

LETTERS

Activity-dependent dynamics and sequestration of proteasomes in dendritic spines

Baris Bingol¹ & Erin M. Schuman¹

The regulated degradation of proteins by the ubiquitin proteasome pathway is emerging as an important modulator of synaptic function and plasticity^{1–15}. The proteasome is a large, multi-subunit cellular machine that recognizes, unfolds and degrades target polyubiquitinated proteins. Here we report NMDA (*N*-methyl-D-aspartate) receptor-dependent redistribution of proteasomes from dendritic shafts to synaptic spines upon synaptic stimulation, providing a mechanism for local protein degradation. Using a proteasome-activity reporter and local perfusion, we show that synaptic stimulation regulates proteasome activity locally in the dendrites. We used restricted photobleaching of individual spines and dendritic shafts to reveal the dynamics that underlie proteasome sequestration, and show that activity modestly enhances the entry rate of proteasomes into spines while dramatically reducing their exit rate. Proteasome sequestration is persistent, reflecting an association with the actin-based cytoskeleton. Together, our data indicate that synaptic activity can promote the recruitment and sequestration of proteasomes to locally remodel the protein composition of synapses.

To examine whether synaptic activity regulates proteasome localization at synapses, we expressed the 19S proteasome subunit Rpt1 fused to green fluorescent protein (GFP) in cultured hippocampal neurons¹⁶. Biochemical analyses showed that the Rpt1-GFP subunit was incorporated into the endogenous proteasome complex at

~77% of the efficiency of another endogenous proteasome subunit (Supplementary Fig. S1a). Under control conditions, Rpt1-GFP was diffusely distributed in both dendrites and spines (Fig. 1a). After depolarization (60 mM KCl, 1.5 min), Rpt1-GFP moved from dendritic shafts into spines within minutes (Fig. 1a–c). Although there was no significant change in the total amount of Rpt1-GFP fluorescence after KCl stimulation, there was on average a ~90% increase in the spine Rpt1-GFP signal (20 min after KCl) (Fig. 1c, d). The increase in spine proteasome signal was persistent, typically lasting for at least 1 h. Most of the Rpt1-occupied spines showed overlap with a presynaptic protein, bassoon (Supplementary Fig. S3a and Supplementary Table S1), indicating that these spines are the sites of functional synapses.

The depolarization-induced redistribution of proteasomes does not reflect bulk movement of proteins into spines (Supplementary Fig. S1b–e), nor can it be explained by an increase in the number or area of spines (Supplementary Figs S1b–g and S2). However, redistribution into spines was also observed with a fluorescently tagged 20S proteasome subunit, α 4, indicating that both 19S and 20S proteasome subunits undergo activity-dependent trafficking (Supplementary Fig. S1f, g).

Depolarization with KCl leads to the activation of many voltage-gated channels, including NMDA-type glutamate receptor channels, which are critical for the initiation of synaptic plasticity in the

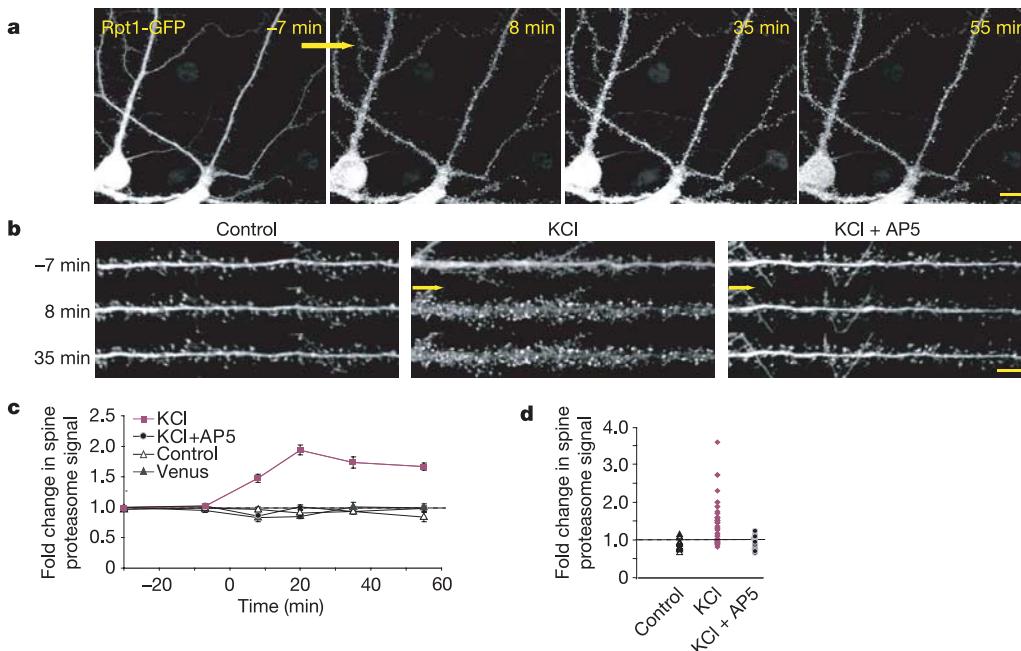


Figure 1 | A GFP-labelled proteasome subunit, Rpt1-GFP, moves into spines upon depolarization. **a**, Time-lapse imaging of Rpt1-GFP-labelled neurons. Arrow indicates time of stimulation with KCl. Scale bar, 11 μ m. **b**, Higher-resolution images of dendrites under the indicated conditions. Scale bar, 6 μ m. **c**, Summary data showing quantification of mean spine signal intensity (from top to bottom, $n = 37, 21, 11$ and 21 cells from 7 independent experiments). The KCl group differs significantly from all others starting at $t = 8$ min ($P < 0.01$). **d**, Change in signal level in spines (at $t = 35$ min) for each individual experiment. Error bars indicate s.e.m.

¹Division of Biology, Howard Hughes Medical Institute, California Institute of Technology, 114-96, Pasadena, California 91125, USA.

central nervous system¹⁷. The NMDA receptor antagonist AP5 ($D(-)$ -2-amino-5-phosphonovaleric acid) blocked KCl-induced movement of both Rpt1-GFP (Fig. 1c, d) and α 4-venus fusion proteins (Supplementary Fig. S1g), indicating that NMDA receptor activity is required. We also observed that direct stimulation with NMDA (20 μ M, 3 min) was sufficient to drive the redistribution of Rpt1-GFP into spines (mean per cent increase in spine signal $35.6 \pm 2.8\%$; $n = 10$ cells per group; $P < 0.05$). Together, these data suggest that NMDA receptor activation specifically recruits proteasomes from dendritic shafts into spines.

We next examined whether endogenous proteasomes show a similar activity-dependent redistribution. First, we observed that most Rpt1-GFP-labelled spines also showed positive immunolabelling for core (20S) endogenous proteasome subunits (Supplementary Fig. S3b and Supplementary Table S1) and actinin (data not shown). We also observed that stimulation (60 mM KCl, 1.5 min) caused a significant increase in the mean intensity of endogenous synaptic proteasome particles, without changing their area or number (Fig. 2a, b). However, the total intensity of the proteasome signal, including all shafts and spines, did not change. Synaptosomes prepared from stimulated hippocampal slices (60 mM KCl, 6 min) revealed a specific increase in the level of synaptic proteasomes, whereas other synaptic proteins showed no change (Fig. 2c, d). Thus, endogenous proteasomes move into synapses during stimulation in both cultured hippocampal neurons and hippocampal slices.

Is proteasome recruitment accompanied by a change in the level of ubiquitinated synaptic proteins? To investigate this, we used an antibody that recognizes poly- and monoubiquitinylated proteins

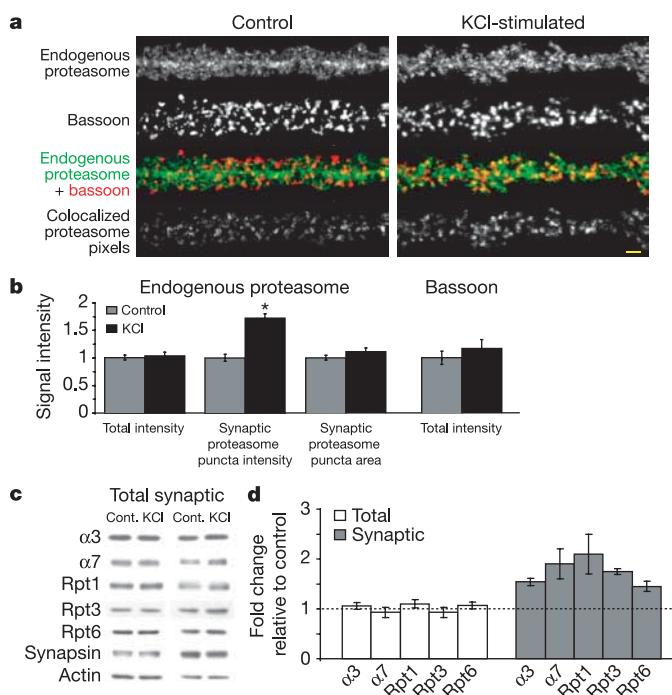


Figure 2 | Endogenous proteasomes move into spines. **a**, Stimulation with KCl also increases endogenous synaptic proteasomes. Immunostaining was performed (12 min after stimulation) using two different antibodies against proteasome components and a presynaptic marker (against bassoon). Scale bar, 3 μ m. **b**, Analysis of endogenous proteasomes (control, $n = 17$ cells; KCl, $n = 24$ cells from 3 independent experiments; $P < 0.05$ for the mean particle intensity), with mean intensity and area of individual proteasome puncta shown. **c**, Western blot analysis of proteasome subunits in total lysate and synaptic fractions. Enrichment of endogenous proteasomes can be detected in synaptosomes prepared from KCl-stimulated slices. Synapsin and actin were used as controls. **d**, Group data for the slice experiment shown in **c** ($n = 3$ experiments; $P < 0.05$). Error bars indicate s.e.m.

but not free ubiquitin (clone FK2)^{2,18}. Depolarization caused an initial $\sim 67\%$ increase in ubiquitin levels in the spine and shaft 10 min after stimulation (Supplementary Fig. S4a, b). At later time points, there was a proteasome-inhibitor-sensitive decrease in ubiquitinated protein levels in spines. Note that the time at which the maximum increase in spine proteasome signal was observed (Fig. 1c) coincides with the time at which we observed the decrease in the ubiquitinated proteins (Supplementary Fig. S4a, b).

We also examined whether stimulation with KCl can locally regulate proteasome activity using a well-characterized, GFP-based proteasomal degradation reporter that harbors a ubiquitin degradation signal (Ub^{G76V} -GFP; refs 19, 20) and loses fluorescence upon degradation. Stimulation of neurons (60 mM KCl, 1.5 min) caused a

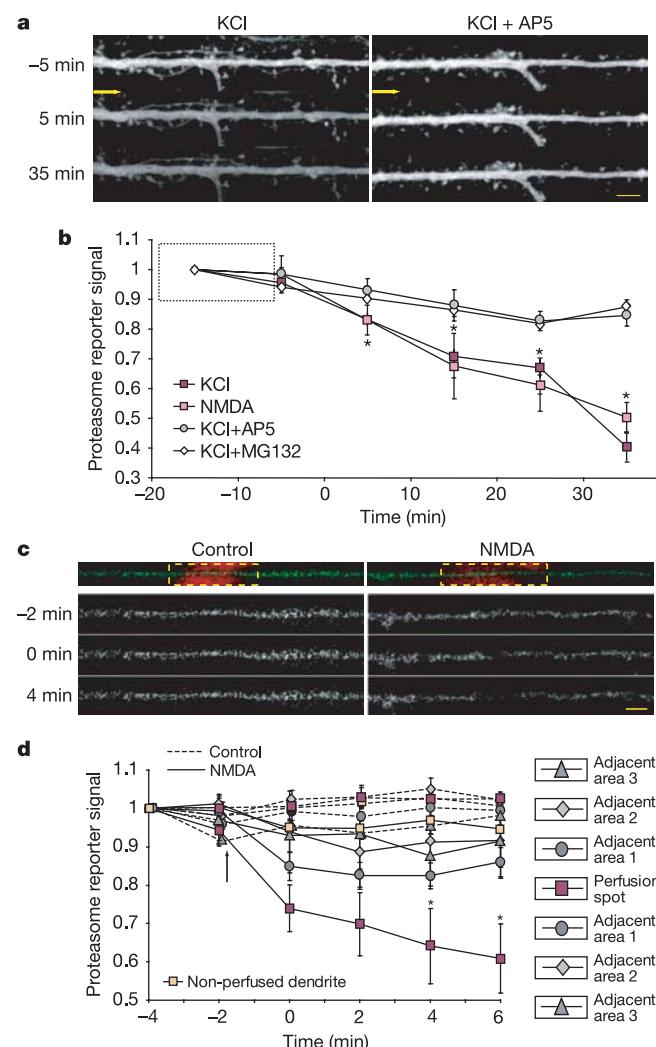


Figure 3 | KCl stimulation increases proteasome activity. **a**, Time-lapse images of dendrites from neurons expressing Ub^{G76V} -GFP. Bath application of KCl (arrow) resulted in an NMDA-receptor-dependent loss of fluorescence, reflecting reporter degradation. Scale bar, 7.5 μ m. **b**, Addition of KCl or NMDA caused a significant drop in fluorescence that was prevented by AP5 or proteasome inhibitor MG132 ($n = 8$ cells per group, 4 independent experiments; $P < 0.05$ at $t = 5$ min and thereafter). The dashed box refers to the region of interest in Supplementary Fig. 5b, c. **c**, Time-lapse images of dendrites from neurons expressing Ub^{G76V} -GFP, locally perfused with NMDA or control solution. The position of the local perfusion is shown in red (yellow box). Perfusion starts at 0 time point. See also Supplementary Fig. S5d, e. Scale bar, 3.5 μ m. **d**, Change in reporter signal at the perfused, adjacent and non-perfused dendrites ($n = 4$ cells per group, 3 independent experiments; $P < 0.05$ at $t = 4$ min and thereafter). Error bars indicate s.e.m.

rapid proteasome-inhibitor-sensitive (Supplementary Fig. S5a) and NMDA-receptor-dependent decrease in the reporter signal, indicating proteasome activity during depolarization (Fig. 3a, b). Consistent with this, direct activation of NMDA receptors (20 μ M NMDA, 3 min) was sufficient to decrease the reporter signal (Fig. 3b). Closer inspection indicated a more rapid loss of fluorescence in the spines (within \sim 2 min) than in the shaft (Supplementary Fig. S5b, c). This was followed by apparently equal rates of reporter degradation in the spines and shaft, possibly owing to diffusion of the reporter from the shaft to the spine (where it would be degraded). To examine whether proteasomes can be activated locally, we perfused NMDA locally and monitored reporter fluorescence (Supplementary Fig. S5d, e). There was a spatially restricted decrease in the intensity of the reporter signal at the perfused spot (\sim 60% decrease), whereas adjacent areas showed only very modest reductions (\sim 15%) (Fig. 3c, d) and non-perfused dendrites showed only a 5% decrease (Fig. 3d). In control (no NMDA) perfusions, there was no change in the GFP signal (Fig. 3c, d). These results show that local protein degradation can occur in dendrites and can be activated by NMDA receptor activity.

To understand the dynamics underlying proteasome enrichment in the spines, we monitored Rpt1-GFP fluorescence recovery after photobleaching (FRAP). In the absence of stimulation, individual spines showed \sim 50–65% recovery of the spine Rpt1 signal after a single or multiple bleaching episodes (Fig. 4a, c). To examine how synaptic stimulation alters the dynamics of proteasome localization, we monitored FRAP in the same individual spines before and after stimulation with KCl. Before stimulation, spines showed about 65% fluorescence recovery (Fig. 4b, c). After stimulation with KCl, which

resulted in an increase in the spine Rpt1 signal (Fig. 4b), there was a dramatic decrease in fluorescence recovery, down to \sim 10% (Fig. 4b, c). Control, unbleached spines showed minimal fluorescence during the FRAP experiment. The decreased recovery of Rpt1-GFP in spines can not be explained by a decrease in the shaft source fluorescence as decreasing the shaft source fluorescence deliberately (by bleaching) to a reduced level comparable to that observed following stimulation had no effect on the extent of Rpt1-GFP fluorescence recovery (Fig. 4d). We thus conclude that the immobile fraction of spine proteasomes increases from 35% to 90% upon stimulation, indicating that the proteasome is actively sequestered in spines.

We reasoned that sequestration of proteasomes in spines may be due to a decrease in the rate of proteasome exit from the spines. To test this, we performed fluorescence loss in photobleaching (FLIP) experiments by repeatedly bleaching an area of the dendritic shaft and monitoring the loss of Rpt1 signal in the adjacent spine (and a control spine) after each bleaching episode. Before KCl stimulation, repeated bleaching of the shaft was associated with a dramatic loss of Rpt1 spine fluorescence owing to movement of fluorescently tagged proteasomes out of spines and into the shafts, where they subsequently underwent bleaching (Fig. 4e, f). After stimulation, there was an increase in the Rpt1-GFP signal in spines that was much more resistant to repeated bleaching of the shaft (Fig. 4e, f). Analysis showed that stimulation caused an increase in the immobile fraction of Rpt1 in the spines (from \sim 16% to \sim 90%). Together, our FRAP and FLIP experiments reveal that stimulation with KCl enhances the proteasome entry rate by \sim 1.5-fold and decreases the exit rate by at

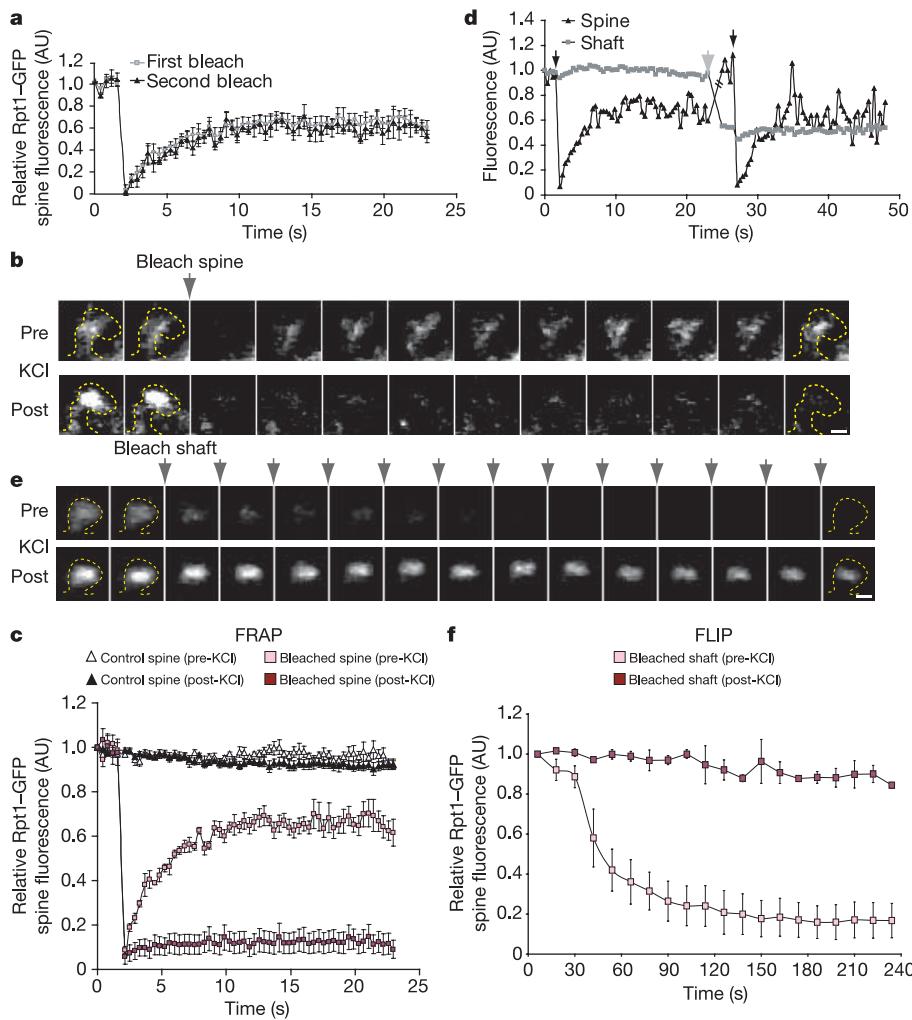
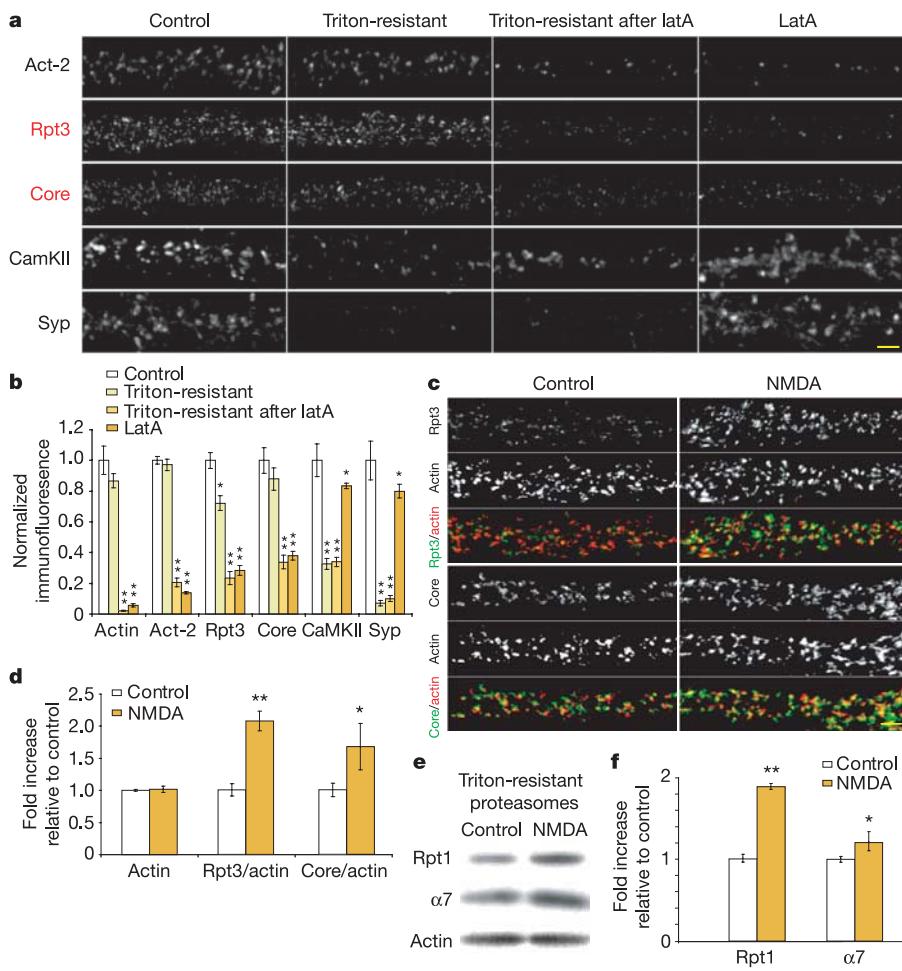


Figure 4 | Photobleaching of Rpt1-GFP indicates the tight association of proteasomes with spines. **a**, In the absence of KCl stimulation, FRAP in spines is similar with repeated bleaching ($n = 9$ cells from 4 independent experiments). Fluorescence levels are shown in arbitrary units (AU). **b**, In a representative FRAP experiment, a single spine was bleached (arrow) before (pre-) and after (post-) KCl stimulation. Fluorescence recovery is greatly reduced after stimulation. Scale bar, 0.75 μ m. **c**, Bleaching of spines was followed by significant recovery of fluorescence before KCl stimulation ($\tau = 2.9 \pm 2.0$ s), but recovery was significantly reduced after stimulation ($\tau = 2.0 \pm 0.5$ s; $n = 9$ cells from 4 experiments; $P < 0.01$). (τ , time constant.) The fluorescence signal in each group is normalized to itself at $t = 0$. **d**, FRAP, monitored in a single spine, was equivalent before and after bleaching (to 50%) of the dendritic shaft ($n = 3$). Parallel diagonal lines on the curve indicate the start of the second FRAP. **e**, Representative FLIP experiment in which Rpt1 fluorescence in a single spine is monitored after repeated bleaching (arrows) of the associated dendritic shaft, before and after KCl stimulation. With KCl stimulation (note the enhanced Rpt1 signal in the spine), there is much less fluorescence loss in the spine after shaft bleaching, indicating reduced mobility of Rpt1. Scale bar, 0.75 μ m. **f**, Analysis of FLIP experiments ($n = 4$ cells from 3 independent experiments; $P < 0.01$). Each group is normalized to itself at $t = 0$. (FLIP pre-stimulation τ , 38.3 ± 2.4 s; post-stimulation, the data were better fit by a linear function.) Error bars indicate s.e.m.

least sixfold. We suggest that the proteasome sequestration induced by stimulation is largely due to a decreased exit rate from spines (possibly due to enhanced protein–protein interactions), and to a much lesser extent due to the increased entry rate.

How do proteasomes become concentrated in spines? To study the potential association of proteasomes with the cytoskeleton, we performed detergent extraction experiments²¹. (Proteins resistant to detergent extraction are more tightly associated with the cytoskeleton.) A large proportion of the proteasomes could not be extracted with detergent: ~70–80% of proteasome labelling remained after detergent extraction compared to non-extracted cultures (Fig. 5a, b). In fact, proteasome subunits more closely resembled the cytoskeleton-associated protein α -actinin-2 than other synaptic proteins²¹ that are less tightly associated with the cytoskeleton (Fig. 5a, b). The partial extractability of the proteasome was also confirmed by western blot analysis (data not shown). To estimate the fraction of proteasomes associated with the actin cytoskeleton in the Triton-resistant population, we incubated neurons with the actin-disrupting agent latrunculin A before detergent extraction²¹. Latrunculin A treatment increased the detergent extractability of proteasomes to a similar extent as α -actinin (Fig. 5a, b), suggesting that the fraction of proteasomes targeted by detergent extraction is mostly associated with the actin cytoskeleton. On the basis of these data, we estimate that ~50% of the proteasomes in dendrites are associated with the actin cytoskeleton. We confirmed this partial association by immunolabelling for proteasome subunits and actin (Supplementary Fig. S3c and Supplementary Table S1). Furthermore, after treatment with latrunculin A alone, we observed that most of the proteasome puncta disappeared (Fig. 5a, b). Latrunculin A treatment affected α -actinin in a similar manner, but did not affect



synaptophysin or calcium/calmodulin-dependent protein kinase II (CaMKII) greatly (Fig. 5a, b). Together, these data suggest that a substantial fraction of the proteasomes in hippocampal neurons is associated with the actin cytoskeleton.

To test whether activity regulates the association of proteasomes with the actin cytoskeleton, we stimulated hippocampal cultures (20 μ M NMDA, 1 min), detergent-extracted and then immunostained for proteasome subunits and actin. NMDA stimulation significantly increased the number of actin-associated proteasome subunits (Fig. 5c, d). Immunoblotting of the detergent-resistant proteasome population also revealed a significant increase in the level of proteasomes that were not extracted (that is cytoskeleton-associated) compared to the unstimulated controls (Fig. 5e, f). These data show that stimulation with NMDA leads to an increase in proteasome association with the actin cytoskeleton, suggesting a plausible mechanism for its sequestration after stimulation. In contrast, there seems to be little involvement of the microtubule-based cytoskeleton (Supplementary Fig S6a, b)^{22,23}.

Recent studies of ubiquitin-proteasome system (UPS) regulation have emphasized the importance of substrate recognition and ubiquitination by the enzymes of the conjugation pathway²⁴. Downstream of ubiquitination, E3 and other proteins²⁵ have been proposed to ‘deliver’ the target proteins to the proteasome. The converse possibility, involving movement of a proteasome to the target protein, is an emerging concept²⁶, with much less direct experimental support. It is worth noting, however, that biochemical studies have shown an apparent interaction of proteasomes with proteins physically proximal to degradation targets²⁶. In addition, movement of proteasomes between the cytoplasm and nucleus has been observed during mitosis and meiosis²⁷.

Figure 5 | The proteasome associates with the actin cytoskeleton. **a**, Immunostaining for proteasome subunits, α -actinin-2 (Act-2), CaMKII and synaptophysin (Syp) in control neurons and neurons exposed to Triton X-100 or latrunculin A alone or sequentially. Scale bar, 4 μ m. **b**, Group data for **a** ($n = 25$ cells per group, 3 independent experiments). Asterisk, $P < 0.05$; two asterisks, $P < 0.01$. **c**, Stimulation with NMDA increases the number of actin-bound proteasome subunits. Only proteasome puncta that are associated with an actin puncta are shown. Scale bar, 4 μ m. **d**, Proteasome colocalization values were normalized to the actin staining within each dendrite ($n = 40$ from 3 independent experiments; $P < 0.01$ for Rpt3 and $P < 0.05$ for core subunits). **e, f**, Stimulation with NMDA increases the pool of proteasomes associated with the actin cytoskeleton ($n = 4$ independent experiments, $P < 0.01$ for Rpt1 and $P < 0.05$ for α 7). Error bars denote s.e.m.

The activity-dependent dynamics described here suggest that proteasomes can be recruited to locally sculpt the protein composition of the synapse, providing on-site degradation rather than serving as something akin to a remote garbage-disposal site. In support of this idea, others have observed the association of proteasomes with the cytoplasmic tails of receptor complexes²⁸, suggesting a capacity for rapid remodelling of membrane-associated receptor complexes^{3,4,7–15}. Here we show dynamic, NMDA receptor-dependent redistribution of proteasomes from dendritic shafts to spines. Together with the detection of more polyribosomes in potentiated spines²⁹, our data suggest the capacity for local remodelling of spines through the coordinated synthesis and degradation of proteins^{13–15}. In our experiments, the long-lasting nature of proteasome recruitment to the spines indicates the possibility of local and persistent remodelling that outlasts the stimulation period.

METHODS

See Supplementary Information for detailed methods.

Viral constructs. Rpt1-GFP was cloned into the pSinRep5 vector (Invitrogen) from the pBS-Rpt1-GFP-HA plasmid¹⁶. Rpt1-GFP-IRES-mRFP was generated from the pSinRep5-Rpt1-GFP clone by inserting IRES and mRFP sequences downstream of the Rpt1-GFP sequence. To generate the α 4-venus Sindbis virus, the α 4 cDNA (MGC accession 2581897) was cloned upstream of venus sequence (pCS2-venus; ref. 30) in pSinRep5. The degradation reporter Ub^{G76V}-GFP construct (refs 19, 20) was cloned into pSinRep5 from the EGFP-N1-Ub^{G76V}-GFP vector.

Cultured hippocampal neurons and live imaging. Dissociated postnatal (P)1–2 rat hippocampal neuronal cultures (18–21 days *in vitro*) were used in all experiments. Images were acquired on a Zeiss LSM 510 microscope ($\times 40$ oil-immersion objective) for tagged proteasome experiments and on an Olympus IX50 microscope ($\times 40$ objective) for proteasome activity experiments.

Statistical analysis. Unpaired, two-tailed Student's *t*-tests and one-way analysis of variance (ANOVA) were used for statistical analyses. All data were tested for normal distribution.

Received 28 December 2005; accepted 30 March 2006.

- Hegde, A. N. et al. Ubiquitin C-terminal hydrolase is an immediate-early gene essential for long-term facilitation in *Aplysia*. *Cell* **89**, 115–126 (1997).
- Campbell, D. S. & Holt, C. E. Chemotropic responses of retinal growth cones mediated by rapid local protein synthesis and degradation. *Neuron* **32**, 1013–1026 (2001).
- Burbea, M., Dreier, L., Dittman, J. S., Grunwald, M. E. & Kaplan, J. M. Ubiquitin and AP180 regulate the abundance of GLR-1 glutamate receptors at postsynaptic elements in *C. elegans*. *Neuron* **35**, 107–120 (2002).
- Ehlers, M. D. Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. *Nature Neurosci.* **6**, 231–242 (2003).
- Zhao, Y., Hegde, A. N. & Martin, K. C. The ubiquitin proteasome system functions as an inhibitory constraint on synaptic strengthening. *Curr. Biol.* **13**, 887–898 (2003).
- Pak, D. T. & Sheng, M. Targeted protein degradation and synapse remodeling by an inducible protein kinase. *Science* **302**, 1368–1373 (2003).
- Patrick, G. N., Bingol, B., Weld, H. A. & Schuman, E. M. Ubiquitin-mediated proteasome activity is required for agonist-induced endocytosis of GluRs. *Curr. Biol.* **13**, 2073–2081 (2003).
- Colledge, M. et al. Ubiquitination regulates PSD-95 degradation and AMPA receptor surface expression. *Neuron* **40**, 595–607 (2003).
- Bingol, B. & Schuman, E. M. A proteasome-sensitive connection between PSD-95 and GluR1 endocytosis. *Neuropharmacology* **47**, 755–763 (2004).

- van Roessel, P., Elliott, D. A., Robinson, I. M., Prokop, A. & Brand, A. H. Independent regulation of synaptic size and activity by the anaphase-promoting complex. *Cell* **119**, 707–718 (2004).
- Juo, P. & Kaplan, J. M. The anaphase-promoting complex regulates the abundance of GLR-1 glutamate receptors in the ventral nerve cord of *C. elegans*. *Curr. Biol.* **14**, 2057–2062 (2004).
- Dreier, L., Burbea, M. & Kaplan, J. M. LIN-23-mediated degradation of β -catenin regulates the abundance of GLR-1 glutamate receptors in the ventral nerve cord of *C. elegans*. *Neuron* **46**, 51–64 (2005).
- Steward, O. & Schuman, E. M. Compartmentalized synthesis and degradation of proteins in neurons. *Neuron* **40**, 347–359 (2003).
- Bingol, B. & Schuman, E. M. Synaptic protein degradation by the ubiquitin proteasome system. *Curr. Opin. Neurobiol.* **15**, 536–541 (2005).
- Yi, J. J. & Ehlers, M. D. Ubiquitin and protein turnover in synapse function. *Neuron* **47**, 629–632 (2005).
- Ennenkel, C., Lehmann, A. & Kloetzel, P. M. GFP-labeling of 26S proteasomes in living yeast: insight into proteasomal functions at the nuclear envelope/rough ER. *Mol. Biol. Rep.* **26**, 131–135 (1999).
- Isaac, J. T. Postsynaptic silent synapses: evidence and mechanisms. *Neuropharmacology* **45**, 450–460 (2003).
- Fujimuro, M., Sawada, H. & Yokosawa, H. Production and characterization of monoclonal antibodies specific to multi-ubiquitin chains of polyubiquitinated proteins. *FEBS Lett.* **349**, 173–180 (1994).
- Lindsten, K., Menendez-Benito, V., Masucci, M. G. & Dantuma, N. P. A transgenic mouse model of the ubiquitin/proteasome system. *Nature Biotechnol.* **21**, 897–902 (2003).
- Dantuma, N. P., Lindsten, K., Glas, R., Jelline, M. & Masucci, M. G. Short-lived green fluorescent proteins for quantifying ubiquitin/proteasome-dependent proteolysis in living cells. *Nature Biotechnol.* **18**, 538–543 (2000).
- Allison, D. W., Gelfand, V. I., Spector, I. & Craig, A. M. Role of actin in anchoring postsynaptic receptors in cultured hippocampal neurons: differential attachment of NMDA versus AMPA receptors. *J. Neurosci.* **18**, 2423–2436 (1998).
- Kurz-Isler, G. Induction of paracrystalline arrays by vincristine in the synaptic formations of the teleost retina. *Cell Tissue Res.* **191**, 75–82 (1978).
- Setou, M. et al. Glutamate-receptor-interacting protein GRIP1 directly steers kinesin to dendrites. *Nature* **417**, 83–87 (2002).
- Fang, S. & Weissman, A. M. A field guide to ubiquitylation. *Cell. Mol. Life Sci.* **61**, 1546–1561 (2004).
- Miller, J. & Gordon, C. The regulation of proteasome degradation by multi-ubiquitin chain binding proteins. *FEBS Lett.* **579**, 3224–3230 (2005).
- Glickman, M. H. & Ravich, D. Proteasome plasticity. *FEBS Lett.* **579**, 3214–3223 (2005).
- Gordon, C. The intracellular localization of the proteasome. *Curr. Top. Microbiol. Immunol.* **268**, 175–184 (2002).
- Ferrell, K., Wilkinson, C. R., Dubiel, W. & Gordon, C. Regulatory subunit interactions of the 26S proteasome, a complex problem. *Trends Biochem. Sci.* **25**, 83–88 (2000).
- Ostroff, L. E., Fiala, J. C., Allwardt, B. & Harris, K. M. Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. *Neuron* **35**, 535–545 (2002).
- Nagai, T. et al. A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. *Nature Biotechnol.* **20**, 87–90 (2002).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank the Kloetzel, Masucci and Kennedy laboratories for providing the Rpt1(CIM5)-GFP, Ub^{G76V}-GFP and mRFP clones, respectively. We also thank members of the Schuman laboratory, especially C.-Y. Tai and S. Kim, and former member G. Patrick for discussions. E.M.S. is an Investigator of the Howard Hughes Medical Institute.

Author Information Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to E.M.S. (schumane@caltech.edu).