Phosphatidylinositol-(3,4,5)-trisphosphate (PIP$_3$) is among the most elusive phosphoinositides. Basal levels of PIP$_3$ are extremely low, owing to a tight spatial and temporal regulation of PIP$_3$ synthesis (Vanhaesebroeck et al., 2001). Nevertheless, PIP$_3$ is enriched in specific subcellular compartments, such as the tip of growing neurites (Aoki et al., 2005; Ménager et al., 2004) and thin protrusions from dendritic spines known as spinules (Ueda and Hayashi, 2013). The synthesis of PIP$_3$ by phosphatidylinositol 3-kinas (PI3Ks) at the postsynaptic terminal is necessary for sustaining synaptic function, possibly by maintaining AMPA receptors (AMPARs) at the postsynaptic membrane (Arendt et al., 2010). In addition, PI3K activity has been linked to long-term potentiation (LTP) (Arendt et al., 2010; Man et al., 2003; Opazo et al., 2003; Sanna et al., 2002). Conversely, we have shown that the phosphatase activity of PTEN (phosphatase and tensin homolog deleted on chromosome ten), which antagonizes PI3K signaling by decreasing PIP$_3$ levels (Maehama and Dixon, 1999), depresses synaptic transmission and is specifically required for NMDA receptor (NMDAR)-dependent long-term depression (LTD) (Jurado et al., 2010). Therefore, upregulation of the PIP$_3$ pathway is generally associated with synaptic potentiation, whereas downregulation of the pathway is linked to synaptic depression (Knafo and Esteban, 2012; Peineau et al., 2007). However, this bidirectional correlation is far from clear. For example, a recent report suggests that PIP$_3$ levels actually decrease during LTP induction owing to a local redistribution (Ueda and Hayashi, 2013). Moreover, PI3K activity has also been linked to LTD (Kim et al., 2011). Therefore, the local dynamics of PIP$_3$ at synaptic compartments during plasticity are still unresolved.

One difficulty in studying the regulation of PIP$_3$ and its relation to synaptic function has been to visualize its compartmentalization and dynamics in living neurons. Overexpression of PIP$_3$-specific pleckstrin homology (PH) domains fused to GFP has been used to monitor changes in PIP$_3$ levels or distribution upon exogenous cell-wide activation of PI3K (Gray et al., 1999; Varnai et al., 2005), but they are not sensitive enough to detect basal PIP$_3$ levels or small local changes. The development of a PIP$_3$-specific PH domain tagged with a fluorescence resonance energy transfer (FRET) reporter has allowed real-time imaging of changes in PIP$_3$ levels in subcellular compartments (Aoki et al., 2005; Sato et al., 2003), including dendritic spines (Ueda and Hayashi, 2013). Using this reporter, together with biochemical experiments, we now show that PIP$_3$ undergoes a constant turnover at dendritic spines under basal conditions. Surprisingly, we found that both LTD and LTP trigger a similar increase in PIP$_3$ synthesis, which is dampened specifically during LTD because of the phosphatase activity of PTEN.
RESULTS
Detection of PIP₃ turnover under basal conditions
To investigate the basal distribution and dynamics of PIP₃ in living neurons, we used the fllip-pm reporter, an intramolecular FRET construct that undergoes FRET when bound to PIP₃ (Sato et al., 2003). We expressed fllip-pm in organotypic hippocampal slice cultures (see Materials and Methods) and carried out live confocal microscopy imaging experiments. Fllip-pm was expressed throughout CA1 pyramidal neurons (Fig. 1A), including the cell body, dendrites and dendritic spines (Fig. 1A,B). YFP and CFP fluorescence upon CFP excitation was quantified from spines and adjacent dendrites, and FRET efficiency was estimated as the YFP:CFP ratio after background subtraction (see representative examples in Fig. 1C). Average values of YFP:CFP ratios in dendritic spines are shown in Fig. 1D. To note, absolute acceptor:donor emission ratios (YFP:CFP) are not numerically equivalent to FRET efficiencies, because of the optical bleed-through between the channels. However, changes in FRET will be reflected in changes in YFP:CFP ratio, as is commonly used (Lam et al., 2012; Tsutsui et al., 2008). To confirm that the YFP:CFP ratio obtained under our optical conditions reflected a FRET reaction, we carried out similar experiments with a modified fllip-pm construct lacking the YFP FRET acceptor (ΔYFP). The YFP:CFP value obtained with ΔYFP was significantly lower than that obtained with fllip-pm (Fig. 1D, P<0.0001), indicating a net contribution of FRET to the YFP:CFP ratio obtained with fllip-pm.

We then evaluated the basal synthesis and turnover of PIP₃ in dendritic spines. To this end, we pharmacologically inhibited PTEN, the lipid phosphatase that converts PI(3,4,5)P₃ into phosphatidylinositol 4,5-bisphosphate [PI(4,5)P₂], with 15 nM bpV(HOpic) [dipotassium bisperoxo(5-hydroxypyridine-2-carboxyl)oxovanadate], a specific PTEN inhibitor (Schmid et al., 2004). As shown in Fig. 1D (“bpV”), PTEN inhibition produced a significant increase in FRET values in spines (P<0.0001; see a representative example in Fig. 1C). Conversely, inhibition of the PIP₃-synthesizing enzyme PI3K with 10 μM LY294002 (LY; a specific PI3K inhibitor Vanhaesebroeck et al., 2001) resulted in a significant decrease in FRET signal in the spine, as compared to the baseline value (Fig. 1D, P=0.01; see

![Image](https://example.com/image.png)

**Fig. 1.** PIP₃ levels in dendritic spines are detectable by FRET and undergo basal turnover. (A) Representative example of a CA1 pyramidal neuron expressing fllip-pm. Images of CFP and YFP fluorescence upon CFP excitation are shown. (B) Schematic of YFP and CFP fluorescence (fluoresc.) quantification at spines and dendritic shafts obtained from images taken using confocal microscopy. FRET signal is approximated as the ratio of YFP:CFP fluorescence after background subtractions. a.u., arbitrary units. (C) Example of FRET images calculated as pixel-by-pixel ratios from YFP and CFP fluorescence images. Fllip-pm-expressing neurons were left untreated (left-most image) or were treated with bpV(HOpic) (15 nM) or LY294002 (10 μM), as indicated, for 1 h before imaging. Second left-most image was obtained from a neuron expressing ΔYFP. These ratio images are shown for visualization purposes – comparisons of FRET values for statistical purposes were always carried out using integrated YFP and CFP intensities, from which the YFP:CFP ratio was then calculated. (D) Average spine and dendrite values of YFP:CFP ratios from slices expressing ΔYFP (black column) or fllip-pm without treatment (white column) or with 15 nM bpV(HOpic) (light gray) or 1-h treatment with 10 μM LY294002 (dark gray). For comparison across conditions, green and red lines show baseline YFP:CFP values for Fllip-PM and ΔYFP, respectively. n, number of spines. Data show the mean+s.e.m.; statistical significance was calculated according to the Mann–Whitney test.
representative spine in Fig. 1C). To note, this value cannot be lower than the YFP:CFP ratio with ΔYFP, which represents the lowest possible ratio in the absence of FRET (Fig. 1D, red line). These results confirm the interpretation that the flip-pm FRET signal is indeed reporting PIP3 levels in spines. In addition, these data indicate that basal PIP3 in spines undergoes a constant metabolic turnover in resting (not stimulated) neurons, under the action of synthetic (PI3K) and degradative (PTEN) activities.

**Transient increase in PIP3 synthesis during LTD induction and modulation by PTEN**

Induction of NMDAR-dependent LTD has been shown to inhibit the downstream PI3K effector Akt, and consequently to activate GSK3β (Peineau et al., 2007). In agreement with these biochemical observations, both PTEN (Jurado et al., 2010) and GSK3β (Peineau et al., 2007) are required for LTD. By contrast, a specific isoform of PI3K has been recently shown to be required for LTD (Kim et al., 2011). Therefore, we decided to directly monitor PIP3 levels in dendritic spines during LTD. Induction of NMDAR-dependent LTD was carried out with a 5-min application of 20 μM NMDA – a protocol termed ‘chemical LTD’ (cLTD). This is a well-established protocol to induce a form of NMDAR-dependent LTD that electrophysiologically and biochemically mimics synaptically induced LTD (Fernández-Monreal et al., 2012; Lee et al., 1998) (supplementary material Fig. S1A). Example images of spines before (baseline), during (cLTD) and after (washout) cLTD induction are shown in Fig. 2A (upper panels). Quantification of YFP:CFP ratios from these images revealed no significant change in the spine or dendrite throughout the experiment (scatter plots of spine populations in Fig. 2C, left panels; average FRET values in spines and dendrites in Fig. 2D, gray columns). These results suggest that LTD induction proceeds without net changes in PIP3 levels.

We have previously reported that the PIP3 phosphatase PTEN is recruited to the postsynaptic membrane upon NMDAR activation during LTD induction, and is required for NMDAR-dependent LTD expression (Jurado et al., 2010). These results suggest that PTEN might participate in the modulation of PIP3.
levels during LTD. To directly test this hypothesis, we repeated the cLTD imaging experiments with slices pretreated for 1 h with the specific PTEN inhibitor bpV(HO)pic (15 nM; the inhibitor was also present during LTD induction and washout). As shown in Fig. 2B, in the absence of PTEN activity, LTD induction led to a significant increase in PIP$_3$ levels in both spines and the adjacent dendritic shaft, with respect to baseline values (see representative examples in Fig. 2A, middle and lower panel rows). It should be noted that this increase occurs on top of the already elevated baseline levels generated upon PTEN inhibition (Fig. 1D). These changes were transient as PIP$_3$ levels gradually decayed to baseline values after the LTD induction (Fig. 2B). This increase in PIP$_3$ signal was detectable in most spines as demonstrated with population scatter plots (Fig. 2C, upper right panel), where FRET values are plotted for each individual spine, with the x-axis representing the YFP:CFP ratio at baseline and the y-axis representing the corresponding value at different time points during the LTD experiment. The population left-shift from the diagonal at 5 min cLTD indicates that most spines had increased FRET values with respect to their baseline at this time point. This left-shift is transient, as the population is equally scattered at both sides of the diagonal by the end of the washout following LTD induction (Fig. 2C, lower right panel). Interestingly, this increase in PIP$_3$ is associated with the spine. As shown in Fig. 2D (white column), there was no significant increase in PIP$_3$ signal in the dendritic shaft sampled away from spines (>2 μm). Taken together, these results indicate that induction of NMDAR-dependent LTD does not decrease PIP$_3$ levels. On the contrary, LTD induction has the potential to increase PIP$_3$ in the spines and the immediately adjacent dendritic shaft, but this increase is normally masked by the activity of PTEN.

**Transient increase in PIP$_3$ levels during LTP induction**

Another major form of NMDAR-dependent synaptic plasticity in the hippocampus is LTP. Several reports have suggested a role for PI3K in both the induction and expression of LTP (Arendt et al., 2010; Chen et al., 2005; Horwood et al., 2006; man et al., 2003; Opazo et al., 2003; Sanna et al., 2002). However, it has been recently reported that LTP induction triggers a redistribution of PIP$_3$ within spines, without net synthesis or degradation (Ueda and Hayashi, 2013).

We have explored this issue by expressing flipl-pm in organotypic slice cultures and measuring PIP$_3$-induced FRET changes in spines and adjacent dendrites while inducing LTP using a pharmacological protocol (cLTP). This protocol drives global neuronal bursting, resulting in robust synaptic potentiation (Kopec et al., 2006; Otmakhov et al., 2004; supplementary material Fig. S1B). Images were taken before (baseline), during (cLTP) and after (washout) LTP induction. Fluorescence signals were analyzed as described above and FRET values were normalized to the average baseline values for each spine and dendrite. Example images of the change in FRET signals of spines undergoing cLTP are shown in Fig. 3A.

As shown in Fig. 3B, LTP induction produced a small but significant increase in PIP$_3$ signal in dendritic spines (P < 0.001) and adjacent dendritic shafts (P = 0.02), which gradually decayed to baseline values shortly after induction (Fig. 3B). In contrast to LTD, PTEN inhibition did not significantly alter the extent or duration of PIP$_3$ accumulation in response to LTP induction (Fig. 3B, blue; shown only for spines, for simplicity). Population scatter plots indicate that the increase in PIP$_3$ was a generalized effect across most spines (leftward shift from the diagonal in Fig. 3C, upper left panel) and was transient (equivalent scatter across the diagonal in Fig. 3C, lower left panel). Again, a similar behavior was observed in the presence of the PTEN inhibitor (Fig. 3C, right panels). The increase in PIP$_3$ was still localized to the vicinity of dendritic spines, as dendritic regions away from the base of spines (>2 μm) did not display a significant change in PIP$_3$ during LTP induction (Fig. 3D, white column).

Importantly, this increase in PIP$_3$ signal was dependent on NMDAR activation and PI3K activity, as demonstrated by the blockade of the YFP:CFP increase when LTP was induced in the presence of either the NMDAR antagonist APV (DL-2-amino-5-phosphonopentanoic acid, 0.1 mM) or the PI3K inhibitor LY294002 (10 μM) (Fig. 3D, gray columns). These inhibitors were present in the perfusion solution during baseline, LTP induction and washout. These results indicate that the accumulation of PIP$_3$ upon NMDAR activation is due to net synthesis by PI3K and not to PIP$_3$ redistribution.

As a control, similar experiments carried out with ΔYFP did not show changes in YFP:CFP ratio (Fig. 3D, purple columns), indicating that the observed increase with flipl-pm is not due to changes in CFP optical bleed-through. Additionally, we also monitored potential changes in intrinsic YFP fluorescence by directly exciting the YFP fluorophore in flipl-pm with a different laser line (514 nm). As shown in Fig. 3E, no changes in YFP fluorescence were observed during cLTP experiments. These two important controls support our interpretation that the LTP-induced changes in YFP:CFP ratio are due to PIP$_3$-driven FRET and not to optical or spectral changes in CFP or YFP fluorescence.

**Upregulation of the PIP$_3$ pathway during LTP induction**

Our observation of increased PIP$_3$ levels upon LTP induction contrasts with previously published results (Ueda and Hayashi, 2013). Therefore, we decided to validate these data with an independent biochemical approach. To this end, we performed enzyme-linked immunosorbent assay (ELISA) quantifications of PIP$_3$ on synaptosomal extracts from organotypic slices during cLTP experiments. Owing to the sensitivity of the technique, a large number of slices (35–45) are required for each synaptosomal fractionation (see Materials and Methods). Therefore, an n value of 1 in this experiment represents the average of 35–45 slices. As shown in Fig. 4A, PIP$_3$ levels increased during cLTP induction and returned to basal levels after washout. This result supports the FRET data, indicating that PIP$_3$ levels transiently increase in dendritic spines (Fig. 3) and in synaptosomal extracts (Fig. 4A) during cLTP induction. In addition, this biochemical method reports absolute levels of PIP$_3$ (see legend for Fig. 4A). Therefore, the approximately twofold increase in PIP$_3$ observed with this assay more likely reflects the actual change in PIP$_3$ with LTP, which is then translated into a 10% change in FRET signal (Fig. 3B,D) under our optical conditions.

An increase in PIP$_3$ levels upon LTP induction would be expected to activate downstream effectors of this pathway, such as Akt. To verify this observation in our experimental system, we performed immunoblotting analysis with antibodies against phosphorylated Akt. As shown in Fig. 4B, phosphorylation of Akt at both Ser473 and Thr308 was significantly increased during cLTP induction. These data show that downstream effectors of the PIP$_3$ pathway are transiently activated during LTP induction.
It is important to keep in mind that synaptosomal extracts are expected to contain both pre- and postsynaptic elements. In addition, synaptosomal fractionations do not yield pure synaptic compartments and, therefore, some degree of extrasynaptic membrane is also likely to be present in these preparations. Nevertheless, taken together, these data strengthen our interpretation that LTP induction is accompanied by a rapid upregulation of PIP3 levels at synaptic compartments and activation of downstream signaling effectors.

**DISCUSSION**

In this study we have reported, for the first time, that PIP3 is transiently upregulated at dendritic spines during synaptic plasticity induction upon NMDAR activation. It should be noted that this effect is likely due to the activation of PI3K and not to a transient inhibition of PTEN or redistribution of PIP3 within the cell, because these changes are observed in the presence of PTEN inhibitors and are blocked by inhibitors of PI3K activity. Interestingly, in the case of NMDAR-dependent LTD, the potential increase in PIP3 is specifically quenched by the activity of PTEN.

Concerning LTP, our results contrast with a recent publication where PIP3 concentration was found to decrease upon induction of LTP (Ueda and Hayashi, 2013). The authors concluded that this decrease was not due to PIP3 degradation but, rather, was due to a non-enzymatic dilution of PIP3 upon spine enlargement during structural plasticity. Our pharmacological protocol for LTP induction produces a more modest increase in spine size than the glutamate uncaging protocol used in the previous study (Ueda and Hayashi, 2013). Therefore, it is possible that we were able to detect a net increase in PIP3 levels owing to a lesser volume dilution in our system. In addition, using synaptosomal fractionations and biochemical detection, we confirmed the increase in PIP3 levels and the activation of downstream effectors of this pathway (Akt phosphorylation) upon LTP induction.

What are the mechanisms by which both LTP and LTD induction would trigger PI3K activation? It has been shown that activation of NMDARs during LTP leads to the activation of Ras (Harvey et al., 2008) and downstream signaling cascades (Kim et al., 2003; Komiyama et al., 2002; Li et al., 2006; Schmitt et al., 2005; Zhu et al., 2002). In turn, Ras can activate PI3K by direct...
interaction with the p110 catalytic subunit of class I PI3Ks, leading to the activation of its lipid kinase activity and synthesis of PI3P (Suire et al., 2002). Downstream effectors of PI3P would lead to the activation of Akt and ERK pathways (Knafo and Esteban, 2012; Perkinton et al., 2002; Sutton and Chandler, 2002; Sweatt, 2004). Growing evidence indicates that key elements of this pathway are localized in dendritic spines (Majumdar et al., 2011; Man et al., 2003; Peineau et al., 2007), supporting the notion that both PI3P synthesis and downstream signaling might be highly compartmentalized for the local control of postsynaptic function during plasticity.

Does this scenario apply to LTD? Interestingly, two distinct Ca2+-dependent Ras activators, RASGRF1 and RASGRF2, have been implicated in LTD and LTD, respectively, by coupling to different MAPK pathways (Li et al., 2006). However, it has also been reported that Ras activity is not required for LTD (Zhu et al., 2001), which is also an effector of AMPAR internalization (Inoue et al., 2012; Perkinton et al., 2002; Sutton and Chandler, 2002; Sweatt, 2004). Growing evidence indicates that key elements of this pathway are localized in dendritic spines (Majumdar et al., 2011; Man et al., 2003; Peineau et al., 2007), supporting the notion that both PI3P synthesis and downstream signaling might be highly compartmentalized for the local control of postsynaptic function during plasticity.

In conclusion, we have reported here that PI3P is tightly regulated by the activation of its lipid kinase activity and synthesis of PI3P (Suire et al., 2002). Downstream effectors of PI3P would lead to the activation of Akt and ERK pathways (Knafo and Esteban, 2012; Perkinton et al., 2002; Sutton and Chandler, 2002; Sweatt, 2004). Growing evidence indicates that key elements of this pathway are localized in dendritic spines (Majumdar et al., 2011; Man et al., 2003; Peineau et al., 2007), supporting the notion that both PI3P synthesis and downstream signaling might be highly compartmentalized for the local control of postsynaptic function during plasticity.
Expression of recombinant proteins

The flip-pm construct was generously provided by Yoshio Umezawa (University of Tokyo, Japan). AYFP was derived by removing part of the PH domain and YFP from the original flip-pm. These constructs were recloned into pSinRep5 for expression using Sindbis virus (Schlesinger and Dubensky, 1999). Recombinant proteins were expressed in organotypic hippocampal slice cultures (Gerges et al., 2005). Briefly, hippocampal slices were prepared from young rats (postnatal days 5–7) and placed in culture on semipermeable membranes. After 4–5 days in culture, the recombinant gene was delivered into the slice using Sindbis virus. This method led to robust expression of the recombinant proteins after 16–24 h, when imaging experiments were carried out.

Antibodies

Antibodies used were against total Akt (Cell Signaling Technology, catalog number 8805; RRID, AB_331163) and phospho-Ser473 Akt (Cell Signaling Technology, catalog number 4060; RRID, AB_331163) and phospho-Ser473 Akt (Cell Signaling Technology, catalog number 4060; RRID, AB_331163).

Fluorescence resonance energy transfer

Fluorescence images were collected by confocal microscopy using a 405-nm Blue Diode laser as the light source for excitation. CFP and YFP fluorescence emission were collected on separate photomultipliers using band-pass filters. The sensitivity of the photomultipliers was constant throughout all experiments to allow comparison of YFP:CFP ratios across different experiments. Laser power was varied to compensate for variability in expression levels of the construct. Digital images were analyzed using NIH ImageJ or Zeiss Zen software. FRET efficiency was then estimated as the ratio of YFP:CFP fluorescence. Under this optical configuration, a fraction of the CFP signal ‘leaked’ into the YFP channel (as evidenced with the ΔYFP construct, Fig. 1). This implies that the YFP:CFP ratio would be larger than zero even in the absence of FRET. This signal bleed-through was purely optical and constant irrespective of FRET conditions. Therefore, we did not attempt to correct for it.

Pharmacological treatments during live imaging

Imaging experiments were performed on organotypic slice cultures at 5–10 days in vitro after expression of the recombinant protein for 24 h. Images of dendritic branches and spines were taken using the optical settings described above. For acute manipulations of PIP3 levels, slices were treated for cLTP induction as described above and were then homogenized in a Dounce glass homogenizer with a buffer containing 0.32 M sucrose, 1 mM MgCl2, 0.5 mM CaCl2, 10 mM HEPES, 1 mM EGTA, 1 mM dithiothreitol and a cocktail of protease inhibitors from Roche (Complete Mini EDTA-free). Proteins from this homogenate were quantified by using the Bradford assay and the same quantity of protein (around 1 mg) for the different conditions was spun down at 1400 g for 10 min at 4°C. The supernatant was kept and the pellet was resuspended in the same homogenization buffer and spun again at 710 g for 10 min at 4°C. Both supernatants were mixed and spun down at 11,600 g for 12 min at 4°C. The pellet (crude synaptosomal fraction) was resuspended in cold 8% trichloroacetic acid and centrifuged at 1500 g for 5 min. The pellet was then washed with a solution of 5% trichloroacetic acid and 1 mM EDTA, vortexed to resuspend and centrifuged again (215 g, 5 min). To remove neutral lipids, we added 1 ml of methanol:CHCl3 (2:1), vortexed at room temperature and centrifuged at 215 g for 5 min. The supernatant was discarded and acidic lipids were then extracted from the pellet by resuspending in 1 ml of methanol:CHCl3:12 M HCl (80:40:1), vortexing at room temperature and centrifuging (215 g, 5 min). The supernatant was then mixed with 333 μl of CHCl3 and 666 μl of 0.1 M HCl, vortexed and centrifuged at 215 g for 5 min. The organic (lower) phase was collected and dried in a clean vial using a vacuum dryer. Dried lipids were used for PIP3 quantification using the Mass ELISA Kit K-2500 (Echelon) according to the manufacturer’s instructions.

Acknowledgements

We thank the personnel at the fluorescence microscopy facility of the CBMSO (SMOC) for their expert technical assistance, and members of the Esteban laboratory for their critical reading of the manuscript. We also thank Yoshio Umezawa (University of Tokyo, Japan) for his generous gift of the flip-pm construct.

Competing interests

The authors declare no competing interests.

Author contributions

K.L.A. and M.B. carried out most of the experimental work. A.L. performed the electrophysiology experiments and J.E.D. carried out some of the imaging experiments. M.M. prepared some of the reagents. K.L.A., M.B. and J.A.E. designed research, analyzed data and wrote the paper.

Funding

This work was supported by grants from the Spanish Ministry (grant numbers CSD-2010-00045 and SAF-2011-24730) and Fundación Ramón Areces to J.A.E. M.B. is the recipient of a postdoctoral contract from the Instituto de Salud Carlos III; and a research award from the Fondation Bettencourt-Schuller (France).
Supplementary material

Supplementary material available online at http://jcs.biologists.orglookup/suppl/doi:10.1242/jcs.156554/-/DC1

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Fig. S1. Electrophysiological recordings of chemical LTD and LTP. (A) Time course of the amplitude of evoked excitatory postsynaptic currents (EPSC) during a clTD experiment. clTD is induced by a 5 min bath application of 20 μM NMDA (black bar). Responses are normalized to the average baseline response (before NMDA application). ‘n’ represents number of cells (only one cell is recorded per slice). Statistical significance is calculated with a paired non-parametric test (Wilcoxon test), comparing the last 5 min of the recordings with the baseline, for each cell. Representative traces averaged from baseline (thin lines) or from the last 5 min of recording (thick lines) are plotted above the time course. (B) Time course of the slope of field excitatory postsynaptic potentials (fEPSPs). fEPSPs are employed in this case to avoid washout of intracellular factors during baseline. clTP is induced by 15 min perfusion with ACSF lacking MgCl₂ and 2-chloroadenosine and containing 0.1 μM rolipram and 50 μM forskolin (black bar). Synaptic stimulation is resumed 20 min after returning to regular ACSF perfusion. ‘n’ represents number of slices. Statistical significance is calculated with a paired non-parametric test (Wilcoxon test), comparing the last 5 min of the recordings with the baseline, for each slice. Representative traces averaged from baseline (thin lines) or from the last 5 min of recording (thick lines) are plotted above the time course. Note the appearance of the population spike after clTP induction. This was present in most recordings after LTP, and is also indicative of synaptic potentiation. Data show mean±s.e.m.