the divided-patch case, although the only change from the occluded condition is the contrast of the superimposed cross. This is consistent with the divided-patch stimulus being perceived as four discrete parts that are not amodally completed, as in Bregman's compelling demonstration with the broken Bs (ref. 12). Thresholds decrease further in the multiple-patch case when the patches are maximally separated, indicating that proximity is also a factor.

These results show that image parsing affects the integration of local speed signals across space. The most dramatic demonstration of this effect is the significant increase in thresholds (19.7%) from the divided to the occluded condition, despite identical moving regions in both stimuli. A control experiment examined whether this increase was due to an increase in cross contrast *per se*. Simply increasing cross contrast from 10 to 30% caused no significant change in thresholds ($P = 0.41$, $n = 4$). For the four observers tested in both experiments, going from the divided to the occluded condition caused an average threshold increase 3.9 times larger than that seen in the increased-contrast control. The increase in thresholds from the divided to the occluded condition, and from the in-phase to the banana condition, in conjunction with our earlier results[6], suggests that speed is estimated by pooling speed samples and that effectively only one independent sample is available from each image region that has been parsed as a distinct entity.

It has been shown that the detection of the trajectory of a moving dot embedded in noise can be disrupted if the trajectory is periodically broken by brownian motion, and is not restored by segregating the intervening brownian motion by colour or depth[13]. Although trajectory detection is sufficiently different from speed discrimination to make a direct comparison difficult, one interpretation of this result that is consistent with ours is that the brownian motion in the occluder does not affect local motion estimates in the non-occluded regions, but rather interferes with factors that would otherwise link parts of the motion trajectory together.

Several factors that potentially explain our results can be ruled out. Bounding contour length has little effect as thresholds remain largely unaffected by increasing the circumference of a patch (Fig. 1*c*) or by superimposing an occluder (Fig. 2) Furthermore, we have shown previously[6] that eye movements, uncertainty about stimulus location, and variation in perceived speed with eccentricity[14], are unlikely to cause the high thresholds in the case of a single patch. However, proximity and phase do appear to influence speed discrimination: bringing the patches closer and making their phase relationships consistent with a single grating cause thresholds to increase (Fig. 2). The grouping of different parts of the image into a single entity appears to make multiple local speed estimates inaccessible, in much the same way that observers are unable to access component speed when viewing coherently moving plaids[15,16]. How might this occur? If the responses of speed-tuned units to multiple patches become increasingly correlated as the patches are brought closer or fused, the benefit of pooling speed information from multiple patches would be reduced[17,18]. Alternatively, inhibitory pooling from surrounding units, either by subtractive[19–21] or divisive[22,23] mechanisms, could decrease the response to extended stimuli, lowering the signal-to-noise ratio and thus increasing thresholds.

The fact that the thresholds in our experiment remain unchanged in the presence of partial occlusion is consistent with the results of He and Nakayama[24,25], who advocate an early parsing of the image into surfaces. Our results extend this view by showing that the parsing of multiple patches, even on a single surface, affects speed perception. Furthermore, there is mounting physiological evidence that neurons in early visual processing areas such as V1 (ref. 26) and MT (ref. 27) are sensitive to image segmentation cues located outside their classical receptive field. Our data provide complementary psychophysical evidence that has important implications for the interaction between local neural mechanisms that code speed. The effect of both proximity and segmentation on speed discrimination suggest that local speed mechanisms do not act in isolation, but rather in assemblies that have both neighbouring and long-range interactions. □

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Bidirectional modification of CA1 synapses in the adult hippocampus *in vivo*

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MEMORIES are believed to be stored by synaptic modifications. One type of activity-dependent synaptic modification, long-term potentiation (LTP), has received considerable attention as a possible memory mechanism, particularly in hippocampus[1]. However, use-dependent decreases in synaptic strength can store information as well. A form of homosynaptic long-term depression (LTD) has been described and widely studied in the CA1 region of the developing hippocampus *in vitro*[2–4]. However, the relevance of this model of LTD to memory has been questioned because of failures to replicate it in the adult brain *in vitro*[5] and, more recently, *in vivo*[6]. Here we re-examine this important issue and find that homosynaptic LTD can in fact be elicited in the adult hippocampus *in vivo*, that it has all the properties described in immature CA1 *in vitro*, and that LTD and LTP are reversible modifications of the same Schaffer collateral synapses. Thus homosynaptic LTD is not peculiar to brain slices, nor is it only of developmental significance. Rather, our data suggest that the mechanisms of LTP and LTD may be equal partners in the mnemonic operations of hippocampal neural networks.

We emulated *in vivo* the precise stimulation and recording configuration that has been used successfully to elicit homosynaptic LTD *in vitro* (Fig. 1*a*). Low-frequency stimulation (LFS; 900 pulses at 1–3 Hz) of the ipsilateral Schaffer collaterals resulted in

a significant ($P < 0.01$) and stable depression of the evoked extracellular excitatory postsynaptic potential (EPSP) (Fig. 1*b*, *c*). The initial slope of the field EPSP 30 min after LFS was $84.4 \pm 5.3\%$ (mean $\pm$ s.e.m.) of the average baseline value for 1 Hz stimulation ($n = 9$) and $82.9 \pm 3.5\%$ for 3 Hz stimulation ($n = 4$).

To assess whether the LTD was restricted only to the synapses that received LFS, we monitored responses to stimulation of a second pathway originating in the contralateral CA1. We found that LFS of the ipsilateral Schaffer collaterals produced LTD only of the responses to stimulation of this input (Fig. 1*c*). Thus, just as in immature hippocampal slices, LFS of the Schaffer collaterals can produce homosynaptic (input-specific) LTD *in vivo*. Figure 1*c* also shows that there is a limit to the magnitude of LTD produced by repeated episodes of LFS.

We assessed the dependence of LTD on stimulation frequency *in vivo* by applying 900 pulses at 10 Hz, a frequency that slice studies suggest produces little net long-term synaptic modification[2]. Our data confirm that 10 Hz stimulation produces no significant change in synaptic response *in vivo*, even if it is delivered following induction of LTP (Fig. 1*d*).

High-frequency stimulation (HFS) after establishment of LTD produced LTP (Fig. 2*a*). However, because the responses are population EPSPs, this finding alone cannot support the conclusion that depressed synapses can subsequently be potentiated. To address this important issue, we first saturated LTD by applying three 1 Hz trains to the Schaffer collaterals (EPSP slope $= 67.6 \pm 4.5\%$ of initial baseline; $n = 3$), and then we produced potentiation relative to this depressed baseline by applying HFS (Fig. 2*b*). Subsequent application of LFS returned the response to the LTD saturation level ($65.1 \pm 7.7\%$ of initial baseline). The simplest interpretation for the observation that HFS unsaturates LTD is that depressed synapses can be potentiated.

We also did the complementary experiment in which LTP was first saturated by applying repeated episodes of HFS (EPSP slope $= 135.7 \pm 10.8\%$ of baseline; $n = 3$), followed by induction of LTD relative to this potentiated baseline (Fig. 2*c,d*). Subsequent application of HFS restored the response to the LTP saturation level ($130 \pm 11.6\%$ of initial baseline). Taken together, these findings strongly suggest that Schaffer collateral synapses in adult CA1 are bidirectionally modifiable *in vivo*.

The characteristics of the homosynaptic LTD we have observed in adult CA1 *in vivo* are identical to those described in a number of slice studies[2-4], suggesting that they are explained by common mechanisms. The mechanism for LFS-induced LTD *in vitro* involves NMDA (*N*-methyl D-aspartate) receptors. Therefore, we did experiments to determine whether LTD *in vivo* is sensitive to NMDA receptor blockade (Fig. 3). Application of 1 Hz conditioning stimulation following administration of the competitive NMDA-receptor antagonist CPP ((±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid; 10 mg kg^{-1} injected i.p.) failed to produce LTD ($100.7 \pm 2.3\%$ of baseline; $n = 4$). As expected, CPP-treated animals also failed to show LTP after HFS[7]. Thus, bidirectional modifications of synapses using low- and high-frequency stimulation in CA1 require activation of NMDA receptors.

LTP has attracted interest as a memory mechanism partly because of its longevity. To investigate the duration of homosynaptic LTD, in several experiments we monitored responses for > 5 h after a single episode of LFS (1 Hz, 900 pulses). In all cases, LTD remained stable for as long as the preparation was viable. An example of stable, long-lasting homosynaptic LTD is shown in Fig. 4*a*. Thus LTD, like LTP, can store information long enough to contribute to a hippocampal memory store.

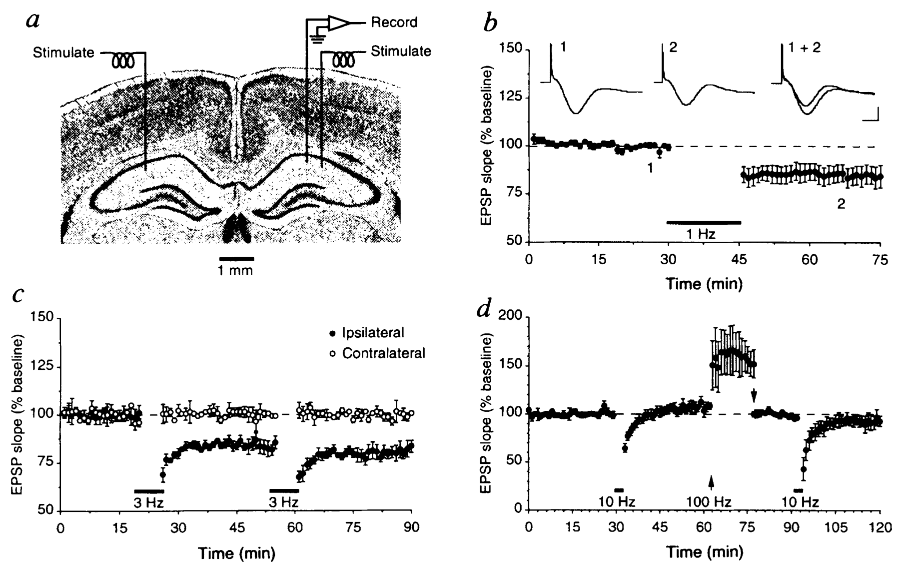

FIG. 1 Induction of homosynaptic LTD using low-frequency conditioning stimulation. *a*, Recording and stimulating electrode configuration in dorsal hippocampus. *b*, 900 pulses delivered at 1 Hz results in LTD of the EPSP slope ($n = 9$). Field potentials (average of 10 consecutive sweeps) are from one case at times indicated by numerals (scale bars: 5 ms, 2.5 mV). *c*, LFS-induced LTD is input-specific and saturable ($n = 5$). *d*, Homosynaptic LTD is frequency dependent. 900 pulses delivered at 10 Hz produces no lasting effect on EPSP slope, irrespective of whether conditioning stimulation is applied 'de novo' or following induction of LTP by high-frequency stimulation (indicated by upward arrow; $n = 5$). For ease of comparison, data have been renormalized after LTP induction (indicated by downward arrow).

METHODS. Adult, male Sprague-Dawley rats (250–500 g) were anaesthetized with sodium pentobarbital (65 mg kg^{-1}, i.p.), placed in a stereotaxic frame, and maintained at 37 ± 0.5 °C. A monopolar recording electrode was positioned in the CA1 stratum radiatum region of the hippocampus of one hemisphere (coordinates in mm from bregma and the midline: 3.5 posterior; 2.3–2.5 lateral). A monopolar stimulating electrode was positioned ~0.2 mm lateral to the recording electrode in order to directly activate the ipsilateral Schaffer collaterals. In some animals a second monopolar stimulating electrode was positioned in a homotopic site in the contralateral hippocampus. All electrodes were constructed from Teflon-insulated stainless-steel wire (75 µm) cut flat at the tip. Final depths of recording and stimulating electrodes were adjusted to optimize the magnitude of the evoked response. Screws inserted into the skull overlying the cerebellum served as recording ground and stimulus anode. Field EPSPs were elicited using stimuli of 0.2 ms duration. Evoked responses were amplified and filtered at 0.1 and 3.0 kHz (1/2 amplitude), digitized at 20 kHz and stored on a computer for later analysis. The initial slope of the field EPSP was used as a measure of the magnitude of the response. At the beginning of each experiment a full input–output curve was generated, and a stimulus strength eliciting an EPSP slope of 50–65% of maximum was used for the remainder of experimentation (30–65 µA). Baseline measurements were collected using single stimuli applied every 15–30 s. LTD was induced using LFS, consisting of 900 pulses delivered at 1 or 3 Hz. To induce LTP, two 1-s bursts of 100 Hz separated by 10 s were applied. At the end of experimentation, rats were killed, their brains removed and sectioned and stained with thionin to verify recording and stimulating electrode placements. Data are expressed as a percentage of the mean response magnitude recorded during the baseline period. Paired or independent two-tailed *t*-tests were used to assess statistical significance. In a small number of cases (< 10%), LFS failed to produce LTD. In these same animals, HFS was also ineffective in producing LTP; these cases were therefore excluded from analysis.

Previous attempts to induce LTD in adult CA1 *in vivo* using LFS have failed[6,8]. In these studies, synaptic responses were evoked in CA1 by stimulating the commissural inputs instead of the ipsilateral Schaffer collaterals. To investigate whether this procedural difference underlies the discrepancy between laboratories, in an additional series of experiments we examined the effects of LFS of the contralateral input. We confirmed that 1 Hz stimulation produces little LTD of the commissural input ($96.2 \pm 1.4\%$ of baseline, $n = 4$; Fig. 4*b*). High-frequency stimulation, however, was still able to produce LTP in these synapses.

Studies of Schaffer collateral LTD in hippocampal slices prepared from adult animals have yielded conflicting results (compare refs 2 and 5). The finding that LTD can be induced in the brain *in situ* is therefore of considerable importance as it demonstrates that LTD is not peculiar to particular conditions *in vitro*. We believe our success in eliciting LTD in Schaffer collateral synapses, both *in vivo* and *in vitro*, is attributable to the proximity of the stimulation and recording electrodes and the use of a restricted range of stimulation intensities[9].

Many studies *in vitro* suggest that homosynaptic LTD results from a critical level of NMDA receptor activation and postsynaptic Ca^{2+} entry[4]. Therefore, it is reasonable to expect that types of stimulation other than the LFS used here may satisfy the conditions for homosynaptic LTD induction. Indeed, repetitive

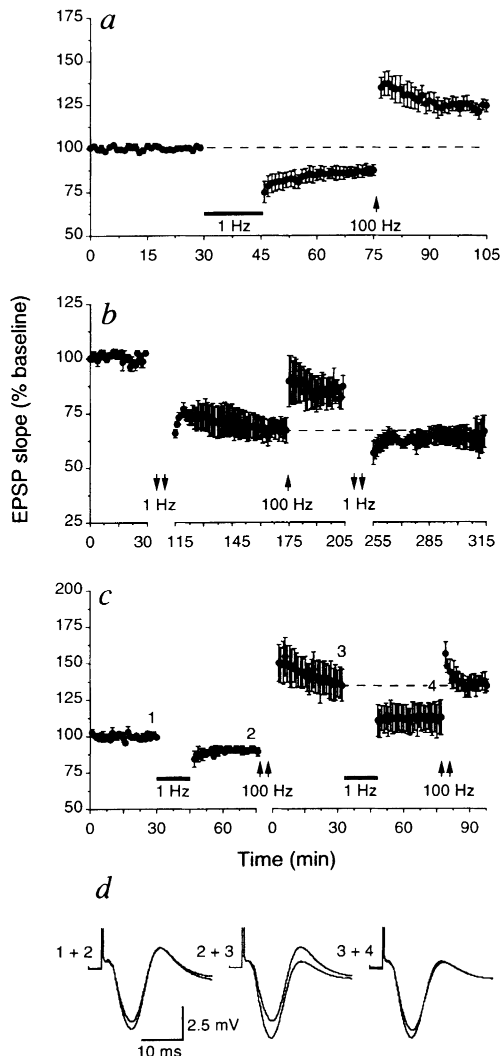

FIG. 2 Bidirectional plasticity in CA1 *in vivo*. *a*, Homosynaptic LTD induced by LFS (EPSP slope = $87.1 \pm 3.4\%$ of baseline) can be fully reversed and replaced with LTP ($123.3 \pm 3.8\%$ of initial baseline) following application of HFS (indicated by upward arrow; $n = 5$). *b*, LTD can be unsaturated by HFS. LTD was saturated by application of three 900-pulse trains of 1 Hz stimulation (downward arrows). The response could be potentiated by HFS (upward arrow) and returned to the LTD saturation level by subsequent application of LFS ($n = 3$). *c*, LTP can be unsaturated by LFS. Following LTD induction, LTP was saturated by application of HFS (3–6 1-sec trains of 100 Hz). The response could then be depressed by application of 900 pulses at 1 Hz and returned to the LTP saturation level by subsequent applications of HFS (2–4 trains, $n = 3$). *d*, Field potentials (average of 10 consecutive sweeps) from one case at times indicated by numerals in *c*.

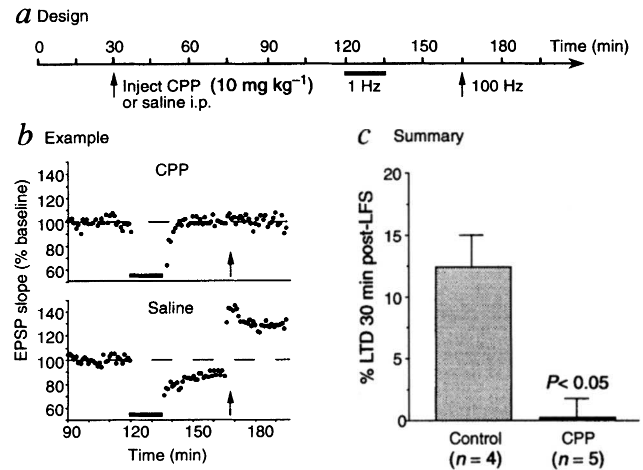

FIG. 3 The NMDA receptor antagonist CPP blocks homosynaptic LTD induction. *a*, Time line depicting experimental design. Animals received CPP ($10\,mg\,kg^{-1}$ i.p.) or an equal volume of saline following 30 min of baseline recording. 90 min following drug administration, 1 Hz conditioning stimulation was applied, followed 30 min later by HFS. *b*, Examples from one animal receiving CPP and a saline control animal. Note the lack of both LTD and LTP in animal receiving CPP. *c*, Data summary.

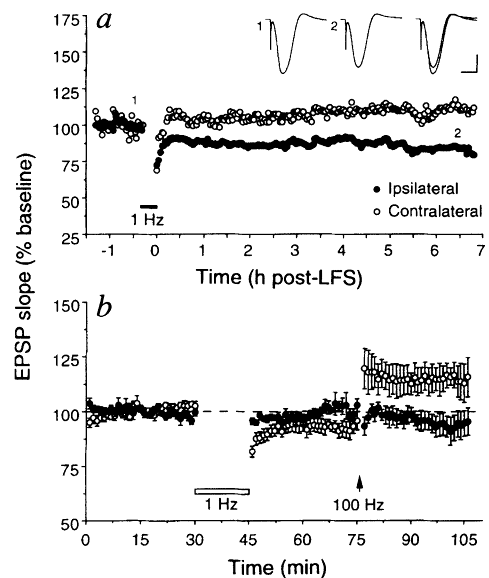

FIG. 4 *a*, Homosynaptic LTD induced by a single episode of LFS (1 Hz, 900 pulses) is stable for as long as the preparation is viable. In this example, LFS of the ipsilateral Schaffer collaterals produced LTD that was stable for > 6.5 h. Displayed field potentials were evoked by stimulation of the ipsilateral Schaffer collaterals (averages of 10 consecutive sweeps) at times indicated by numerals. Scale bars: 2 mV, 10 ms. *b*, Responses evoked by contralateral stimulation of CA1 support little LTD after LFS, but show robust LTP after 100 Hz stimulation ($n = 4$).

paired-pulse stimulation of the commissural input reportedly can produce LTD in CA1 *in vivo*[8]. Although issues of input specificity and bidirectional plasticity were not directly addressed in that study, the LTD was sensitive to NMDA receptor blockade. Thus, commissural LTD and Schaffer collateral LTD may require different types of stimulation patterns, but use the same mechanisms.

Our findings show that homosynaptic LTD is not peculiar to the brain slice preparation, and that synapses in adult CA1 support long-term, bidirectional modifications. Demonstration of bidirectional synaptic plasticity in the adult hippocampus has significant impact on hypotheses concerning how hippocampal neural networks store information. Many CA1 neurons are selective for positions in space, and this selectivity shifts as animals learn new spatial environments[10,11]. Shifts in selectivity require that neurons acquire responsiveness to new stimuli and lose responsiveness to previously effective stimuli[12]. The mechanisms of homosynaptic LTP and LTD, functioning together, are ideally suited to account for this type of receptive field plasticity. □

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A role for the proteasome regulator PA28α in antigen presentation

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Cytotoxic T cells recognize viral proteins as peptide fragments which are produced in the cytosol and transported on major histocompatibility complex (MHC) class I proteins to the cell surface[1]. Viral peptides that meet the stringent binding characteristics of class I proteins are generated by the 20S proteasome[2,3]. The interferon (IFN)-γ-inducible activator of the 20S proteasome, PA28 (refs 4–6), strongly influences the proteasomal cleavage pattern *in vitro*[7]. This led us to investigate whether changes in cellular levels of PA28 affect the efficiency of viral antigen processing. A mouse fibroblast line expressing the murine cytomegalovirus pp89 protein was transfected with either the human or murine gene encoding the PA28α subunit, which is sufficient to activate the peptide-hydrolysing activity of the 20S proteasome *in vitro*. Here we report that enhanced expression of PA28α at a level similar to that obtained after IFN-γ induction resulted in a marked enhancement of recognition by pp89-specific cytotoxic T cells; the presentation of influenza nucleoprotein was also significantly improved. These results demonstrate a fundamental *in vivo* function for PA28α in antigen processing.

Two subunits, PA28α and PA28β, with an apparent relative molecular mass of about 29,000 (M_r 29K) are found in human PA28 activator complexes of the 20S proteasome[8,9]. They form ringlike hexa- or heptameric complexes of $M_r \sim 200$K that bind reversibly to the cylindrical 20S proteasome and activate its peptide-hydrolysing activity[10]. The subunit stoichiometry of PA28 complexes has not yet been determined. The primary structures of the PA28 α and β subunits have 47% identity, and both are inducible by IFN-γ to a comparable extent[11,12]. Only PA28α contains KEKE motifs, which are putatively involved in the interaction between proteasome and PA28 (ref. 13). Complexes of recombinant PA28α protein display biochemical properties and peptide-hydrolysing activity very similar to native, human erythrocyte PA28 complexes[8], so we constitutively overexpressed PA28α in the mouse fibroblast clone B8, which expresses the pp89 immediate early gene product of murine cytomegalovirus (MCMV). The efficiency of antigen processing was monitored by cytotoxic T cells specific for the pp89-derived peptide YPHFMPTNL, presented by the class I allele H-2L^d (ref. 14). Enhanced expression of the human PA28α protein in B8 cells resulted in a markedly enhanced recognition by pp89 peptide-specific cytotoxic T cells. To achieve the same lysis in PA28α transfectants, as compared to B8 wild-type cells, about 20-fold fewer cytotoxic T cells were required. A representative result obtained with B8 wild-type cells and human PA28α transfectants, designated BPαh.1 and BPαh.5, respectively, is shown in Fig. 1*a*. To verify this finding in a homologous system, we cloned the murine PA28α complimentary DNA (A.S., unpublished data) for overexpression in B8 cells. Enhanced expression of the murine protein, which is 94% identical to its human homologue, also led to a similar increase in susceptibility to pp89-specific T cell-mediated lysis (see clones BPαm.4 and BPαm.5 in Fig. 1*a*). This increase in specific lysis was due to the PA28α overexpression only, because transfection of the puromycin-resistance gene alone, or together with LMP2 and LMP7 proteins expressed from the same expression vector, had no effect in cytotoxicity assays (Fig. 1*b*). The deficiency in pp89 antigen presentation of B8 wild-type cells was due to a lack of pp89 nonamer peptide, as exogenous peptide enhanced the recognition of B8 cells to levels obtained with PA28α transfectants (Fig. 1*c*).

To quantify the expression of human and murine PA28α in the recipient clone B8 and in transfectants, quantitative western-blot analysis was performed. Total lysates were blotted and probed with a PA28α-specific polyclonal antibody, and subsequent to stripping the blots were reprobed with an antibody specific for the constitutive proteasome subunit C3. Densitometric scans of the blots shown in Fig. 2*a* yielded expression of PA28α in all clones tested that was threefold higher than in B8 cells after normalization on total protein or C3 expression. PA28α expression in the transfectants was found to be comparable with that following stimulation of B8 cells with IFN-γ for 72 h (Fig. 2*b*). To exclude an effect of PA28α transfection on pp89 expression, we quantified the expression of pp89 protein in B8 cells and PA28α transfectants. Immunoprecipitation of pp89 from lysates of metabolically labelled cells demonstrated that the amount of pp89 expression was identical in B8 cells and its PA28α transfectants (Fig. 2*c*). The PA28α-mediated increase in antigen presentation was not due to a higher pp89 turnover, as no obvious enhancement of pp89 degradation was found for PA28α transfectants in pulse–chase experi-