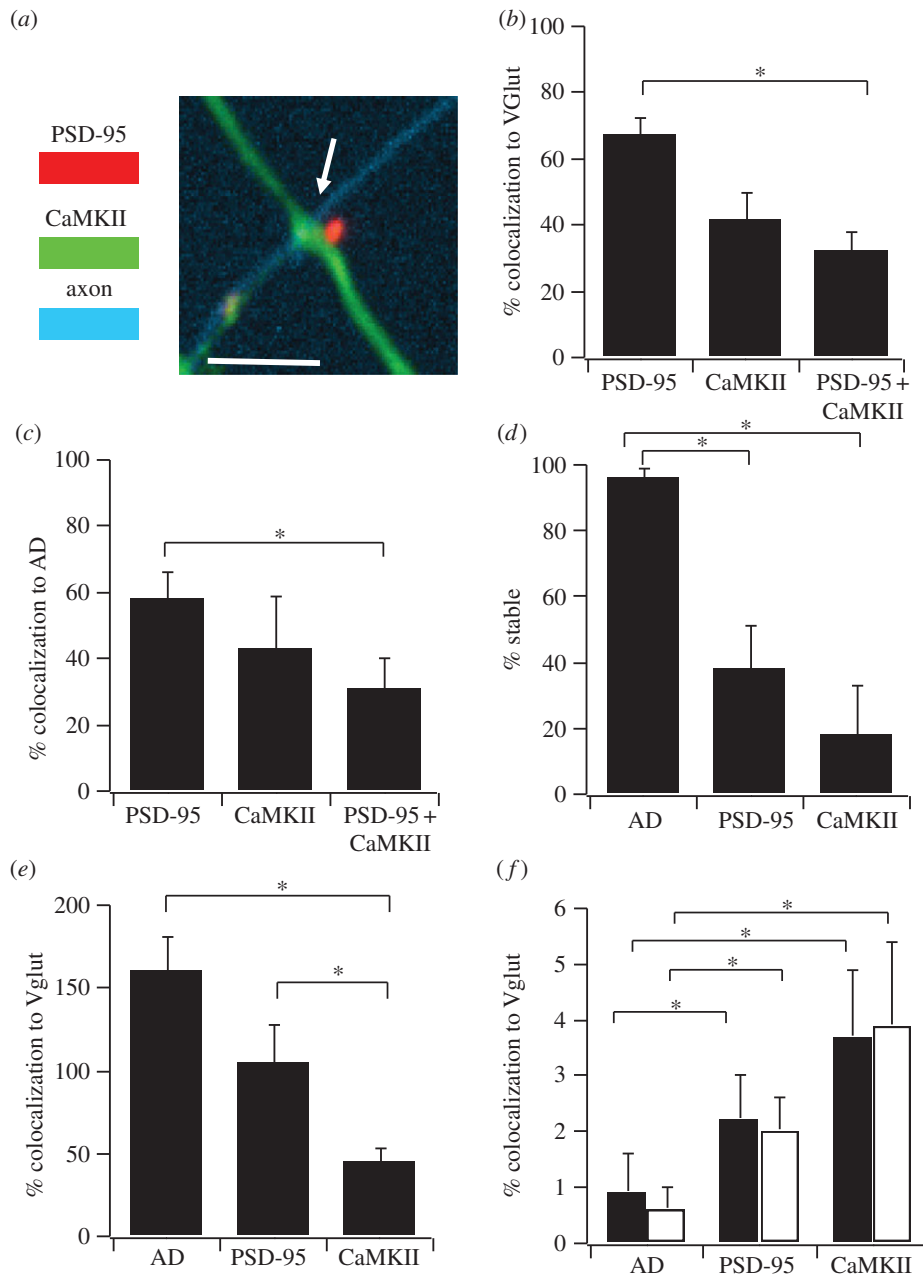


Figure 1. Axodendritic (AD) contacts are more stable than their constituent proteins. (a) An example of an AD contact; the axon (blue) is contacting a dendrite expressing CaMKII (green) and PSD-95 (red). In this example, the AD contact accumulates CaMKII but not PSD-95. Scale bar, 5 μm . (b) Static colocalization rates of indicated marker to VGlut1; $N = 7$ neurons. (c) Colocalization rates, (d) percentage of stable contacts, (e) average contact lifetimes and (f) rates of gain and loss for the indicated markers; $n = 5$ pre–post pairs of neurons. Asterisk (*) denotes $p < 0.05$, ANOVA followed by corrected t -tests for the comparisons indicated by brackets.

had longer lifetimes than CaMKII puncta contacts (figure 1*d,e*), consistent with our static colocalization data (figure 1*b*).

To examine turnover of AD and puncta contacts, we next quantified the rates of contact gain and loss. Consistent with our previous work [14], we found that rates of gain and loss were well balanced for each contact type, suggesting that overall synapse number and composition were relatively stable. When we compared across contact type, we saw that AD contacts have significantly lower turnover than either PSD-95 or CaMKII puncta, which is consistent with their higher stability and longer contact lifetimes (figure 1*f*). Thus, physical AD contacts are much more stable than their constituent postsynaptic proteins, which can move in and out of existing AD contacts.

Next we asked whether the behaviour of PSD-95 and CaMKII puncta were different at stable and transient AD

contacts. Figure 2*a* shows two examples of AD contact formation and the accompanying protein dynamics. The top panel illustrates an axon making contact with a preexisting dendritic site at which PSD-95 and CaMKII are colocalized. The bottom panel illustrates a dendritic PSD-95 punctum that moves towards the axon and makes a contact that persists until the end of the experiment. In figure 2*b*, two examples of stable AD contacts are illustrated: the top panel illustrates a stable AD contact that remains colocalized with both PSD-95 and CaMKII throughout the imaging session, and the bottom panel illustrates a contact that transiently accumulates CaMKII but not PSD-95 (note that the nearby PSD-95 punctum does not colocalize with the site of contact or CaMKII accumulation). We observed a great variety of such dynamics at both stable and transient AD contacts; to quantify this behaviour we calculated the percentage of transient and stable AD contacts

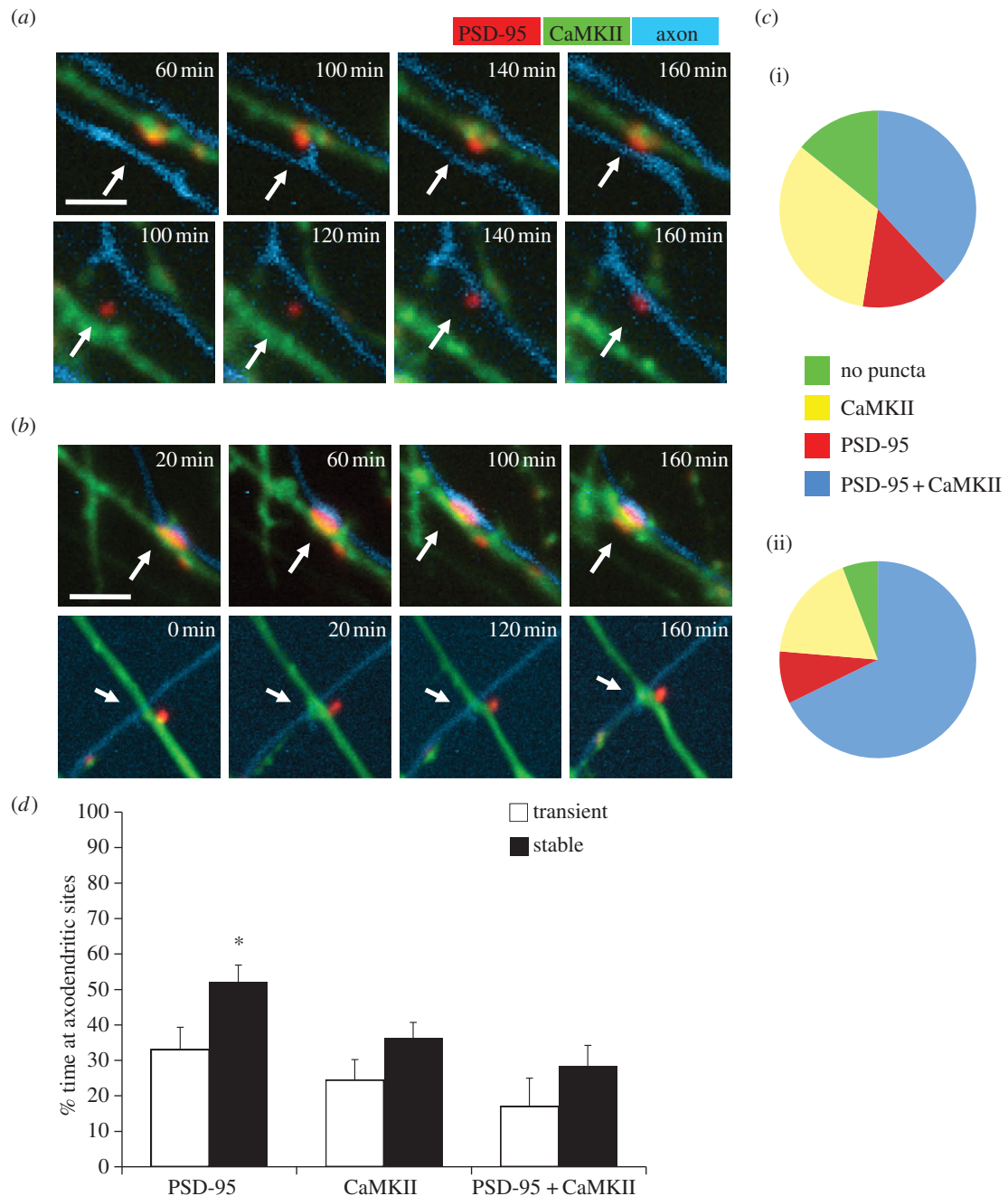


Figure 2. PSD-95 spends more time at stable than labile AD contacts. (a) Examples of transient AD contacts, with varying dynamics of PSD-95 and CaMKII proteins. (b) Examples of stable AD contacts, with varying dynamics of PSD-95 and CaMKII. Scale bar, 5 μm . (c) Protein content of (i) transient versus (ii) stable contacts; fractions represent the percentage of AD contacts that have one or both proteins present at some point during the 3 h imaging session. (d) Percentage of time that PSD-95, CaMKII and PSD-95 + CaMKII spend at stable versus transient AD sites. $N = 44$ transient puncta and 80 stable puncta from five pairs of neurons. Asterisk (*) denotes $p < 0.05$, unpaired t -test between transient and stable populations.

containing one or both synaptic proteins at any point during the experiment, as well as the percentage that did not significantly accumulate either. Strikingly, stable contacts were much more likely to have both synaptic proteins present at some point during the experiment (figure 2c). To quantify this further, we calculated the percentage of time that each puncta type spends at stable and transient AD sites (figure 2d). PSD-95 spent significantly more time at stable than at transient AD contacts, and also spent significantly more time at stable AD contacts than either CaMKII or colocalized PSD-95 and CaMKII puncta. Though there was a trend for more CaMKII and colocalized PSD-95 + CaMKII at stable contacts, this was not statistically significant. Thus, PSD-95 spends significantly more time at stable AD sites than at transient sites. This raises

the possibility that PSD-95 plays a role in the stabilization of those AD sites.

Previously, we found that activated CaMKII enhances connectivity between pairs of neurons by increasing the rate of new contact formation [13,14]. Thus, while there was no significant difference in dwell-time of CaMKII at stable or transient connections (figure 2d), the important variable might be the activation state of CaMKII rather than absolute protein levels. To address this, cultures were fixed after time-lapse imaging of CaMKII and PSD-95 and probed using an antibody specific for phospho-T286 (figure 3a). The area that encompassed the PSD-95 or CaMKII punctum was used as a mask and overlaid onto the phospho-T286 staining, to allow us to correlate the intensity of phospho-T286 with

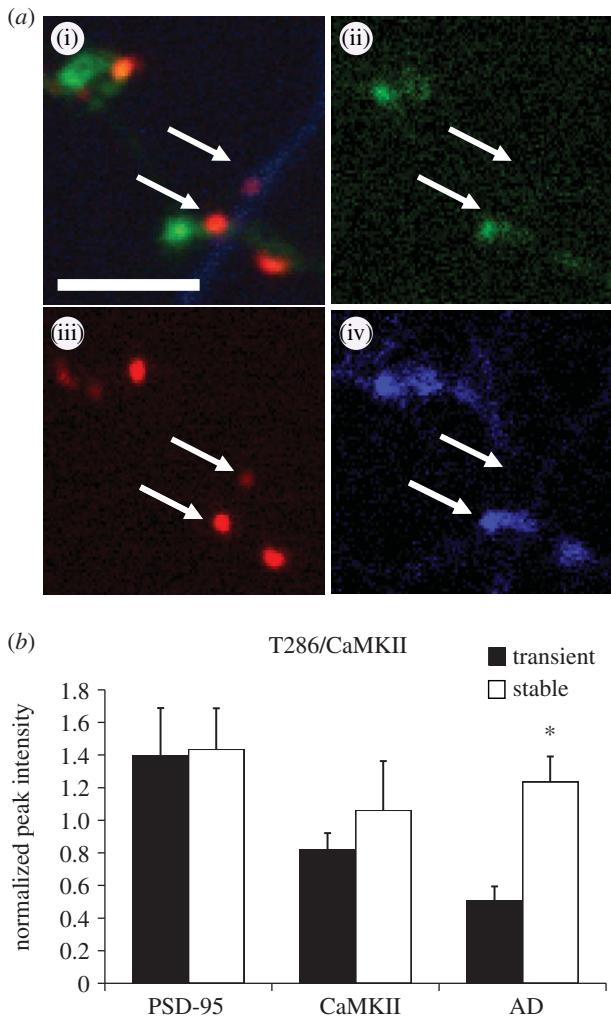


Figure 3. Post hoc staining of neuron pairs for phospho-T286CaMKII. (a) Example showing AD contacts containing PSD-95 at the end of the experiment (i); post hoc imaging of CaMKII (ii) and PSD-95 (iii) after staining for phospho-T286 (iv). Scale bar, 5 μm . (b) The ratio of phospho to total CaMKII at stable and transient contacts; $N = 12$ transient and $n = 36$ stable contacts, from five pairs of neurons. Asterisk (*) denotes $p < 0.05$, unpaired t -test between transient and stable populations.

the behaviour of either the CaMKII or PSD-95 punctum. For the AD contacts, a mask was made that encompassed the total area of contact and this was dilated by 1 pixel in order to account for puncta that were partially overlapping the site of AD contact. Interestingly, the ratio of activated to total CaMKII was lower at transient than at stable AD contacts (figure 3b). By contrast, this ratio was not correlated with the behaviour of PSD-95 or CaMKII puncta at AD contacts (figure 3b). Unfortunately, this post hoc approach did not give us access to the activation state of CaMKII just prior to contact gain or loss, so our data cannot rule out a transient enhancement of activated CaMKII just prior to formation of new contacts. These data show that, under our experimental conditions, there is more activated CaMKII at stable AD contacts.

We have shown so far that stable AD contacts have a higher ratio of active to inactive CaMKII and an enhanced accumulation of PSD-95. The synaptic localization of PSD-95 increases developmentally and with time *in vitro* [28,29], and there is ample evidence that PSD-95 overexpression can enhance the number of synapses in immature neurons [20,21,23,35], suggesting an important role for PSD-95 in

some aspect of excitatory synapse formation. However, it is not known whether PSD-95 exerts its effects by enhancing the rate of synapse formation, or by enhancing the stability of newly formed contacts. We thus wished to assess whether overexpression of synaptic PSD-95 affects the turnover and stabilization of synaptic contacts.

In order to simultaneously follow a large number of pre-synaptic and postsynaptic sites on individual neurons with or without overexpressed PSD-95, we used live imaging of GluA2-EYFP to image synaptic receptor clusters [33], and FM4-64 dye loading to follow presynaptic contacts onto GluA2-EYFP expressing neurons; at the end of each imaging session a second round of FM loading was performed to identify newly formed presynaptic sites. As there is considerable surface dendritic GluA2, we were also able to follow dendritic structures with this technique (figure 4a). We have extensively characterized the effects of GluA2 expression on synaptic function and have found no effects on synaptic transmission or on synapse formation [32,33], so this method allows us to simultaneously image pre- and postsynaptic structures with minimal impact on synapse formation. Using this method, we were able to track sites where pre- and postsynaptic structures were stable throughout the experiment, and sites where pre- and postsynaptic contacts were either gained or lost (figures 4a and 5a).

In order to test the role of PSD-95 in promoting stabilization of AD contacts, we first quantified overall GluA2 dynamics regardless of their presynaptic association under baseline conditions and compared that to conditions where PSD-95 was overexpressed. To assess whether the effects of PSD-95 change as synapses mature, these dynamics were examined at two ages: DIV 7 and DIV 14. Between DIV 7 and DIV 14 GluA2 puncta became more stable; further, PSD-95 overexpression in young but not older neurons mimicked this increase in stability (figure 4b). Similarly, the average lifetime of GluA2 puncta was significantly longer at DIV 14 than at DIV 7, and this was also mimicked by overexpression of PSD-95 in young neurons (figure 4c). A similar effect of PSD-95 was observed when we quantified the rates of loss and gain for colocalized GluA2 and FM4-64 puncta, which are putative sites of synaptic contact (figure 5b). These rates were higher at DIV 7 than at DIV 14. Further, PSD-95 overexpression reduced turnover at DIV 7 but not at DIV 14. Interestingly, PSD-95 overexpression did not affect the ratio of contact gain to loss, as this ratio remained close to unity across ages and PSD-95 expression levels (figure 5b). These data show that PSD-95 overexpression in young neurons enhances the stability of putative synaptic contacts, while having little impact on older synapses that express more endogenous PSD-95 [29].

4. Discussion

In this study we used simultaneous time-lapse imaging of two important synaptic proteins, PSD-95 and CaMKII, to probe the dynamics of protein composition at young synapses. Our data reveal that this composition is highly dynamic, with both proteins moving into and out of the same synapse independently, so that synapses cycle rapidly between states in which they have none, one, or both proteins present. Despite this dynamism, both PSD-95 and CaMKII spent more time at stable than at labile synaptic contacts. Further, we found that stable contacts have a higher ratio of

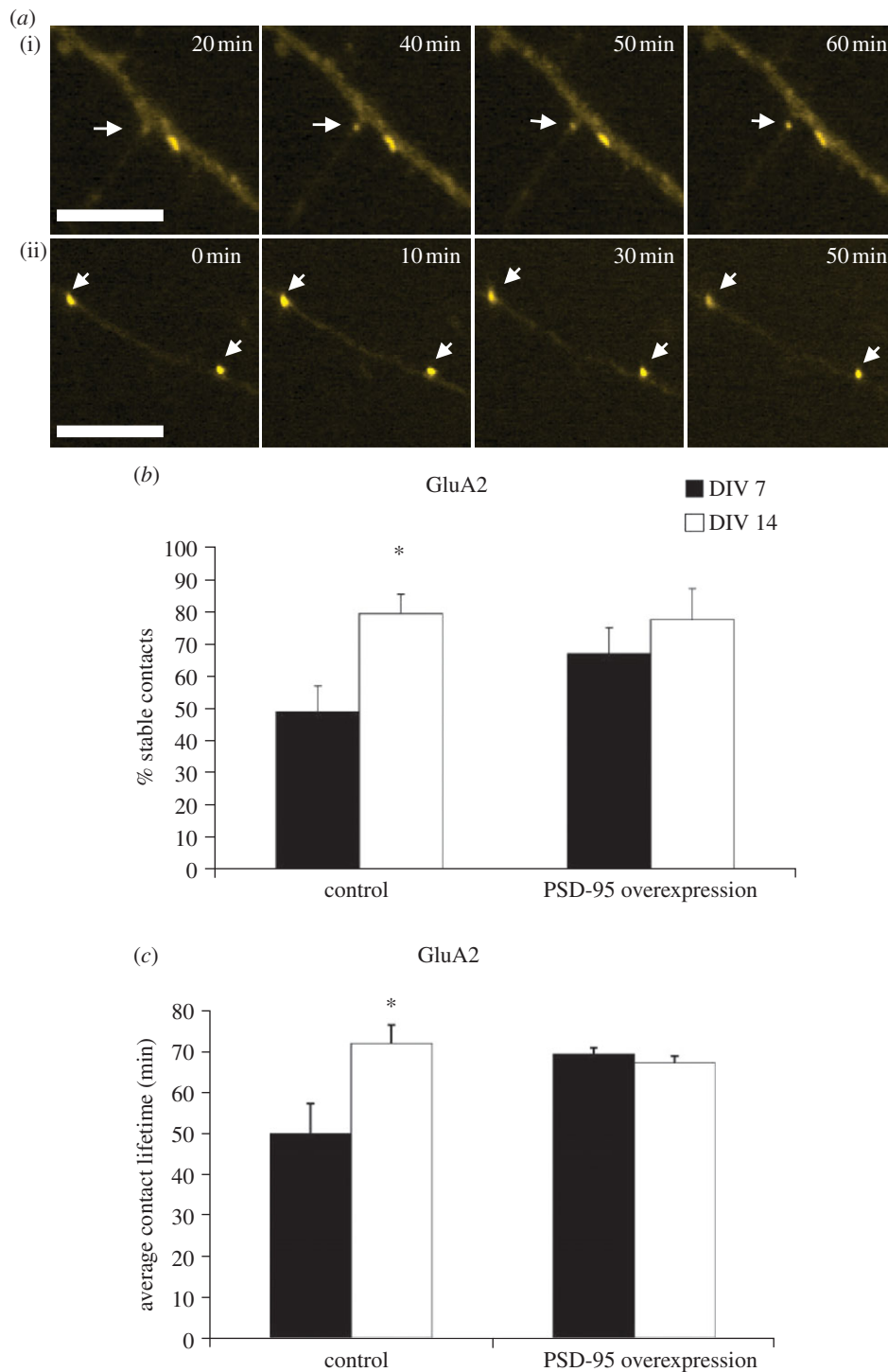


Figure 4. GluA2 puncta are more stable in older neurons, and this is mimicked by PSD-95 overexpression in young neurons. (a) Example images of labile (i), appeared or disappeared) and stable (ii) GluA2 puncta. Scale bar, 15 μm . PSD-95 overexpression significantly increases (b) the percentage of stable GluA2 puncta and (c) average lifetime at DIV 7 but not DIV 14. $N = 5$ neuron each condition. Asterisk (*) denotes $p < 0.05$, unpaired t -test between DIV 7 and DIV 14 conditions.

activated to total CaMKII than do labile contacts. Finally, we found that overexpression of PSD-95 in young neurons (where expression levels are low) was able to reduce contact turnover and stabilize pre- and postsynaptic structures. In contrast, in older neurons with more stable contacts PSD-95 overexpression had no impact on synapse stability. Taken together, these data suggest that PSD-95 promotes synapse maturation and stabilization.

Consistent with previous reports [3–5,7], we found that PSD-95 and CaMKII are quite dynamic at AD contacts, moving into and out of these sites over the course of our 3 h experiment. Less than 20% of PSD-95 and CaMKII puncta were stable over this period of time, and the average lifetime of contacts

was about 100 min for PSD-95 and 40 min for CaMKII; as a consequence putative synaptic sites cycle quickly between expressing one or the other or both of these proteins. These striking dynamics suggests that synapses have a highly variable protein composition over time, though the purpose and regulation of this variability are not clear. The majority of stable contacts have at least a transient expression of both PSD-95 and CaMKII such that PSD-95 spends significantly more time at stable AD contacts. This could be because the prolonged presence of PSD-95 helps to stabilize AD contacts, or because stable AD contacts are better able to accumulate PSD-95. The former interpretation is favoured by the observation that overexpression of PSD-95 increased the fraction of stable contacts.

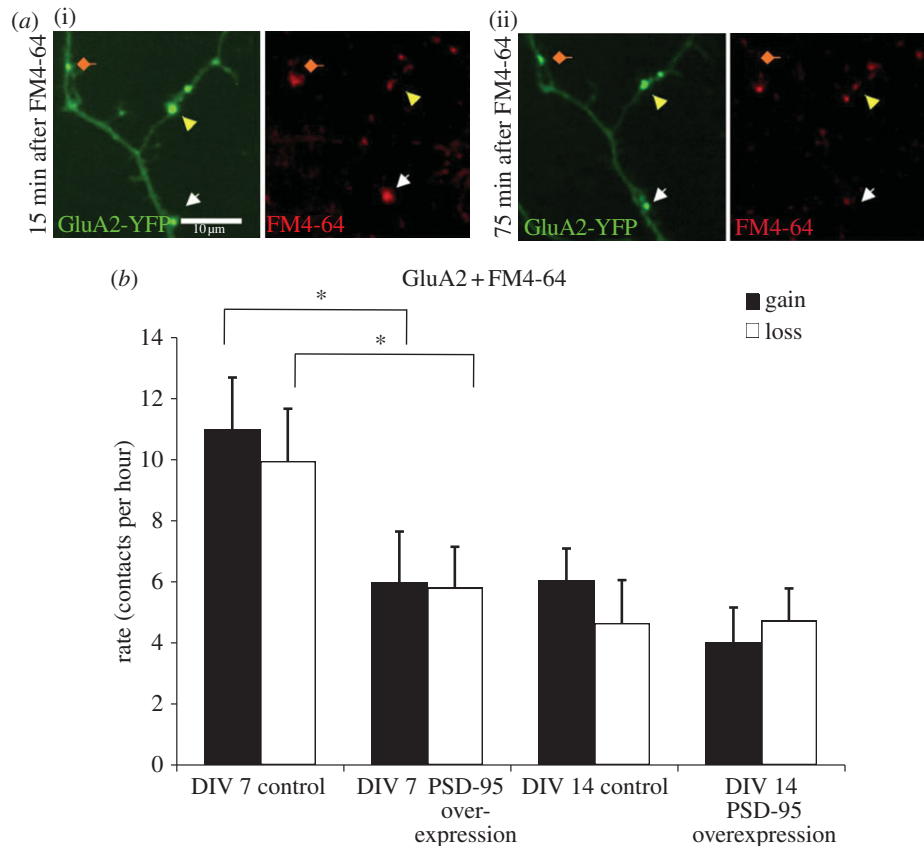


Figure 5. Colocalized pre- and postsynaptic markers are more stable in older neurons, and this is mimicked by PSD-95 overexpression. (a) Example of live GluA2 and FM imaging of pre- and postsynaptic sites simultaneously, 15 min (i) and 75 min (ii) after FM labelling; orange arrow indicates non-colocalized site, while arrowheads indicate sites of colocalization. Scale bar, 15 μ m. (b) Rates of contact gain and loss for the two ages; PSD-95 overexpression significantly reduced these rates at DIV 7 but not at DIV 14. $N = 5$ neuron each condition. Asterisk (*) denotes $p < 0.05$, ANOVA followed by corrected t -tests for the comparisons indicated by brackets.

PSD-95 binds indirectly to α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPA), regulates synaptic strength, and has been proposed to serve as a 'slot' protein that directly determines synaptic AMPAR content [36–39]. Manipulations of PSD-95 can dramatically alter evoked synaptic transmission [23,25,37,40–43], but we found previously that PSD-95 overexpression or knockdown in this same culture system did not significantly increase AMPA quantal amplitude [29], in keeping with other reports of only modest effects [5,23,37, 40,41,44]. The modest impact of PSD-95 on quantal amplitude, coupled with the substantial impact on synapse stability shown here and suggested previously [31], suggest that the effects of PSD-95 overexpression on evoked transmission are primarily due to an enhancement of the number of stable, functional synapses.

Interestingly, the effects of PSD-95 on synapse stabilization appear to be developmentally regulated, as PSD-95 overexpression stabilized young but not older synapses. This is consistent

with our previous finding that PSD-95 knockdown reduces metabotropic excitatory postsynaptic current frequency in old but not young neurons [29]. Between DIV 7 and DIV 14 the levels of synaptic PSD-95 increase substantially [28,29], and it has been shown that postsynaptic densities share and compete for PSD-95 [5,7]. As the acquisition of PSD-95 is probably regulated by changes in activity and experience [5,45,46] when PSD-95 levels are low early in development, competition for PSD-95 could help determine which synapses are maintained and which are lost. Later in development PSD-95 levels are high enough that they are probably no longer limiting, and so this form of competition may be restricted to very young synapses. This could explain why overexpression of PSD-95 stabilizes young synaptic contacts, but has no effect in older neurons.

Acknowledgements. We thank L. Wang for technical assistance, and R. Nicoll, D. Bredt and M. N. Waxham for providing DNA constructs.

Funding statement. This work was supported by NIH grant NS036853.

References

- Yoshihara Y, De Roo M, Muller D. 2009 Dendritic spine formation and stabilization. *Curr. Opin. Neurobiol.* **19**, 146–153. (doi:10.1016/j.conb.2009.05.013)
- Krueger DD, Tuffy LP, Papadopoulos T, Brose N. 2012 The role of neuroligins and neuroligins in the formation, maturation, and function of vertebrate synapses. *Curr. Opin. Neurobiol.* **22**, 412–422. (doi:10.1016/j.conb.2012.02.012)
- Okabe S, Kim HD, Miwa A, Kuriu T, Okado H. 1999 Continual remodeling of postsynaptic density and its regulation by synaptic activity. *Nat. Neurosci.* **2**, 804–811. (doi:10.1038/12175)
- Marrs GS, Green SH, Dailey ME. 2001 Rapid formation and remodeling of postsynaptic densities in developing dendrites. *Nat. Neurosci.* **4**, 1006–1013. (doi:10.1038/nn717)
- Gray NW, Weimer RM, Bureau I, Svoboda K. 2006 Rapid redistribution of synaptic PSD-95 in the

- neocortex *in vivo*. *PLoS Biol.* **4**, e370. (doi:10.1371/journal.pbio.0040370)
6. Kuriu T, Inoue A, Bito H, Sobue K, Okabe S. 2006 Differential control of postsynaptic density scaffolds via actin-dependent and -independent mechanisms. *J. Neurosci.* **26**, 7693–7706. (doi:10.1523/JNEUROSCI.0522-06.2006)
 7. Tsuriel S, Geva R, Zamorano P, Dresback T, Boeckers T, Gundelfinder ED, Garner CC, Ziv NE. 2006 Local sharing as a predominant determinant of synaptic matrix molecular dynamics. *PLoS Biol.* **4**, e271. (doi:10.1371/journal.pbio.0040271)
 8. Wu GY, Cline HT. 1998 Stabilization of dendritic arbor structure *in vivo* by CaMKII. *Science* **279**, 222–226. (doi:10.1126/science.279.5348.222)
 9. Zou DJ, Cline HT. 1999 Postsynaptic calcium/calmodulin-dependent protein kinase II is required to limit elaboration of presynaptic and postsynaptic neuronal arbors. *J. Neurosci.* **19**, 8909–8918.
 10. Lisman J, Schulman H, Cline H. 2002 The molecular basis of CaMKII function in synaptic and behavioral memory. *Nat. Rev. Neurosci.* **3**, 175–190. (doi:10.1038/nrn753)
 11. Kazama H, Morimoto-Tanifuji T, Nose A. 2003 Postsynaptic activation of calcium/calmodulin-dependent protein kinase II promotes coordinated pre- and postsynaptic maturation of *Drosophila* neuromuscular junctions. *Neuroscience* **117**, 615–625. (doi:10.1016/S0306-4522(02)00923-5)
 12. Yamagata Y *et al.* 2009 Kinase-dead knock-in mouse reveals an essential role of kinase activity of Ca²⁺/calmodulin-dependent protein kinase II α in dendritic spine enlargement, long-term potentiation, and learning. *J. Neurosci.* **29**, 7607–7618. (doi:10.1523/JNEUROSCI.0707-09.2009)
 13. Pratt KG, Watt AJ, Griffith LC, Nelson SB, Turrigiano GG. 2003 Activity-dependent remodeling of presynaptic inputs by postsynaptic expression of activated CaMKII. *Neuron* **39**, 269–281. (doi:10.1016/S0896-6273(03)00422-7)
 14. Pratt KG, Taft CE, Burbea M, Turrigiano GG. 2008 Dynamics underlying synaptic gain between pairs of cortical pyramidal neurons. *Dev. Neurobiol.* **68**, 143–151. (doi:10.1002/dneu.20577)
 15. Sugiyama Y, Kawabata I, Sobue K, Okabe S. 2005 Determination of absolute protein numbers in single synapses by a GFP-based calibration technique. *Nat. Methods* **2**, 677–684. (doi:10.1038/nmeth783)
 16. Rao A, Cha EM, Craig AM. 2000 Mismatched appositions of presynaptic and postsynaptic components in isolated hippocampal neurons. *J. Neurosci.* **20**, 8344–8353.
 17. Ahmed R, Zha XM, Green SH, Dailey ME. 2006 Synaptic activity and F-actin coordinately regulate CaMKII α localization to dendritic postsynaptic sites in developing hippocampal slices. *Mol. Cell. Neurosci.* **31**, 37–51. (doi:10.1016/j.mcn.2005.08.020)
 18. Gerrow K, Romorini S, Nabi SM, Colicos MA, Sala C, El-Husseini A. 2006 A preformed complex of postsynaptic proteins is involved in excitatory synapse development. *Neuron* **49**, 547–562. (doi:10.1016/j.neuron.2006.01.015)
 19. De Roo M, Klausner P, Mendez P, Pogliani L, Muller D. 2008 Activity-dependent PSD formation and stabilization of newly formed spines in hippocampal slice cultures. *Cereb. Cortex* **18**, 151–161. (doi:10.1093/cercor/bhm041)
 20. Garner CC, Nash J, Huganir RL. 2000 PDZ domains in synapse assembly and signaling. *Trends Cell Biol.* **10**, 274–280. (doi:10.1016/S0962-8924(00)01783-9)
 21. Prange O, Murphy TH. 2001 Modular transport of postsynaptic density-95 clusters and association with stable spine precursors during early development of cortical neurons. *J. Neurosci.* **21**, 9325–9333.
 22. Matsuzaki M, Ellis-Davies GC, Nemoto T, Miyashita Y, Iino M, Kasai H. 2001 Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nat. Neurosci.* **4**, 1086–1092. (doi:10.1038/nn736)
 23. El-Husseini AE, Schnell E, Chetkovich DM, Nicoll RA, Brecht DS. 2000 PSD-95 involvement in maturation of excitatory synapses. *Science* **290**, 1364–1368.
 24. El-Husseini AE *et al.* 2002 Synaptic strength regulated by palmitate cycling on PSD-95. *Cell* **108**, 849–863. (doi:10.1016/S0092-8674(02)00683-9)
 25. Beïque JC, Lin DT, Kang MG, Aizawa H, Takamiya K, Huganir RL. 2006 Synapse-specific regulation of AMPA receptor function by PSD-95. *Proc. Natl Acad. Sci. USA* **103**, 19 535–19 540. (doi:10.1073/pnas.0608492103)
 26. Irie M, Hata Y, Takeuchi M, Ichtchenko K, Toyoda A, Hirao K, Takai Y, Rosahl TW, Südhof TC. 1997 Binding of neuroligins to PSD-95. *Science* **277**, 1511–1515. (doi:10.1126/science.277.5331.1511)
 27. Scheiffele P, Fan J, Choi J, Fetter R, Serafini T. 2000 Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. *Cell* **101**, 657–669. (doi:10.1016/S0092-8674(00)80877-6)
 28. Sans N, Petralia RS, Wang YX, Blahos 2nd J, Hell JW, Wenthold RJ. 2000 A developmental change in NMDA receptor-associated proteins at hippocampal synapses. *J. Neurosci.* **20**, 1260–1271.
 29. Sun Q, Turrigiano GG. 2011 PSD-95 and PSD-93 play distinct signaling/scaffolding roles in synaptic scaling up and down. *J. Neurosci.* **31**, 6800–6808. (doi:10.1523/JNEUROSCI.5616-10.2011)
 30. Trachtenberg JT, Chen BE, Knott GW, Feng G, Sanes JR, Welker E, Svoboda K. 2002 Long-term *in vivo* imaging of experience-dependent synaptic plasticity in adult cortex. *Nature* **420**, 88–94. (doi:10.1038/nature01273)
 31. Ehrlich I, Klein M, Rumpel S, Malinow R. 2007 PSD-95 is required for activity-driven synapse stabilization. *Proc. Natl Acad. Sci. USA* **104**, 4176–4181. (doi:10.1073/pnas.0609307104)
 32. Wierenga CJ, Ibata K, Turrigiano GG. 2005 Postsynaptic expression of homeostatic plasticity at neocortical synapses. *J. Neurosci.* **25**, 2895–2905. (doi:10.1523/JNEUROSCI.5217-04.2005)
 33. Ibata K, Sun Q, Turrigiano GG. 2008 Rapid synaptic scaling induced by changes in postsynaptic firing. *Neuron* **57**, 819–826. (doi:10.1016/j.neuron.2008.02.031)
 34. Shen K, Meyer T. 1999 Dynamic control of CaMKII translocation and localization in hippocampal neurons by NMDA receptor stimulation. *Science* **284**, 162–166. (doi:10.1126/science.284.5411.162)
 35. Kim E, Sheng M. 2004 PDZ domain proteins of synapses. *Nat. Rev. Neurosci.* **5**, 771–781. (doi:10.1038/nrn1517)
 36. Schnell E, Sizemore M, Karimzadegan S, Chen L, Brecht DS, Nicoll RA. 2002 Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number. *Proc. Natl Acad. Sci. USA* **99**, 13 902–13 907. (doi:10.1073/pnas.172511199)
 37. Ehrlich I, Malinow R. 2004 Postsynaptic density 95 controls AMPA receptor incorporation during long-term potentiation and experience-driven synaptic plasticity. *J. Neurosci.* **24**, 916–927. (doi:10.1523/JNEUROSCI.4733-03.2004)
 38. Elias GM, Nicoll RA. 2007 Synaptic trafficking of glutamate receptors by MAGUK scaffolding proteins. *Trends Cell Biol.* **17**, 343–352. (doi:10.1016/j.tcb.2007.07.005)
 39. Keith D, El-Husseini A. 2008 Excitation control: balancing PSD-95 function at the synapse. *Front. Mol. Neurosci.* **1**, 4. (doi:10.3389/neuro.02.004.2008)
 40. Beïque JC, Andrade R. 2003 PSD-95 regulates synaptic transmission and plasticity in rat cerebral cortex. *J. Physiol.* **546**, 859–867. (doi:10.1113/jphysiol.2002.031369)
 41. Stein V, House DR, Brecht DS, Nicoll RA. 2003 Postsynaptic density-95 mimics and occludes hippocampal long-term potentiation and enhances long-term depression. *J. Neurosci.* **23**, 5503–5506.
 42. Elias GM, Funke L, Stein V, Grant SG, Brecht DS, Nicoll RA. 2006 Synapse-specific and developmentally regulated targeting of AMPA receptors by a family of MAGUK scaffolding proteins. *Neuron* **52**, 307–320. (doi:10.1016/j.neuron.2006.09.012)
 43. Xu W, Schluter OM, Steiner P, Czervionke BL, Sabatini B, Malenka RC. 2008 Molecular dissociation of the role of PSD-95 in regulating synaptic strength and LTD. *Neuron* **57**, 248–262. (doi:10.1016/j.neuron.2007.11.027)
 44. Kelsch W, Lin CW, Lois C. 2008 Sequential development of synapses in dendritic domains during adult neurogenesis. *Proc. Natl Acad. Sci. USA* **105**, 16 803–16 808. (doi:10.1073/pnas.0807970105)
 45. Yoshii A, Sheng MH, Constantine-Paton M. 2003 Eye opening induces a rapid dendritic localization of PSD-95 in central visual neurons. *Proc. Natl Acad. Sci. USA* **100**, 1334–1339. (doi:10.1073/pnas.0335785100)
 46. Steiner P, Higley MJ, Xu W, Czervionke BL, Malenka RC, Sabatini BL. 2008 Destabilization of the postsynaptic density by PSD-95 serine 73 phosphorylation inhibits spine growth and synaptic plasticity. *Neuron* **60**, 788–802. (doi:10.1016/j.neuron.2008.10.014)