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Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors

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Regulation of AMPA receptor (AMPAR) trafficking is important for neural plasticity. Here we examined the trafficking and synthesis of the GluR1 and GluR2 subunits using ReAsH-EDT$_2$ and FlAsH-EDT$_2$ staining. Activity blockade of rat cultured neurons increased dendritic GluR1, but not GluR2, levels. Examination of transected dendrites revealed that both AMPAR subunits were synthesized in dendrites and that activity blockade enhanced dendritic synthesis of GluR1 but not GluR2. In contrast, acute pharmacological manipulations increased dendritic synthesis of both subunits. AMPARs synthesized in dendrites were inserted into synaptic plasma membranes and, after activity blockade, the electrophysiological properties of native synaptic AMPARs changed in the manner predicted by the imaging experiments. In addition to providing a novel mechanism for synaptic modifications, these results point out the advantages of using FlAsH-EDT$_2$ and ReAsH-EDT$_2$ for studying the trafficking of newly synthesized proteins in local cellular compartments such as dendrites.

The mechanisms that control the trafficking of AMPARs toward and away from synapses have received significant attention because of their importance in synaptogenesis, synapse maturation and various forms of synaptic plasticity[1,2]. Several approaches have been taken to examine AMPAR trafficking, including immunocytochemical labeling of endogenous and epitope-tagged recombinant AMPARs, visualizing AMPAR subunits fused to fluorescent reporters, making biochemical measurements by surface biotinylation or membrane fractionation approaches, and making electrophysiological measurements of the synaptic delivery of recombinant receptors (for review, see refs. 1,2). Although these approaches have provided valuable and often complementary information, each also has inherent limitations. It has been difficult to address, for example, questions related to the location and trafficking of newly synthesized AMPAR subunits. In particular, it has not been possible to definitively determine whether local dendritic synthesis of AMPARs occurs and, if so, whether this is modulated by activity. Such local control of AMPAR production could importantly contribute to the various forms of synaptic plasticity that require new protein synthesis[3,4].

A new method for directly examining and comparing the local trafficking of preexisting and recently synthesized proteins in specific subcellular domains uses biarsenical dyes that bind to short sequences containing four cysteine residues[5]. These dyes are nonfluorescent until they bind to the tetracysteine motif, at which point they become strongly green (FlAsH-EDT$_2$) or red (ReAsH-EDT$_2$) fluorescent[5]. The advantages of this approach have been shown by recent experiments that examined the trafficking of connexin43 to gap junctions[6].

Here we used this methodology to examine and compare the dendritic trafficking of preexisting and recently synthesized AMPAR subunits. To determine how activity modifies dendritic AMPAR trafficking, we examined the consequences of chronically blocking synaptic activity, a manipulation that has been shown to enhance synaptic strength due, at least in part, to the accumulation of AMPARs at synapses[7]. Our results provide strong evidence that AMPARs are locally synthesized in dendrites and delivered to synapses, a process that can be regulated by activity and thereby likely contributes to activity-dependent changes in synaptic strength. They also demonstrate that ReAsH-EDT$_2$/FlAsH-EDT$_2$ labeling is a new, powerful technique for addressing critical questions concerning dendritic protein synthesis[3,4].

RESULTS

ReAsH/FlAsH staining distinguishes recently synthesized AMPARs

A tetracysteine motif (EAAAREACCRECCARA) was inserted into the intracellular carboxy (C) termini of GluR1 and GluR2, two AMPAR subunits that have key roles in the activity-dependent trafficking of AMPARs during long-term potentiation (LTP) and depression (LTD)[1,2]. The motif was placed ten amino acids from the C terminus to minimize potential interference with known interactions involving PDZ protein and other binding partners[8] (**Fig. 1a**). These constructs also contained an extracellular, N-terminal HA epitope tag immediately adjacent to a specific cleavage site for the non-cell permeable protease thrombin[9]. This permits visualization of surface-expressed AMPARs in a temporally controlled manner[9].

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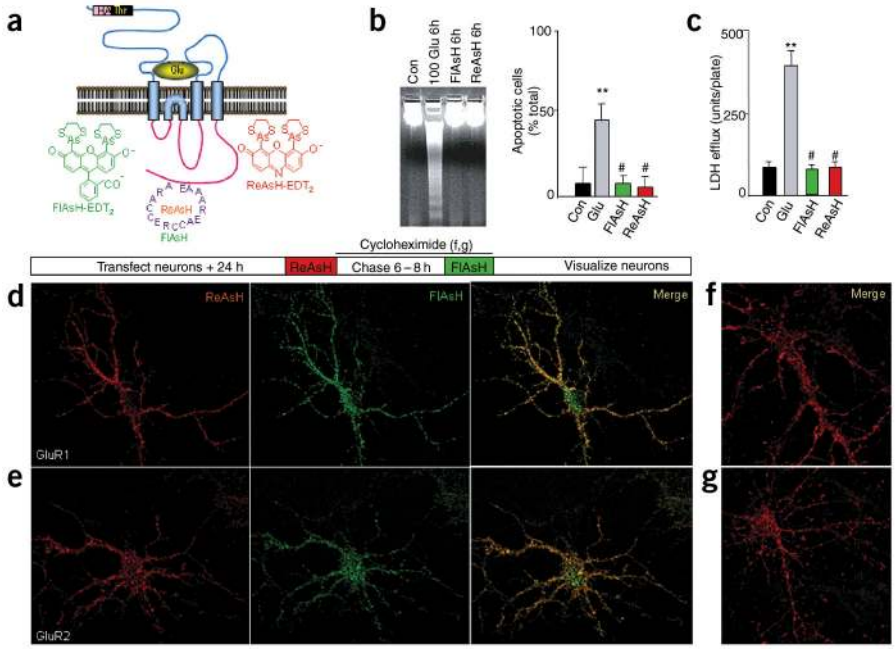

Figure 1 ReAsH-EDT$_2$ and FlAsH-EDT$_2$ are not toxic and specifically stain tetracysteine-tagged GluR1 and GluR2. (**a**) Schematic showing the membrane topology of GluR1/2 and the location of the intracellular tetracysteine and extracellular HA/thrombin tags. (**b**) DNA laddering (indicative of apoptosis) is shown only following 100 μM glutamate stimulation but not following prolonged FlAsH-EDT$_2$ or ReAsH-EDT$_2$ application. Graph shows the percentage of apoptotic cells per field after 100 μM glutamate or prolonged ReAsH-EDT$_2$/FlAsH-EDT$_2$ application. In this and all subsequent figures, error bars represent s.e.m. (**c**) Using LDH release as a marker of necrotic cell death, 100 μM glutamate, but not ReAsH-EDT$_2$/FlAsH-EDT$_2$, evoked significant cell death when compared to controls. (**d**,**e**) Representative confocal images of sequential ReAsH-EDT$_2$ and FlAsH-EDT$_2$ stained cells expressing GluR1-Cys4 (**d**) and GluR2-Cys4 (**e**). (**f**,**g**) Representative examples of merged ReAsH-EDT$_2$ and FlAsH-EDT$_2$ stained cells expressing GluR1-Cys4 (**f**) and GluR2-Cys4 (**g**) in which cycloheximide was applied during the chase period prior to FlAsH application. Note the complete absence of FlAsH-EDT$_2$ staining.

As the biarsenical dyes FlAsH-EDT$_2$ and ReAsH-EDT$_2$ had not previously been applied to mammalian neurons, we first tested for toxic effects. FlAsH-EDT$_2$ and ReAsH-EDT$_2$ (1–2.5 μM) were applied to cultured hippocampal neurons for a time period (6 h) much longer than that used to label and visualize GluR1/2 (15–60 min; see Methods) and toxicity assays were performed. Neither reagent caused an increase in the proportion of cells showing apoptosis (**Fig. 1b**) or necrosis (**Fig. 1c**). Furthermore, after prolonged exposure to FlAsH-EDT$_2$ and ReAsH-EDT$_2$, both control and transfected neurons showed normal morphology, exhibiting no signs of damage such as chromatin condensation, membrane blebbing, or loss of mitochondrial membrane potential (data not shown). These results are consistent with the lack of toxicity of FlAsH-EDT$_2$ when applied to *Drosophila* neuromuscular junctions[10].

We next performed pulse-chase experiments, applying first ReAsH-EDT$_2$ and then 6–8 h later FlAsH-EDT$_2$ to cells expressing tetracysteine-tagged GluR1 (GluR1-Cys4) or GluR2 (GluR2-Cys4). We expected that ReAsH-EDT$_2$ would label all preexisting GluR1/2-Cys4 subunits, while FlAsH-EDT$_2$ would label those AMPAR subunits synthesized during the chase period. The spatial distributions of ReAsH- (red) versus FlAsH- (green) labeled puncta were consistent with this prediction (**Fig. 1d**,**e**). ReAsH puncta were observed throughout dendrites, with modest somatic staining that was concentrated on or near the cell surface. In contrast, most FlAsH puncta were in the soma and proximal dendritic tree in a pattern consistent with recent passage through the secretory pathway. FlAsH puncta were also observed in distal dendrites at distances greater than 500 μM from the soma.

Several observations ensured that ReAsH-EDT$_2$ labeled preexisting AMPAR subunits while FlAsH-EDT$_2$ labeled only those subunits synthesized during the chase period. First, ReAsH-EDT$_2$/FlAsH-EDT$_2$ staining was not observed in untransfected cells. Second, reversing the order of dye application resulted in the appropriate reciprocal stain-

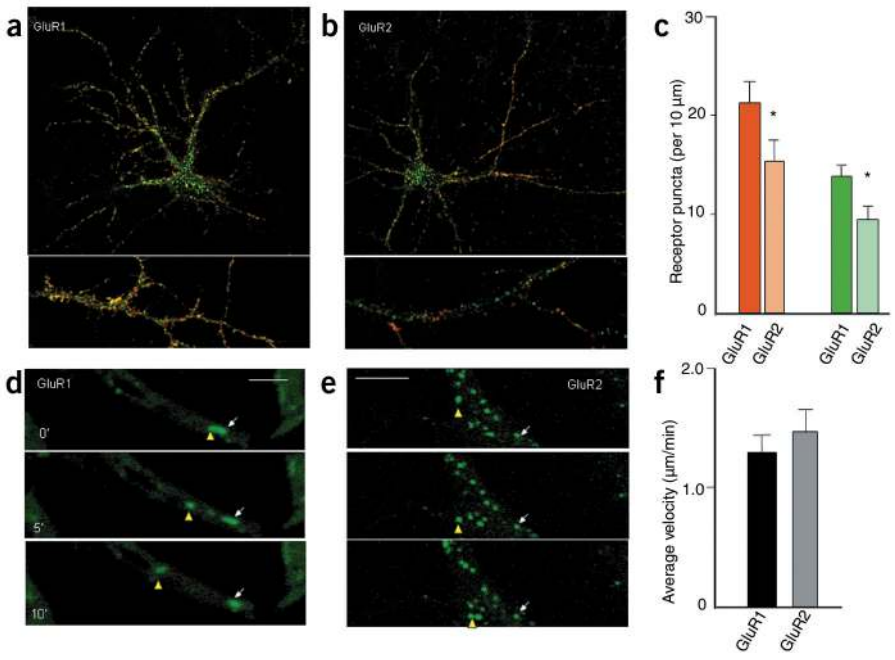

Figure 2 Characterization of basal AMPAR subunit trafficking. (**a**,**b**) Examples of ReAsH and FlAsH stained cells expressing GluR1-Cys4 (**a**) and GluR2-Cys4 (**b**). Higher power views of dendritic processes are shown in lower images. (**c**) Quantitation of ReAsH and FlAsH stained puncta shows different levels of GluR1-Cys4 and GluR2-Cys4 (*$P < 0.05$ compared with corresponding subunit). (**d**,**e**) FlAsH stained GluR1-Cys4 (**d**) and GluR2-Cys4 (**e**) were imaged over the course of 10 min. White arrows point to examples of stationary puncta; yellow arrowheads point to mobile puncta moving in the anterograde direction. Scale bars, 4 μm (**d**) and 10 μm (**e**). (**f**) Quantitation of velocities of mobile FlAsH stained GluR1-Cys4 and GluR2-Cys4 puncta.

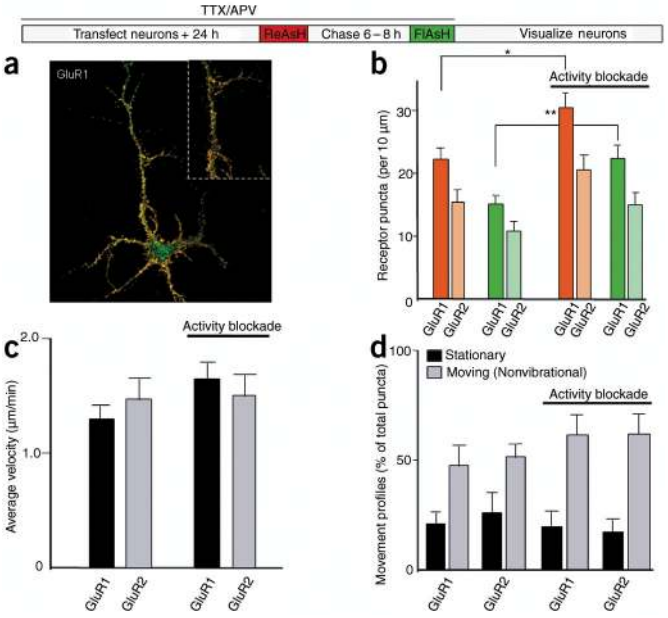

Figure 3 Chronic activity blockade increased dendritic levels, but not the rate of transport, of GluR1. (**a**) Example of ReAsH-EDT$_2$/FlAsH-EDT$_2$ staining of GluR1-Cys4 following chronic activity blockade. Inset shows higher power view of dendritic processes. (**b**) Quantification of ReAsH and FlAsH stained GluR1/2 puncta in control cells and cells after activity blockade. Levels of both preexisting (ReAsH) and recently synthesized (FlAsH) GluR1 were increased by activity blockade ($*P < 0.05$; $**P < 0.01$). (**c**) Quantification of the velocity of mobile FlAsH/ReAsH stained GluR1 and GluR2 receptor puncta under control conditions and following activity blockade. No differences were observed between the groups. (**d**) Quantification of the proportion of stationary and mobile GluR1/GluR2 punta in control cells and following activity blockade. Again, no differences were observed between the groups.

ing pattern (data not shown). Importantly, applying the protein synthesis inhibitor cycloheximide during the chase period before FlAsH-EDT$_2$ application prevented the visualization of any FlAsH-labeled puncta (**Fig. 1f,g**). This indicated that ReAsH-EDT$_2$ likely labeled all preexisting GluR1/2-Cys4 and that subsequent FlAsH-EDT$_2$ application permitted visualization of only those AMPAR subunits synthesized during the chase period. FlAsH-labeled puncta in dendritic processes could be observed with chase periods as short as 1 h (the shortest interval examined), indicating that the trafficking of these proteins into dendrites was constitutive and robust.

Basal trafficking of GluR1-Cys4 and GluR2-Cys4

To compare the basal trafficking patterns of GluR1-Cys4 versus GluR2-Cys4, we counted the number of AMPAR puncta in dendritic processes. There were consistently more preexisting and recently synthesized GluR1-Cys4 than GluR2-Cys4 dendritic puncta (**Fig. 2a–c**; $n = 50, 38$ for GluR1-, GluR2-Cys4, respectively). This was unlikely to be due to differences in the level of expression of the two proteins, as both were under control of the same promoter and were always trans-

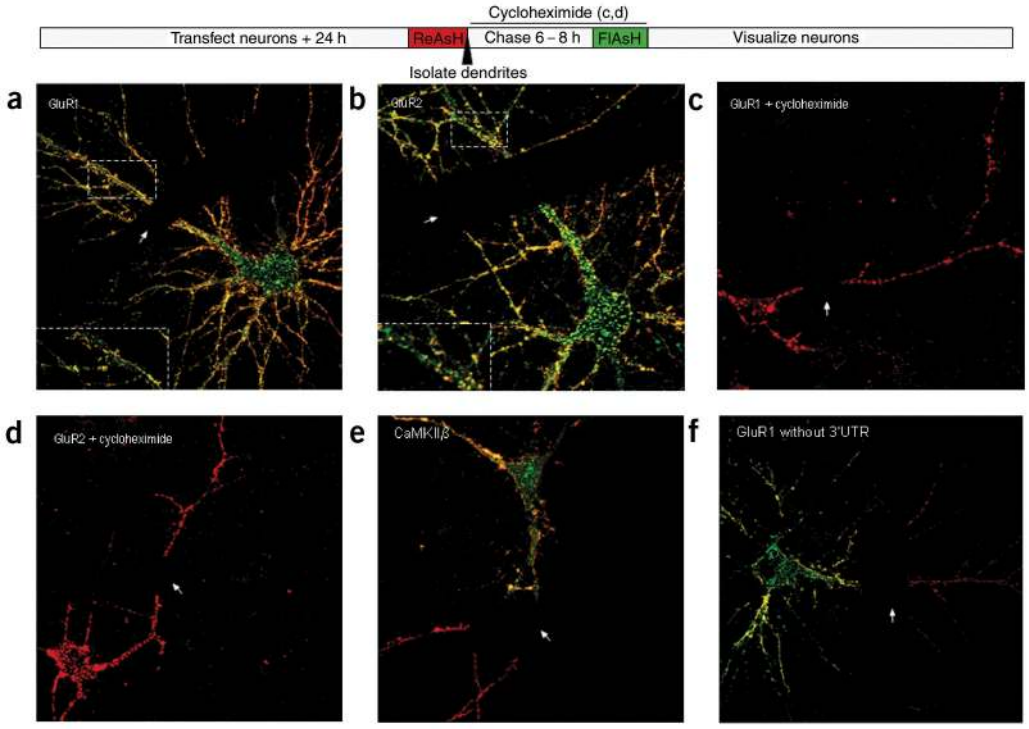

Figure 4 Local synthesis of AMPAR subunits occurs in isolated dendrites. (**a**,**b**) Representative examples of ReAsH/FlAsH stained cells transfected with GluR1-Cys4 (**a**) and GluR2-Cys4 (**b**). Transection of dendrites (indicated by small white arrows) preceded the application of FlAsH-EDT$_2$. Insets show higher power view of transected dendrites with clear FlAsH stained puncta. (**c**,**d**) Representative examples demonstrating that cycloheximide application (100 µM during the chase period and period of FlAsH-EDT$_2$ application) completely prevented FlAsH staining in both the soma and physically isolated dendrites. (**e**) Example of cell transfected with tetracysteine-tagged βCaMKII showing that it is not locally synthesized in dendrites. Note robust ReAsH and FlAsH staining of βCaMKII in the soma and attached proximal portion of the isolated dendrites but only ReAsH staining in the transected dendrites. (**f**) Example of cell transfected with GluR1-Cys4 lacking its 3′UTR. As in **e**, note robust ReAsH and FlAsH staining of GluR1 in the soma and attached dendrites but only ReAsH staining in the transected dendrites.

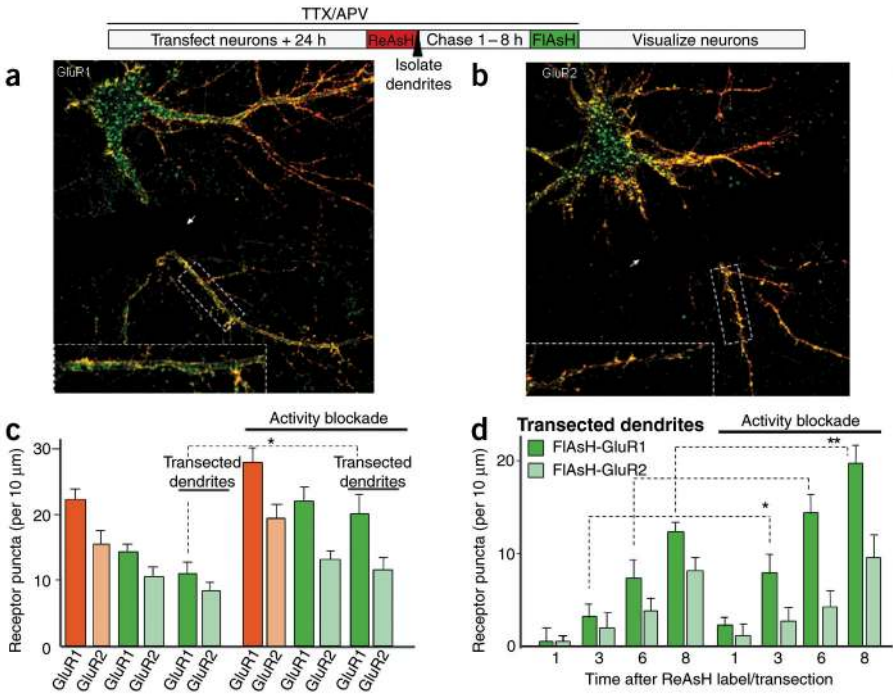

Figure 5 Chronic activity blockade enhances local dendritic synthesis of GluR1. (**a**,**b**) Examples of ReAsH/FlAsH stained cells expressing GluR1-Cys4 (**a**) and GluR2-Cys4 (**b**) following chronic activity blockade and transection of dendrites. Insets show higher power views of segments from the transected dendrites. (**c**) Quantification of the effects of activity blockade on the levels of GluR1 and GluR2 in transected dendrites. Activity blockade increased the local synthesis of GluR1 in transected dendrites (*$P < 0.05$ compared to control conditions) but not of GluR2. (**d**) Time course of the increase in GluR1 in transected dendrites following activity blockade. Activity blockade increased amount of FlAsH labeled GluR1, but not GluR2, at 3, 6 and 8 h after transection of dendrites (*$P < 0.05$, **$P < 0.01$).

fected in parallel in 'sister' cultures of neurons from the same preparation. Furthermore, comparisons of total protein expression both by western blotting (using HA antibodies) and by total HA immunofluorescence revealed that expression levels were nearly identical (data not shown).

To examine whether there were differences in the rate of transport of GluR1-Cys4 versus GluR2-Cys4 puncta, we performed live-cell imagining of ReAsH- and FlAsH-labeled puncta. For both receptor subunits, 30–40% of the FlAsH and ReAsH puncta remained stationary during the course of the live cell imaging experiments, whereas approximately 60% of ReAsH and FlAsH puncta moved continuously in the anterograde (~40%) or retrograde (~20%) direction at 1–2 µm/min (**Fig. 2d–f**; $n = 20, 24$ for GluR1-, GluR2-Cys4). Individual particles could shift directions several times during the imaging period (15–30 min). Remaining puncta either were stable during the entire imaging period or exhibited rapid vibrational movements. These movements are similar to those described previously for endogenous GluR2 subunits[11] and for overexpressed receptor subunits[12]. Importantly, there were no detectable differences in the movements of GluR1-Cys4 and GluR2-Cys4 puncta nor between preexisting and recently synthesized puncta.

Effects of activity blockade on dendritic AMPAR trafficking

Neurons appear to use homeostatic mechanisms to elicit changes in strength at excitatory synapses in response to prolonged (>24 h) increases or decreases in activity[7]. However, little is known about the detailed mechanisms by which this occurs. We therefore used ReAsH-EDT_2 /FlAsH-EDT_2 labeling to examine the effects of chronic blockade of activity on the trafficking of GluR1-Cys4 and GluR2-Cys4, a manipulation that increases the number of AMPARs at synapses[13,14]. Compared to sister control cultures, chronic blockade of activity caused an increase in the amount of both preexisting (35% increase) and recently synthesized (43% increase) GluR1-Cys4 in dendrites (**Fig. 3a**,**b**; $n = 48$ and 42 for control and activity blockade conditions, respectively). Surprisingly, although GluR2-Cys4 in dendrites seemed to slightly rise, these changes were not statistically significant (**Fig. 3b**; $n = 48$ and 52 for control and activity blockade conditions).

What might account for the increase in dendritic GluR1-Cys4? One possibility is that the rate of delivery of AMPARs from soma to dendrites was enhanced. We therefore measured the rate of anterograde transport of ReAsH- and FlAsH-labeled puncta in cultures that had experienced activity blockade as well as the proportion of vesicles that exhibited continual (>5 min) movement. Neither of these measurements was affected when compared to sister control cultures (**Fig. 3c**,**d**; $n = 22, 26$ for control and activity blockade conditions), suggesting that the rate of transport of AMPARs is not affected by this manipulation of activity.

Local synthesis of AMPAR subunits in isolated dendrites

An intriguing possibility is that AMPARs are locally synthesized in dendrites and that activity can modify this process. Based on molecu-

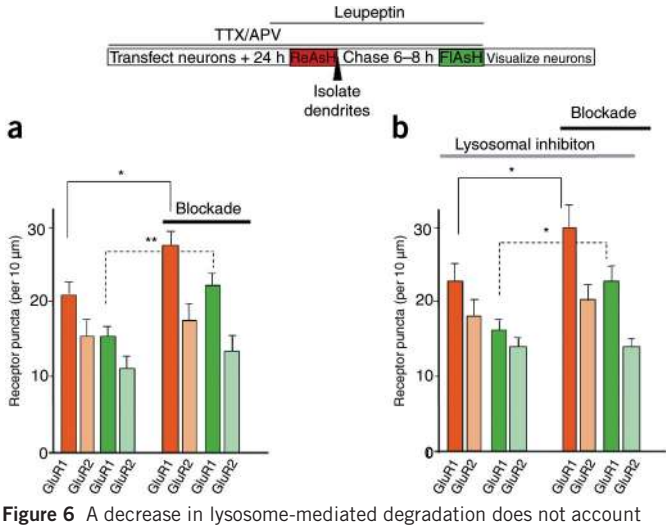

Figure 6 A decrease in lysosome-mediated degradation does not account for the increase in GluR1 following activity blockade. (**a**,**b**) Quantitation of ReAsH and FlAsH staining in isolated dendrites expressing GluR1-Cys4 or GluR2-Cys4 under control conditions and activity-blocked conditions in the absence (**a**) or presence (**b**) of leupeptin (50 µM; 36 h treatment prior to ReAsH/FlAsH staining) (*$P < 0.05$; **$P < 0.01$).

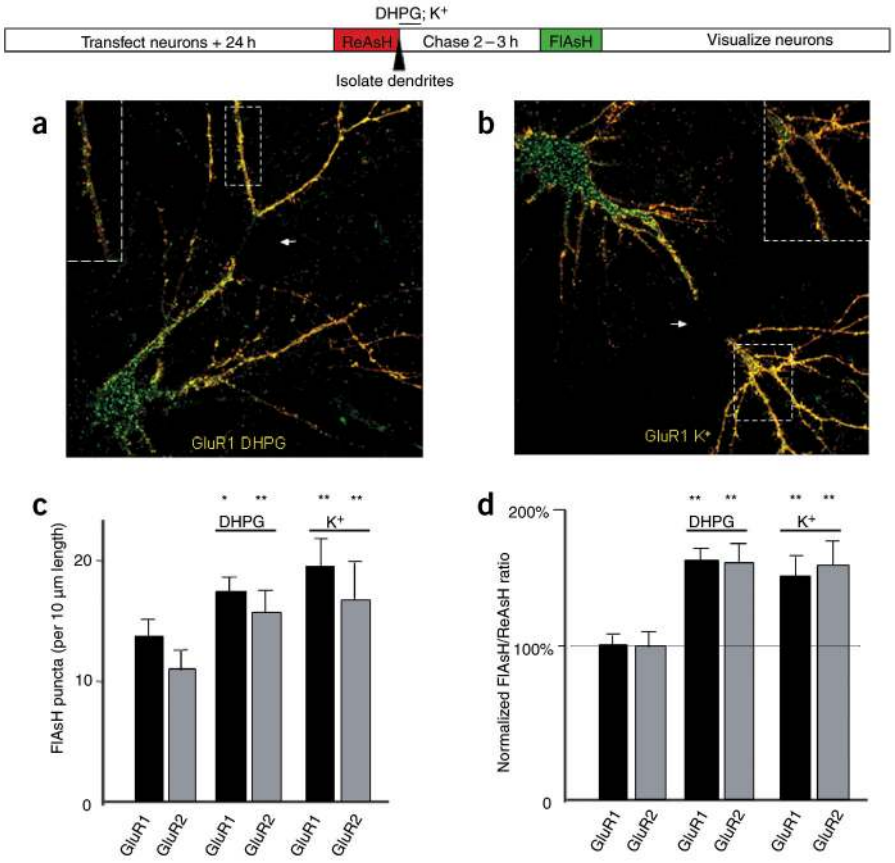

Figure 7 Acute treatments increase local dendritic synthesis of both GluR1 and GluR2. (**a**,**b**) Examples of ReAsH/FlAsH stained cells expressing GluR1-Cys4 in which the mGluR1 agonist DHPG (25 µM for 15 min); (**a**) or high K^+ (90 mM for 1 min applied three times over 5 min); (**b**) was applied after transection of dendrites and immediately prior to FlAsH staining. Images were acquired 1 h after FlAsH staining. Insets show higher power views of segments of the isolated dendrites. (**c**) Quantitation of the amount of FlAsH stained GluR1 and GluR2 puncta in transected dendrites following DHPG or high K^+ treatment ($^*P < 0.05$; $^{**}P < 0.01$). (**d**) Histograms showing values depicted in panel **c** normalized to their own ReAsH labeled puncta in isolated dendrites ($^{**}P < 0.01$).

lar profiling of isolated neurites, it has been suggested that dendrites contain mRNAs for AMPAR subunits[15]. Furthermore, transfection of GluR2 mRNA into isolated dendrites resulted in the surface expression of AMPARs[16]. There are, however, risks of contamination using the first approach[3], and although showing that dendrites contain the machinery necessary for synthesizing and transporting integral membrane proteins is important, it does not address the issue of whether AMPAR subunit mRNA can be transported into dendrites. Sequential ReAsH-EDT_2/FlAsH-EDT_2 staining provided a technique that permits a test of whether AMPAR mRNA can be transported to dendrites and locally synthesized. If AMPARs were synthesized only in the soma (or very proximal dendrites) and were then transported to distal dendritic sites, labeling of preexisting Glu1/2-Cys4 with ReAsH-EDT_2 followed by physical transection of dendrites would prevent any subsequent FlAsH-EDT_2 labeling of AMPAR subunits in dendrites. In contrast, if dendritic synthesis of GluR1 or 2 did occur, then FlAsH-EDT_2 staining should still be present even when it is applied after ReAsH-EDT_2 and the physical isolation of dendrites.

In nearly every cell examined (>95%, $n > 50$), following ReAsH-EDT_2 application and transection of individual dendrites, application of FlAsH-EDT_2 clearly labeled GluR1-Cys4 and GluR2-Cys4 puncta in the physically isolated dendrites (**Fig. 4a**,**b**). The interpretation of this result depended on the assumption that the initial ReAsH-EDT_2 application irreversibly stained all preexisting Cys4-tagged AMPAR subunits. To test that this assumption holds true when examining isolated dendrites, we again applied the protein synthesis inhibitor cycloheximide after ReAsH-EDT_2 application and transection of dendrites. In every cell examined ($n = 24$), inhibiting protein synthesis prevented any FlAsH-EDT_2 staining in both intact and isolated dendrites (**Fig. 4c**,**d**). This result indicates that the FlAsH-labeled puncta in the isolated dendrites were the result of local dendritic synthesis of the Cys4-tagged AMPARs.

A limitation of this approach, however, is that by necessity we examined recombinant rather than endogenous proteins. It is therefore conceivable that the overexpression of GluR1/2-Cys4 mRNAs artifactually induced their transport into dendrites. Several controls were used to address this possibility. First, we expressed a Cys4-tagged form of the β isoform of calcium/calmodulin-dependent protein kinase II (βCaMKII), the mRNA of which is not found in dendrites[17]. βCaMKII-Cys4-expressing cells that had dendritic processes transected after ReAsH-EDT_2 application showed robust staining for both ReAsH and FlAsH in the soma and connected dendrites, but exhibited only ReAsH staining in the physically isolated dendrites (**Fig. 4e**; $n = 12$). This demonstrated that despite its robust expression, βCaMKII was not synthesized in dendrites, and suggested that the dendritic synthesis of GluR1/2-Cys4 was unlikely to be due to an artifact of its overexpression. Second, we also Cys4-tagged αCaMKII (containing its 3′ and 5′ UTRs) and in contrast to βCaMKII-Cys4, it showed robust synthesis in transected dendrites (data not shown). Thus, two different mRNAs with well-established dendritic (αCaMKII) or non-dendritic (βCaMKII) localizations behaved as expected. Third, as the 3′ UTRs of mRNAs are thought to contain key sequences responsible for mRNA targeting and/or stability within dendrites[3,4], we deleted the 3′ UTR of the GluR1-Cys4 construct and tested whether it still exhibited dendritic synthesis. In contrast to the previous results (**Fig. 4a**,**b**), little (<10% of GluR1 with the 3′UTR region) or no dendritic synthesis of this GluR1-Cys4 was detected, as evidenced by the lack of FlAsH staining in isolated dendrites despite robust ReAsH and FlAsH staining in the soma and physically connected dendrites (**Fig. 4f**, $n = 18$). This indicated that the 3′ UTR of GluR1 mRNA contained specific targeting sequences that are required for its transport to and/or stabilization within dendrites.

Activity influences dendritic synthesis of AMPARs

The preceding experiments suggested that the mRNAs for the AMPAR subunits GluR1 and GluR2 can be transported to dendrites where they are locally synthesized. We next asked whether changes in activity can affect this process by comparing the degree of local synthesis of GluR1-Cys4 and GluR2-Cys4 in transected dendrites from

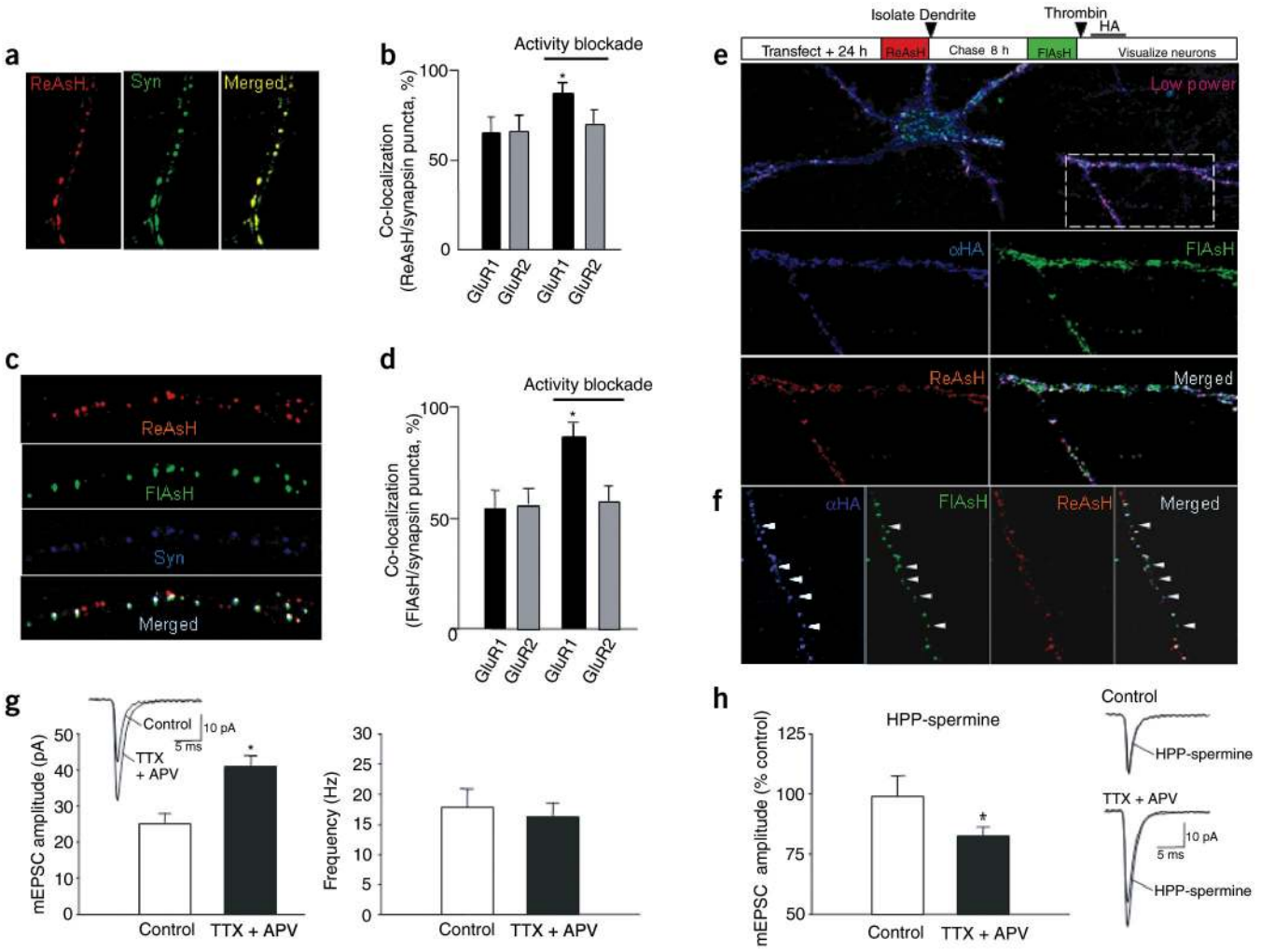

Figure 8 Chronic activity blockade enhances synaptic localization of GluR1 in isolated dendrites and changes the subunit composition of native synaptic AMPARs. (**a**) Example of colocalization of ReAsH-labeled GluR1-Cys4 with the presynaptic marker protein synapsin I in an isolated dendrite. (**b**) Quantitation of synaptic localization of ReAsH-stained GluR1 and GluR2 in control conditions and following activity blockade (*$P < 0.05$). (**c**) Example of ReAsH- and FlAsH-labeled GluR1-Cys4 colocalized with synapsin I in an isolated dendrite. (**d**) Quantitation of synaptic localization of FlAsH-stained GluR1 and GluR2 in control conditions and following activity blockade in isolated dendrites (*$P < 0.05$). (**e**) Example of GluR1-Cys4 transfected neuron with transected dendrites in which soon after FlAsH-EDT$_2$ application thrombin was applied to cleave the extracellular HA epitope from all surface expressed subunits. After 2 h of recovery, neurons were stained with anti-HA to visualize all newly delivered surface AMPARs. Note that a significant number of newly synthesized GluR1-Cys4 (FlAsH labeled) co-localize with HA staining (as well as with ReAsH staining). (**f**) Higher magnification of a physically isolated dendrite showing that there are punctate regions where the HA and FlAsH staining does not colocalize with ReAsH staining (indicated by arrowheads). (**g**) Activity blockade increased the mean amplitude (left graph) but not frequency (right graph) of mEPSCs (*$P < 0.01$). Inset shows averaged mEPSC ($n = 400$–600 events) recorded from typical control and activity-blockaded cells. (**h**) HPP-spermine decreased the mean amplitude of mEPSCs in activity-blockaded cells but not in control cells (*$P < 0.01$). Traces show sample averaged mEPSCs ($n = 400$–600 events) from individual cells before and after application of HPP-spermine.

control cultures with those in which we blocked activity. Consistent with our previous results, chronic activity blockade increased the amount of preexisting and newly synthesized GluR1-Cys4 in dendrites physically connected to the soma, whereas GluR2-Cys4 was not significantly affected (**Fig. 5a–c**; $n = 28, 36$ for control and activity blockade conditions, respectively). The same result was obtained when transected dendrites were examined (**Fig. 5a–c**; $n = 24$ for both control and activity blockade conditions). Specifically, the amount of GluR1-Cys4 synthesized during the chase period following ReAsH staining in transected dendrites was enhanced by activity blockade, whereas the synthesis of GluR2-Cys4 was not significantly affected.

We next compared the time course of local dendritic synthesis of GluR1-Cys4 and GluR2-Cys4 in isolated dendrites from control and activity-blocked cultures. FlAsH-labeled GluR1-Cys4 and GluR2-Cys4 could be detected with a chase period as short as 1 h, and there was a trend toward increased GluR1-Cys4 in the activity-blocked cultures at this time point (**Fig. 5d**; $n = 24, 18$ for control and activity-blockade conditions, respectively). By 3 h, the increase in newly synthesized GluR1-Cys4 in isolated dendrites in activity-blocked cultures was significant, whereas GluR2-Cys4 levels were unaffected (**Fig. 5d**; $n = 24, 22$ for control and activity-blockade conditions).

Although the preceding experiments are consistent with the hypothesis that dendritic synthesis of GluR1-Cys4 is enhanced by blockade of activity, ReAsH and FlAsH fluorescence reflects the net amount of protein, making it possible that blocking activity caused a decrease in the degradation rate of AMPAR subunits. To test this

possibility, we treated cultures with the lyososomal inhibitor leupeptin during the last 36 h of the activity blockade[18]. If the increase in GluR1-Cys4 puncta in activity-blocked cultures was due to decreased lysosomal degradation, then leupeptin should prevent this increase. Sequential ReAsH-EDT$_2$/FlAsH-EDT$_2$ staining of transected dendrites in activity-blocked cultures treated with leupeptin revealed a clear increase in the expression of recently synthesized GluR1-Cys4 compared to control cultures (**Fig. 6a**,**b**; n = 28, 24 for control and activity blockade without leupeptin; n = 24, 26 for control and activity blockade with leupeptin treatment). The magnitude of this increase was the same as that seen in sister cultures not treated with leupeptin (**Fig. 6a**), demonstrating that the increase in GluR1-Cys4 in isolated dendrites from activity-blocked cultures was not due to a decrease in lysosomal mediated degradation.

A number of acute pharmacological manipulations, such as activation of group-I metabotropic glutamate receptors (mGluRs), enhance dendritic synthesis of specific proteins[3,4,19]. We therefore tested whether application of the group-I mGluR receptor agonist (S)-3,5-dihydroxyphenylglycine (DHPG) would also enhance the synthesis of GluR1-Cys4 and GluR2-Cys4 in transected dendrites. In addition, to mimic acute increases in activity, in separate experiments we applied high potassium in a manner that has been reported to induce a form of LTP that involves AMPAR trafficking[20]. Both acute manipulations elicited an increase in recently synthesized GluR1-Cys4, and, in contrast to the effects of chronic activity blockade, also increased GluR2-Cys4 (**Fig. 7**; n = 36, 48 respectively). Because these experiments involved ReAsH-EDT$_2$ staining followed by an acute manipulation and then FlAsH-EDT$_2$ staining, we could calculate a FlAsH/ReAsH ratio. If the acute manipulations increased recently synthesized GluR1-Cys4 and GluR2-Cys4 because of a decrease in their degradation, we would expect such a mechanism to affect both ReAsH and FlAsH stained AMPAR subunits and the ratio would thus not change significantly. Instead this analysis demonstrated that DHPG and K^+ treatments yielded a robust increase in the FlAsH/ReAsH ratio for both AMPAR subunits (**Fig. 7d**). These results suggest that acute pharmacological manipulations can enhance the local dendritic synthesis of both GluR1 and GluR2.

AMPARs synthesized in dendrites traffic to synapses

We have demonstrated that GluR1 and GluR2 can be locally synthesized in dendrites and that this process is modulated by activity. An important question is whether such locally synthesized AMPAR subunits are inserted into the plasma membrane at synapses and have functional effects. To address this issue, we first tested whether Cys4-tagged GluR1 and GluR2 were found at synapses. Consistent with previous studies using recombinant AMPAR subunits[9,21], ~60–70% of ReAsH-labeled GluR1-Cys4 and GluR2-Cys4 puncta colocalized with a presynaptic marker (namely, synapsin I) in transected dendrites (**Fig. 8a**,**b**). Importantly, chronic activity blockade increased the degree of synaptic localization of ReAsH-labeled GluR1-Cys4 but not GluR2-Cys4 (**Fig. 8b**; n = 36 for both GluR1- and GluR2-Cys4 in control conditions; n = 36, 32 for GluR1- and GluR2-Cys4 in activity blockade conditions). We next asked if the increase in synaptic localization of GluR1-Cys4 in activity-blocked cultures also occurred specifically for those AMPAR subunits recently synthesized in transected dendrites. Again, there was a clear increase in the proportion of recently synthesized (that is, FlAsH-labeled) GluR1-Cys4 puncta at synapses as a consequence of activity blockade with no effect on GluR2-Cys4 (**Fig. 8c**,**d**; n = 24, 22 for GluR1- and GluR2-Cys4 in control conditions; n = 22, 24 for GluR1- and GluR2-Cys4 in activity blockade conditions).

Next, we used the thrombin-cleavable HA epitope on our constructs to test whether the dendritically synthesized GluR1-Cys4 was inserted into the plasma membrane. Consistent with previous results[9], application of thrombin effectively cleaved the HA tag, rendering preexisting surface AMPARs undetectable (data not shown). This allowed us to perform experiments in which we (i) applied ReAsH-EDT$_2$ to label preexisting AMPAR subunits, (ii) applied FlAsH-EDT$_2$ following a chase period to label newly synthesized GluR1-Cys4, (iii) immediately applied thrombin to cleave the HA tag from surface expressed ReAsH-labeled subunits, and (iv) applied an HA antibody following an additional (1–4 h) chase period (**Fig. 8e**,**f**). Co-localization of the HA tag with FlAsH thereby identified recently synthesized AMPARs that were inserted into the plasma membrane while colocalization of HA with ReAsH reflected the trafficking of preexisting AMPARs to the surface. In physically attached dendrites, a significant proportion of FlAsH puncta (~40%) stained for HA, demonstrating that recently synthesized AMPAR subunits traffic to the dendritic plasma membrane. This experiment was repeated using transected dendrites and again FlAsH puncta (~35%) also stained for HA (**Fig. 8e**; n = 34).

A limitation of these experiments was that the FlAsH puncta that exhibited HA staining often colocalized with ReAsH-labeled puncta. At these sites it was not possible to determine whether the surface expressed GluR1-Cys4 derived from preexisting ReAsH-labeled GluR1 or newly synthesized FlAsH-labeled GluR1. In many transected dendrites (~50%), however, it was possible to find sites of punctate HA staining that colocalized only with FlAsH and not ReAsH (**Fig. 8f**). This result provides strong evidence that AMPARs locally synthesized in dendrites are inserted into the dendritic plasma membrane. An additional analysis (data not shown) revealed that in control cultures ~50% of the FlAsH-labeled GluR1 and GluR2 puncta colocalized with their ReAsH-labeled counterparts. Activity blockade increased the extent of this colocalization to ~70% for GluR1 but had no detectable effect on GluR2. This suggests that within the time period of these experiments, ~50% of the recently synthesized AMPAR subunits traffic to synapses that already contain AMPARs and that, for GluR1, this proportion is increased by chronic activity blockade.

An important question is whether the recombinant AMPAR subunits that we studied reflect the behavior of native AMPARs. Since activity blockade caused a greater increase in the amount of dendritic GluR1 than GluR2, we predicted that the subunit composition of endogenous synaptic AMPARs would change such that there would be a greater proportion of AMPARs lacking GluR2. We tested this by recording miniature excitatory postsynaptic currents (mEPSCs) from untransfected neurons and examining the effects of HPP-spermine (10 μM), a polyamine that preferentially blocks AMPARs lacking GluR2 or those with lower stoichiometric levels of GluR2[22–24]. Consistent with previous results[7,13,14], mEPSC amplitude (but not frequency) was significantly increased in activity-blockaded cultures (**Fig. 8g**; n = 9, 10 for control and blockaded cultures, respectively). Importantly, HPP-spermine had no significant effect on control mEPSCs but caused a clear (~20%) decrease in mEPSC amplitude in activity-blockaded cultures (**Fig. 8h**, n = 7, 6 for control and blockaded cultures, respectively). This result indicates that the subunit composition of native synaptic AMPARs changed in response to activity blockade in the manner predicted by the ReAsH/FlAsH labeling experiments.

DISCUSSION

Using ReAsH-EDT$_2$ and FlAsH-EDT$_2$, we examined the trafficking of preexisting and newly synthesized AMPAR subunits GluR1 and

GluR2. These dyes were not toxic to neurons and several controls demonstrated that they enabled an accurate identification of AMPARs that had been recently synthesized in a known, finite time period. Most importantly, this technique provided a direct assay for the local synthesis of AMPARs in specific dendritic compartments. Sequential ReAsH-EDT_2/FlAsH-EDT_2 staining revealed that both GluR1 and GluR2 were locally synthesized in dendrites physically isolated from their somas. The lack of dendritic synthesis of βCaMKII as well as the very large (>90%) reduction in the dendritic synthesis of a GluR1 construct lacking its 3′ UTR indicated that this local dendritic synthesis of AMPARs was unlikely to be due to artifactual targeting of overexpressed mRNA into dendrites.

Although RNA amplification techniques suggested that hippocampal dendrites contain mRNAs for AMPAR subunits[3,4,15], *in situ* hybridization studies have not detected these[3,4,25,26]. Possible explanations for these discrepancies include the relative sensitivity of the assays used and that dendritic AMPAR mRNAs may take forms that interfere with their hybridization to probes[3]. Consistent with the former explanation are recent experiments showing that endogenous GluR2 mRNA can be readily detected in the dendrites of hippocampal neurons in culture using high-resolution fluorescence *in situ* hybridization (Grooms, S.Y. *et al.*, *Soc. Neurosci. Abstr.* **28**, 839.4, 2002).

Previous work showed that dendrites often contain the core elements of the secretory pathway necessary for the synthesis and transport of integral membrane proteins, specifically ER and Golgi[3,4,27–30]. Furthermore, isolated dendrites can incorporate sugar precursors indicative of Golgi function[31], and surface AMPARs can be detected after transfection of isolated dendrites with GluR2 mRNA[16]. These results as well as direct imaging of ER-to-Golgi transport in dendrites[30] suggest that integral membrane proteins, and not merely cytoplasmic proteins, can be locally synthesized in dendrites. We have extended these findings by showing that AMPAR subunit mRNAs are targeted to proximal and distal dendrites where AMPARs can be locally synthesized and processed so that they can be incorporated into the plasma membrane at or near synaptic sites. The vast majority (>95%) of physically isolated dendrites exhibited local synthesis of GluR1 and GluR2 and some surface expression of AMPARs. This suggests that ER, Golgi and associated components of the secretory pathway are ubiquitously expressed in dendrites (at least in cultured neurons) and that retrograde transport from dendritic ER to somatic Golgi (see ref. 30) may not be required for the processing of integral membrane proteins that are synthesized in dendrites. That mRNA for a glutamate receptor subunit is found in subsynaptic regions of the fly neuromuscular junction further suggests that the local control of glutamate receptor synthesis is an evolutionarily conserved mechanism[32].

Activity-dependent regulation of dendritic synthesis of proteins is of great interest because it provides an attractive mechanism for the synapse-specific modifications that underlie various forms of synaptic and experience-dependent plasticity[3,4,33]. To test whether activity affected the trafficking and local synthesis of AMPARs, we performed a straightforward manipulation—chronic blockade of activity—that enhances synaptic strength at least in part due to the synaptic accumulation of AMPARs[7,13,14]. We found that such activity blockade increased the amount of dendritic and synaptically localized GluR1 but had much smaller effects on GluR2. Although several lines of evidence suggested that this increase in dendritic GluR1 level was most likely caused by an increase in the local dendritic synthesis of GluR1, we cannot rule out that additional mechanisms, such as an increase in the stability of GluR1 at synapses (both the protein and its mRNA), also contribute to the synaptic modifications caused by this manipulation.

A prediction of our results was that chronic activity blockade should cause a change in the subunit composition and thereby the functional properties of native synaptic AMPARs. Examination of the inhibitory effects of HPP-spermine on mEPSCs confirmed this prediction by providing strong evidence that activity blockade increased the proportion of synaptic AMPARs lacking GluR2 (see also Thiagarajan, T.C. *et al.*, *Soc. Neurosci. Abstr.* **29**, 10.1, 2003). Such AMPARs would provide a source of calcium to dendritic spines in addition to NMDA receptors and calcium channels—a source that may have a profound effect on subsequent activity-dependent changes in synaptic properties[34,35]. Thus our data examining the trafficking and dendritic synthesis of AMPAR subunits suggest homeostatic changes in synaptic strength may differ in important mechanistic ways from the changes in synaptic strength that occur during LTP.

We also found that acute manipulations, specifically application of a mGluR agonist[3,4,19] or application of high K^+ using a protocol that can elicit LTP[20], increased the levels of both GluR1 and GluR2 in isolated dendrites. It was possible that these acute manipulations increased the levels of recently synthesized GluR1 and GluR2 by decreasing their rate of degradation, not by increasing their synthesis. However, when normalized to the level of preexisting GluR1/2 (ReAsH fluorescence), there was still a large increase in the amount of recently synthesized GluR1 and GluR2 (FlAsH fluorescence). This result suggests that the changes in GluR1 and GluR2 following these acute manipulations were indeed due to an increase in their local dendritic synthesis. These results also point out an advantage of using FlAsH/ReAsH staining to measure changes in local protein synthesis over measuring the fluorescent changes of proteins fused to GFP or its variants.

Elucidating the detailed molecular mechanisms responsible for the targeting of GluR1 and GluR2 mRNA to dendrites and the activity-dependent regulation of their local dendritic synthesis will be important topics for future investigation. The approach presented here will also be advantageous to catalog more definitively which sets of synaptic proteins are in fact locally synthesized in dendrites. Sequential staining of Cys4-tagged proteins using ReAsH-EDT_2 and FlAsH-EDT_2 provides, arguably, the most direct measure of local protein synthesis currently available. It provides high sensitivity while retaining high specificity as evidenced by the differences in the staining patterns in isolated dendrites for mRNAs that are targeted to dendrites (GluR1/2, αCaMKII) versus those that are not (βCaMKII). Thus, as cDNAs of key synaptic proteins with their corresponding 3′ and 5′ UTRs become available, it should be possible to determine which ones are synthesized in dendrites and how activity affects this process for each protein. It will also be interesting to see if specific groups of dendritic mRNAs exist such that alterations in activity lead to coordinated local changes in the synthesis of specific protein complexes. Combined with information about the coordinated activity-regulated degradation of postsynaptic proteins[18], such information will prove invaluable for elucidating the molecular mechanisms for the synapse-specific pre- and postsynaptic structural modifications that likely accompany many forms of synaptic plasticity[36,37].

METHODS

Treatment and transfection of primary hippocampal cultures. Hippocampal cultures (see **Supplementary Methods** online for details) were prepared from embryonic (E18) Sprague-Dawley rats according to procedures approved by the IACUC of Stanford University. After 7 days *in vitro* (d.i.v.), neurons were transfected using the calcium phosphate precipitation method as previously described[9]. Briefly, neurons were transfected with 5–8 μg DNA per well (12-well plates) and then stained with FlAsH and/or ReAsH 24–36 h after transfec-

tion (see **Supplementary Methods** online for details of cDNA constructs). All experiments involving drug treatments and comparisons of cDNA constructs were performed at the same time on sister cultures. For chronic blockade experiments, Neurobasal/B27 media was supplemented with TTX (final 1 μM; Sigma) and APV (final 150 μM; Sigma) from days 5–8 *in vitro*. Continuous leupeptin 50 μM (Roche) treatment was carried out on hippocampal neurons for a period of 36 h prior to ReAsH-EDT_2/FlAsH-EDT_2 staining.

FlAsH-EDT_2 and ReAsH-EDT_2 labeling. After establishing that the biarsenical compounds ReAsH-EDT_2 and FlAsH-EDT_2 were not toxic to mammalian neurons (see Results and **Supplementary Methods** online for details), we proceeded to study transfected neurons. Transfected neurons were rinsed twice with Phenol Red-free Hank's Balanced Salt Solution containing 1 g/mL glucose (HBSS; Invitrogen) and then incubated at 37 °C for 1 h with 2.5 μM ReAsH dye suspended in HBSS/25 μM 1,2-ethanedithiol (EDT_2) to label all expressed proteins. Following ReAsH-EDT_2 application, neurons were washed five times with HBSS/250 μM EDT_2, rinsed twice with Dulbecco's PBS (D-PBS; Invitrogen), and then returned to the incubator in full media during the chase periods (typically 6–8 h, but see **Fig. 6**). Following the chase period, neurons were rinsed again with HBSS and incubated with 1.25 μM FlAsH-EDT_2 dye in HBSS/EDT_2 for 1 h. Cells were washed extensively with HBSS/EDT_2, washed in D-PBS and then fixed for 15 min with 4% paraformaldehye/4% sucrose. Cells were then either cleared and mounted for viewing via confocal microscopy or further processed for immunofluorescence staining. Note that control experiments where the order of the dye application was reversed (*i.e.*, FlAsH-EDT_2 followed by ReAsH-EDT_2 staining) resulted in the same staining patterns reported here and was found to be equally effective in terms of specificity ($n = 12$ cells).

Image acquisition and analysis. Labeled neurons were acquired using a 63× objective on a Zeiss LSM510 Axiovert laser-scanning microscope. Typically a *z*-axis projection of a stack of 5–7 images covering an average depth of 3–5 μm acquired at 1024×1024 bits were used for analysis and quantitation. Images for GluR1 and GluR2 (and/or βCaMKII) were collected on the same day using identical pinhole, laser and detector settings. Samples were imaged within 2 d of staining and/or antibody labeling. For counts of individual ReAsH- and FlAsH-labeled puncta per 10 μm length, images were imported into Metamorph (Universal Imaging). Separate channels: red-ReAsH, green-FlAsH were isolated and to avoid 'thresholding' artifact; higher magnification of areas of neuronal processes (typically 2–3 processes per neuron were examined) were thresholded to the same levels for both GluR1 and GluR2 with somas being excluded. Processes were outlined in Metamorph to determine total process area and to ensure that the same sized region of interest was examined in both channels. Individual thresholded puncta were counted for every discrete 10 μm length of process for each channel. Two or three processes per cell and 20–50 cells from four separate cultures each were averaged. For transected processes, individual puncta in transected and intact processes (both proximal and distal to the transection, as well as non-transected processes from the same cell) were used in analyzing differences between the AMPAR constructs. Typically 3–5 transected processes and an equal number of non-transected counterparts were measured from each coverslip and three coverslips from a total of four distinct cultures were used for a total of 24–48 cells per each construct per condition. Analyses were performed blind without knowledge of the treatment history of the coverslip being examined. Statistical analysis was performed between treatment groups of the same construct using one-way ANOVA analysis while those between multiple treatment groups or between different constructs were analyzed using two-way ANOVAs using Analyze-it software (Analyze-it Corp). See **Supplementary Methods** online for details of live cell imaging, synapsin-I staining, colocalization analyses and thrombin cleavage assays.

Dendritic transection. FlAsH-EDT_2 or ReAsH-EDT_2 stained neurons were visualized at 40× with an upright microscope equipped for fluorescent detection. Dendrites were transected using a borosilicate glass micropipette controlled by an MP-285 micromanipulator (Sutter Instrument Co.). Neurons were included in the data only if they remained healthy as defined by several criteria: (i) both the cut dendrites and the remaining soma with its attached processes remained morphologically intact; (ii) there was no leakage of FlAsH-EDT_2 or ReAsH-EDT_2 from any portion of the intact cell or cut dendrites; (iii) there was no sign of membrane blebbing or varicosity formation; (iv) typical punctate FlAsH and ReAsh staining was observed in the soma and attached processes. About 25% of neurons whose dendrites were transected met these criteria and most of these had dendrites cut more than 50 μm from the soma. Typically neurons and transected processes were allowed to recover and synthesize proteins (6–8 h) prior to being relabeled with the complementary dye. For the activity-blockade experiments, cells with transected dendrites were returned to media containing TTX and APV.

Electrophysiological recordings. Whole-cell voltage-clamp recordings were made from hippocampal neurons using an Axopatch 1D amplifier (Axon Instruments) and a cesium based pipette solution. Coverslips containing TTX + APV treated cells or cells from non-treated sister cultures were placed in a recording chamber on a fixed stage of a BX50WI microscope (Olympus). See **Supplementary Methods** online for details.

Note: Supplementary information is available on the Nature Neuroscience website.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Neuroscience* website for details).

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1. Malinow, R. & Malenka, R.C. AMPA receptor trafficking and synaptic plasticity. *Annu. Rev. Neurosci.* **25**, 103–126 (2002).
2. Song, I. & Huganir, R.L. Regulation of AMPA receptors during synaptic plasticity. *Trends Neurosci.* **25**, 578–588 (2002).
3. Steward, O. & Schuman, E.M. Protein synthesis at synaptic sites on dendrites. *Annu. Rev. Neurosci.* **24**, 299–325 (2001).
4. Job, C. & Eberwine, J. Localization and translation of mRNA in dendrites and axons. *Nat. Rev. Neurosci.* **2**, 889–898 (2001).
5. Zhang, J., Campbell, R.E., Ting, A.Y. & Tsien, R.Y. Creating new fluorescent probes for cell biology. *Nat. Rev. Mol. Cell Biol.* **3**, 906–918 (2002).
6. Gaietta, G. *et al.* Multicolor and electron microscopic imaging of connexin trafficking. *Science* **296**, 503–507 (2002).
7. Turrigiano, G.G. & Nelson, S.B. Hebb and homeostasis in neuronal plasticity. *Curr. Opin. Neurobiol.* **10**, 358–364 (2000).
8. Braithwaite, S.P., Meyer, G. & Henley, J.M. Interactions between AMPA receptors and intracellular proteins. *Neuropharmacol.* **39**, 919–930 (2000).
9. Passafaro, M., Piech, V. & Sheng, M. Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. *Nat. Neurosci.* **4**, 917–926 (2001).
10. Marek, K.W. & Davis, G.W. Transgenically encoded protein photoinactivation (FlAsH-FALI): acute inactivation of synaptotagmin I. *Neuron* **36**, 805–813 (2002).
11. Borgdorff, A.J. & Choquet, D. Regulation of AMPA receptor lateral movements. *Nature* **417**, 649–653 (2002).
12. Washbourne, P., Bennett, J.E. & McAllister, A.K. Rapid recruitment of NMDA receptor transport packets to nascent synapses. *Nat. Neurosci.* **5**, 751–759 (2002).
13. Turrigiano, G.G., Leslie, K.R., Desai, N.S., Rutherford, L.C. & Nelson, S.B. Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature* **391**, 892–896 (1998).
14. O'Brien, R.J. *et al.* Activity-dependent modulation of synaptic AMPA receptor accumulation. *Neuron* **21**, 1067–1078 (1998).
15. Miyashiro, K., Dichter, M. & Eberwine, J. On the nature and differential distribution of mRNAs in hippocampal neurites: implications for neuronal functioning. *Proc. Natl. Acad. Sci. USA* **91**, 10800–10804 (1994).
16. Kacharmina, J.E., Job, C., Crino, P. & Eberwine, J. Stimulation of glutamate receptor protein synthesis and membrane insertion within isolated neuronal dendrites. *Proc. Natl. Acad. Sci. USA* **97**, 11545–11550 (2000).
17. Martone, M.E., Pollock, J.A., Jones, Y.Z. & Ellisman, M.H. Ultrastructural localization of dendritic messenger RNA in adult rat hippocampus. *J. Neurosci.* **16**, 7437–7446 (1996).
18. Ehlers, M.D. Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. *Nat. Neurosci,* **6**, 231–242 (2003).
19. Weiler, I.J. & Greenough, W.T. Metabotropic glutamate receptors trigger postsynaptic protein synthesis. *Proc. Natl. Acad. Sci. USA* **90**, 7168–7171 (1993).
20. Pickard, L. *et al.* Transient synaptic activation of NMDA receptors leads to the inser-

tion of native AMPA receptors at hippocampal neuronal plasma membranes. *Neuropharmacol.* **41**, 700–713 (2001).
21. Lissin, D.V. *et al.* Activity differentially regulates the surface expression of synaptic AMPA and NMDA glutamate receptors. *Proc. Natl. Acad. Sci. USA* **95**, 7097–7102 (1998).
22. Dingledine, R., Borges, K., Bowie, D. & Traynelis, S.F. The glutamate receptor ion channels. *Pharmacol. Rev.* **51**, 7–61 (1999).
23. Washburn, M.S., Numberger, M., Zhang, S. & Dingledine, R. Differential dependence on GluR2 expression of three characteristic features of AMPA receptors. *J. Neurosci.* **17**, 9393–9406 (1997).
24. Mainen, Z.F., Jia, Z., Roder, J. & Malinow, R. Use-dependent AMPA receptor block in mice lacking GluR2 suggests postsynaptic site for LTP expression. *Nat. Neurosci.* **1**, 579–586 (1998).
25. Craig, A.M., Blackstone, C.D., Huganir, R.L. & Banker, G. The distribution of glutamate receptors in cultured rat hippocampal neurons: postsynaptic clustering of AMPA-selective subunits. *Neuron* **10**, 1055–1068 (1993).
26. Benson, D.L. Dendritic compartmentation of NMDA receptor mRNA in cultured hippocampal neurons. *Neuroreport* **8**, 823–828 (1997).
27. Spacek, J. & Harris, K.M. Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. *J. Neurosci.* **17**, 190–203 (1997).
28. Gardiol, A., Racca, C. & Triller, A. Dendritic and postsynaptic protein synthetic machinery. *J. Neurosci.* **19**, 168–179 (1999).
29. Pierce, J.P., van Leyen, K. & McCarthy, J.B. Translocation machinery for synthesis of integral membrane and secretory proteins in dendritic spines. *Nat. Neurosci.* **3**, 311–313 (2000).
30. Horton, A.C. & Ehlers, M.D. Dual modes of endoplasmic reticulum-to-Golgi transport in dendrites revealed by live-cell imaging. *J. Neurosci.* **23**, 6188–6199 (2003).
31. Torre, E.R. & Steward, O. Protein synthesis within dendrites: glycosylation of newly synthesized proteins in dendrites of hippocampal neurons in culture. *J. Neurosci.* **16**, 5967–5978 (1996).
32. Sigrist, S.J. *et al.* Postsynaptic translation affects the efficacy and morphology of neuromuscular junctions. *Nature* **405**, 1062–1065 (2000).
33. Steward, O. mRNA at synapses, synaptic plasticity, and memory consolidation. *Neuron* **36**, 338–340 (2002).
34. Jia, Z. *et al.* Enhanced LTP in mice deficient in the AMPA receptor GluR2. *Neuron* **17**, 945–956 (1996).
35. Meng, Y., Zhang, Y. & Jia, Z. Synaptic transmission and plasticity in the absence of AMPA glutamate receptor GluR2 and GluR3. *Neuron* **39**, 163–176 (2003).
36. Lüscher, C., Nicoll, R.A., Malenka, R.C. & Muller, D. Synaptic plasticity and dynamic modulation of the postsynaptic membrane. *Nat. Neurosci.* **3**, 545–550 (2000).
37. Yuste, R. & Bonhoeffer, T. Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Annu. Rev. Neurosci.* **24**, 1071–1089 (2001).